Lettre de la Société Francophone de Chronobiologie

RYTHMES

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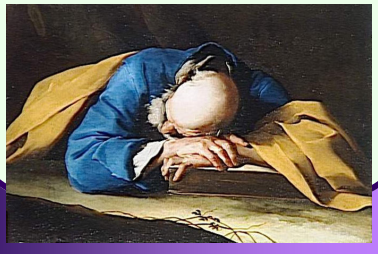
Sommaire



Éditorial

Travailler moins pour dormir plus !

Depuis la rentrée de septembre 2008, la plupart des écoles maternelles et primaires ont adopté la semaine de 4 jours. Ce schéma résultait d'une décision ministérielle, qui laissait cependant une certaine latitude dans son application, et avait été critiqué par différentes instances, dont certains membres de la Société Francophone de Chronobiologie, société ringardisée à l'époque par les pouvoirs publics.

Ce mois de février 2010, la Chronobiologie revient en force sur le devant de la scène à propos de ces rythmes scolaires, sous la forme d'un rapport de l'Académie de Médecine « Aménagement du temps scolaire et santé de l'enfant », rédigé par Yvan Touitou (past président de la SFC) et Pierre Bégué professeur de Pédiatrie. Ce rapport souligne:

- la désynchronisation des enfants, entraînant fatigue et difficultés d'apprentissage,
- le caractère néfaste de la semaine de 4 jours sur les performances et la vigilance des enfants, en particulier pendant les 2 premiers jours de la semaine,
- l'importance de respecter le besoin de sommeil.

Des solutions sont proposées, en particulier celles d'allonger le temps scolaire à 4 jours et demi ou 5 jours, d'augmenter le nombre de jours annuels travaillés mais de diminuer le temps de travail quotidien. Le rapport mentionne que ces propositions doivent s'accompagner d'un aménagement « des temps de vie » des enfants. Ce rapport rejoint l'analyse du groupe d'Odile Rohmer (CEPA-CNRS Strasbourg) de 2005, qui insistait sur l'importance d'un rythme de vie régulier chez l'enfant. A la suite de ce rapport, l'Académie Nationale de Médecine a émis à l'intention des pouvoirs publiques et des parents des recommandations qui, en mettant l'enfant au centre de la réflexion, insistent sur les liens entre temps scolaire et santé de l'enfant.

Les différentes parties prenantes sont désormais au pied du mur, enseignants, parents, responsables des mouvements associatifs, pouvoirs publics et… scientifiques, qui auront donc à refondre l'agenda scolaire dans l'intérêt prédominent des élèves, en envisageant les structures pour accueillir les enfants pendant leur temps libre.

Au-delà de ce problème des rythmes scolaires, purement franco-français, le problè-

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me du sommeil des jeunes générations apparaît particulièrement d'actualité dans le monde Occidental. Ainsi le Journal of Adolescent Health consacre le numéro entier de Février aux habitudes de vie des adolescents et leurs conséquences néfastes sur le sommeil. Environ 70% des élèves du secondaire ne dorment pas suffisamment pendant la semaine. Les étudiants d'aujourd'hui auraient perdu 1 heure et quinze minutes de sommeil en quelques années. Les adolescents et les adultes émergents constituent une population à haut risque de somnolence diurne. Le stress apparaît être un facteur prédictif de troubles du sommeil, tandis que l'exercice physique est un facteur de prévention majeur des effets du stress sur le sommeil.

En écho à une étude présentée lors des rencontres annuelles de l'American Association for the Advancement of Science, le magazine *Science* publie un article intitulé « Lack of sleep is contagious ». Les habitudes de jeunes gens en termes de sommeil et de consommation de substances stupéfiantes y sont appréhendées. L'originalité de l'étude réside dans le fait qu'elle montre que ces habitudes sont perçues à travers le prisme d'une dynamique relationnelle, et notamment l'implication des individus dans des réseaux sociaux sur le web. Un individu a d'autant plus de chance de présenter un temps de sommeil court (de moins de 7h) qu'il est un élément central du réseau. Cartographiés, ces réseaux d'échange et d'influence permettent d'avancer l'hypothèse de l'origine du manque de sommeil par "contagion", sur un modèle de mimesis.

Cette avalanche de littérature dénote l'inquiétude de part le monde des différents partenaires impliqués dans la prévention et le traitement des troubles du sommeil. La pandémie attendue n'était donc pas celle que l'on croyait. A terme, associée au problème de l'obésité, elle ne pourra qu'alimenter l'épidémie qui touche déjà les adultes et les seniors, à savoir l'insomnie chronique.

Bruno Claustrat
Président

In Memoriam Maurice Fontaine 1904 – 2009

Maurice Fontaine était fils d'instituteurs de campagne ; son père était mort au combat pendant la guerre de 1914 ; ces circonstances l'avaient fortement marqué dans son respect des valeurs humaines.

Il s'était fait connaître comme spécialiste des migrations des poissons qui partagent leur existence entre océan et cours d'eau, milieux salés et d'eau douce ; il avait montré un changement dans les mécanismes d'osmorégulation qui précèdent le passage de l'un à l'autre de ces milieux, comportements d'anticipation essentiels en chronobiologie.

On connaît le volume *Physiologie* qu'il a dirigé (1969) dans l'Encyclopédie de la Pléiade. D'un ton tout différent ses *Rencontres insolites d'un biologiste autour du monde* (1991-2001).

Lorsque nous lui avons demandé s'il accepterait de faire partie du Conseil de notre société, et d'en être le président, il était surchargé d'obligations, entre la direction de l'Institut Océanographique et celle du Muséum (incluant le zoo de Vincennes), impliquant, c'était en 1968, les discussions avec nombre de syndicats. Il a accepté sans hésitation.

Il a été le premier président du Groupe d'étude des rythmes biologiques ; il a hébergé au Muséum nos toutes premières assemblées générales. Il présidait notre Conseil avec une affabilité qui l'a imprégné de sérénité scientifique en un temps où il nous fallait prendre de bonnes habitudes. Notre Société lui doit beaucoup.

Lucien Baillaud

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Etienne CHALLET, Secrétaire Général de la SFC
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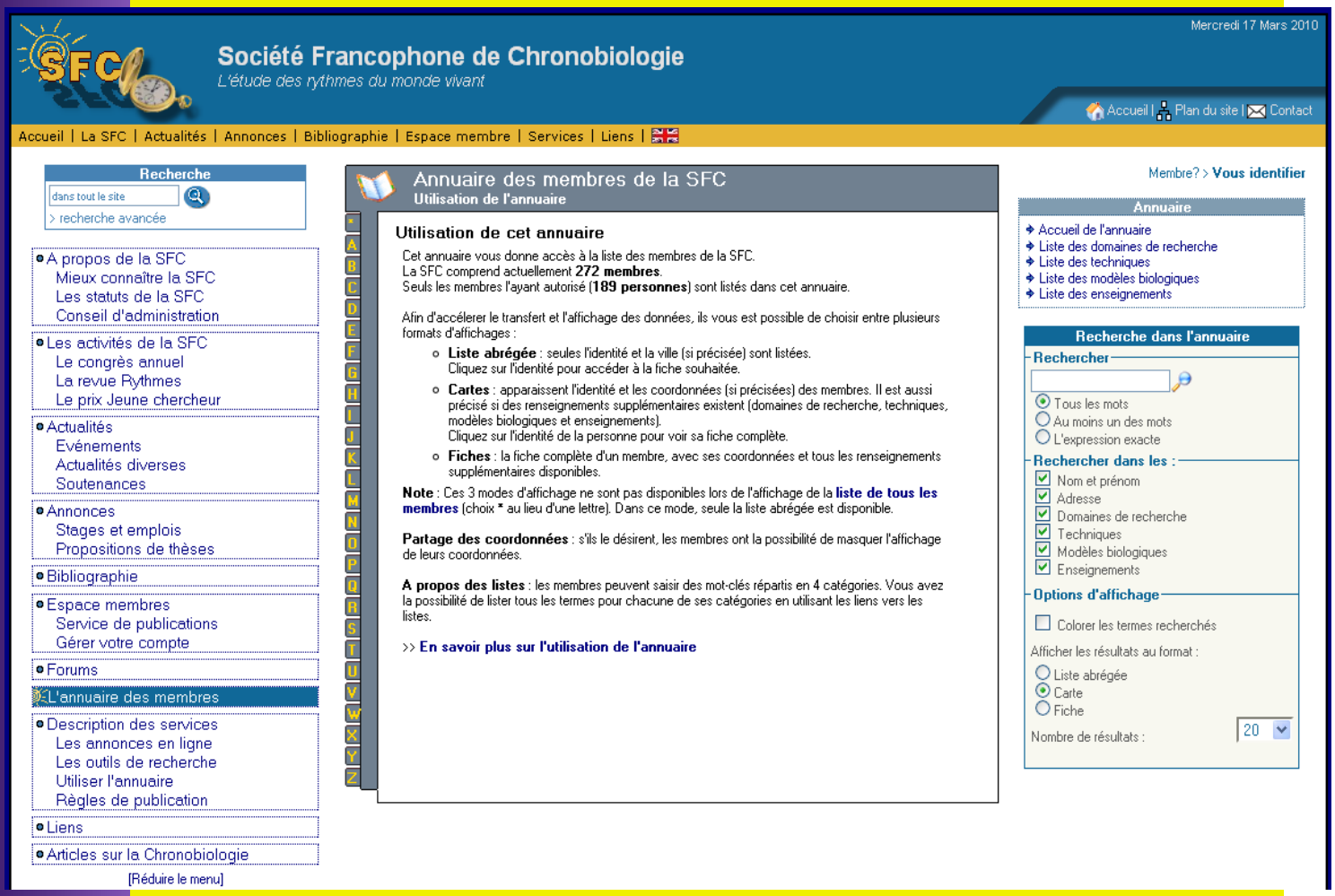
Visitez régulièrement le site Web de la SFC

Le site de la Société Francophone de Chronobiologie est consultable à l'adresse

http://www.sf-chronobiologie.org

Tout comme l'ancien site, il comporte une présentation de la société et de ses activités ainsi qu'un annuaire de ses membres. Chaque membre recevra un courrier avec un nom de login et un mot de passe personnel qui lui donnera un accès personnel pour notamment modifier sa fiche. Le site constitue aussi une riche source d'informations sur la recherche et l'enseignement qui portent sur la chronobiologie, ainsi que sur l'actualité de cette discipline. Je vous laisse explorer le site de manière plus approfondie et compte sur vous tous pour l'alimenter régulièrement et le faire vivre longtemps !

Sophie LUMINEAU



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Message de l'Editrice de RYTHMES

Fabienne AUJARD

Chers amis,

Comme cela a été voté lors de la dernière Assemblée Générale de la SFC qui s'est tenue à Strasbourg en août dernier, des évolutions ont été apportées à la publication de **RYTHMES**. Du fait de l'utilisation de plus en plus massive des moyens de communication informatiques, une grande majorité d'entre vous a opté pour la version électronique du journal. C'est un format que je vous recommande fortement car il est plus économique et plus écologique. En parallèle, une version papier est maintenue afin de satisfaire tous les lecteurs de **RYTHMES**. Des changements vont cependant être apportés afin de faciliter le tirage et l'envoi du journal, dans le but de continuer à respecter la fréquence de publication que nous nous sommes fixée. C'est pourquoi je vous rappelle qu'à partir de ce numéro, les versions papier de **RYTHMES** seront reliées par agrafage et que la cotisation annuelle à la SFC incluant l'inscription automatique à l'EBRS et l'envoi de la version papier de **RYTHMES** est maintenant fixée à **35 €.** La cotisation annuelle incluant l'envoi de **RYTHMES** en version électronique reste à **25 €.**

Différence inter-individuelle face à la privation de sommeil : PER3 un candidat potentiel

Antoine U. Viola

Centre for Chronobiology, Psychiatric Hospital of the University of Basel, Switzerland

Notre physiologie et notre comportement sont deux composantes dépendant de nos rythmes biologiques, affectés, entre autre, par l'alternance jour/nuit. Le bon fonctionnement de ce rythme veille/sommeil est lié au parfait ajustement de nos préférences diurnales ainsi qu'à la qualité du sommeil.

Chez l'homme, ces facteurs sont si variables d'un individu à l'autre qu'il est difficile de distinguer les besoins biologiques des contraintes sociales. Soumises à un contrôle endogène qui demeure partiellement inexpliqué, les différences interindividuelles sont associées à l'alternance jour/nuit ainsi qu'à nos contraintes sociales. Ce cycle temporel veille/sommeil dépend de deux processus oscillants endogènes: l'oscillateur circadien qui est virtuellement indépendant de notre comportement, et l'homéostasie du sommeil (Borbély 1982) qui est principalement sous le contrôle de notre comportement.

Il a été clairement établi que nos besoins en sommeil ou éveil résultent de l'interaction de ces deux processus oscillatoires. Le processus homéostatique, aussi appelé processus S (pour sommeil), dépend directement d'une part de la quantité d'heures d'éveil et d'autre part, de la quantité d'heures de sommeil qui précède (Borbély 1982). Ce processus est ainsi l'expression de la tendance ou du besoin de sommeil. En conséquence, il augmente au fur et à mesure de l'accumulation d'éveil et diminue avec le cumul d'heures de sommeil. Le deuxième processus, nommé Processus C (pour circadien), dépend quant à lui de l'horloge biologique interne et évolue selon un rythme d'environ 24 h (Daan, Beersma et al. 1984). Il représente l'expression ou le besoin circadien de sommeil, et est donc totalement indépendant de la quantité de sommeil ou d'éveil. L'évolution du Processus C se caractérise par une diminution progressive du besoin circadien de sommeil, lequel atteint un minimum vers 16 h. Durant la nuit, la tendance circadienne du sommeil augmente de façon progressive pour atteindre un maximum vers 4 h du matin. L'interaction entre les processus C et S détermine la tendance effective au sommeil d'une personne. Plus précisément, la diminution de la propension circadienne au sommeil (Processus C) durant la journée, vient contrecarrer l'augmentation graduelle de la propension homéostatique du sommeil (Processus S) accumulée durant la journée, ce qui nous permet de rester éveillé durant 16 heures consécutives. De la même manière, l'augmentation de la propension circadienne au sommeil (Processus C) durant la nuit vient contrecarrer la diminution de la propension du Processus S qui survient au fur et à mesure que l'on accumule des heures de sommeil. Ceci nous permet de bénéficier de huit heures de sommeil consolidé. Ce modèle proposé au début des années 80 a subi de nombreuses révisions, mais le principe de base demeure bien établi (Achermann and Borbély 2003; Achermann 2004). Il a été clairement montré que le contrôle central des rythmes circadiens permettant à l'homme de se synchroniser avec son environnement, se situe au niveau du noyau suprachiasmatique (SCN) (Moore 1983). Ainsi, comme illustré dans la figure-1, il est montré que des facteurs tels que notamment la lumière ou l'activité sociale, affectent notre cycle veille-sommeil (Dijk and Lockley 2002). D'importantes variations interindividuelles sont observées au niveau de la préférence diurnale, de la durée de sommeil, de l'activité électrœncéphalographique, et de la sensibilité face au manque de sommeil. Ceci a conduit à mettre en évidence des prédispositions génétiques contrôlant et modifiant tant notre horloge interne que la structure de notre cycle veille-sommeil.

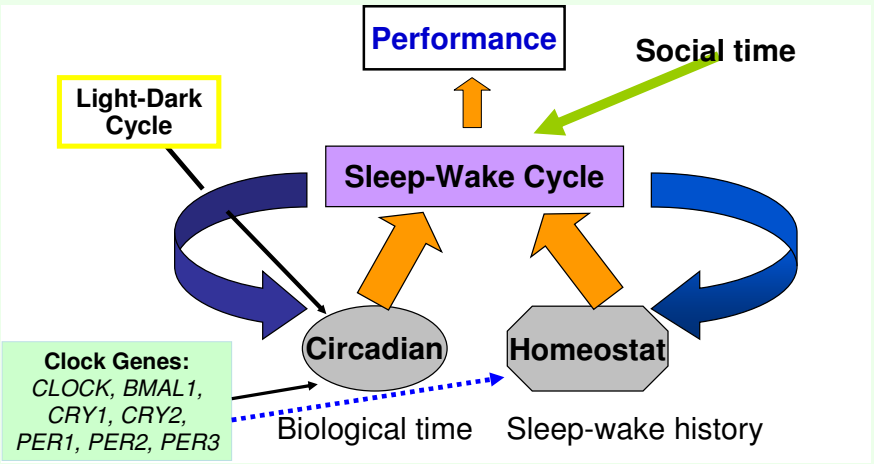

Figure 1 *: Modèle caractérisant la boucle d'autorégulation du rythme veille-sommeil. Modifié selon Dijk and Lockley 2002.*

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Bien que le premier article consacré à la génétique du sommeil publié par Jean-Louis Valatx dans Nature date des années 70, la compréhension des mécanismes génétiques et leurs implications avec les modifications physiologiques n'en sont qu'à leurs débuts.

L'approche moléculaire des rythmes circadiens a permis de caractériser les mécanismes sous-jacents permettant ces adaptations. Il existe ainsi des gènes spécifiques de l'horloge, codant pour des protéines, capables d'exercer un rétrocontrôle sur la transcription de leur propre gène (Schibler 2005). Cette boucle d'autorégulation associée à des modifications post-translationnelles est à la base de la rythmicité, sa durée détermine, quant à elle, la période du rythme. CLOCK et BMAL1, deux protéines de l'horloge appartiennent à la famille des protéines b-HLH (hélice boucle hélice de type basique) à domaine PAS (motif de dimérisation). Des complexes activateurs spécifiques associant une protéine CLOCK ou NPAS2 à une protéine BMAL1 ou BMAL2, qui sous forme hétérodimérique, se lient à des séquences régulatrices de type E-Box présentes dans la région promotrice de gènes spécifiques de l'horloge *PER* et *CRY* mais aussi au niveau du promoteur d'autre gènes cibles. Les complexes inhibiteurs de type *PER:PER* ou *PER:CRY* peuvent rétroagir sur leur propre transcription en empêchant les complexes activateurs d'agir.

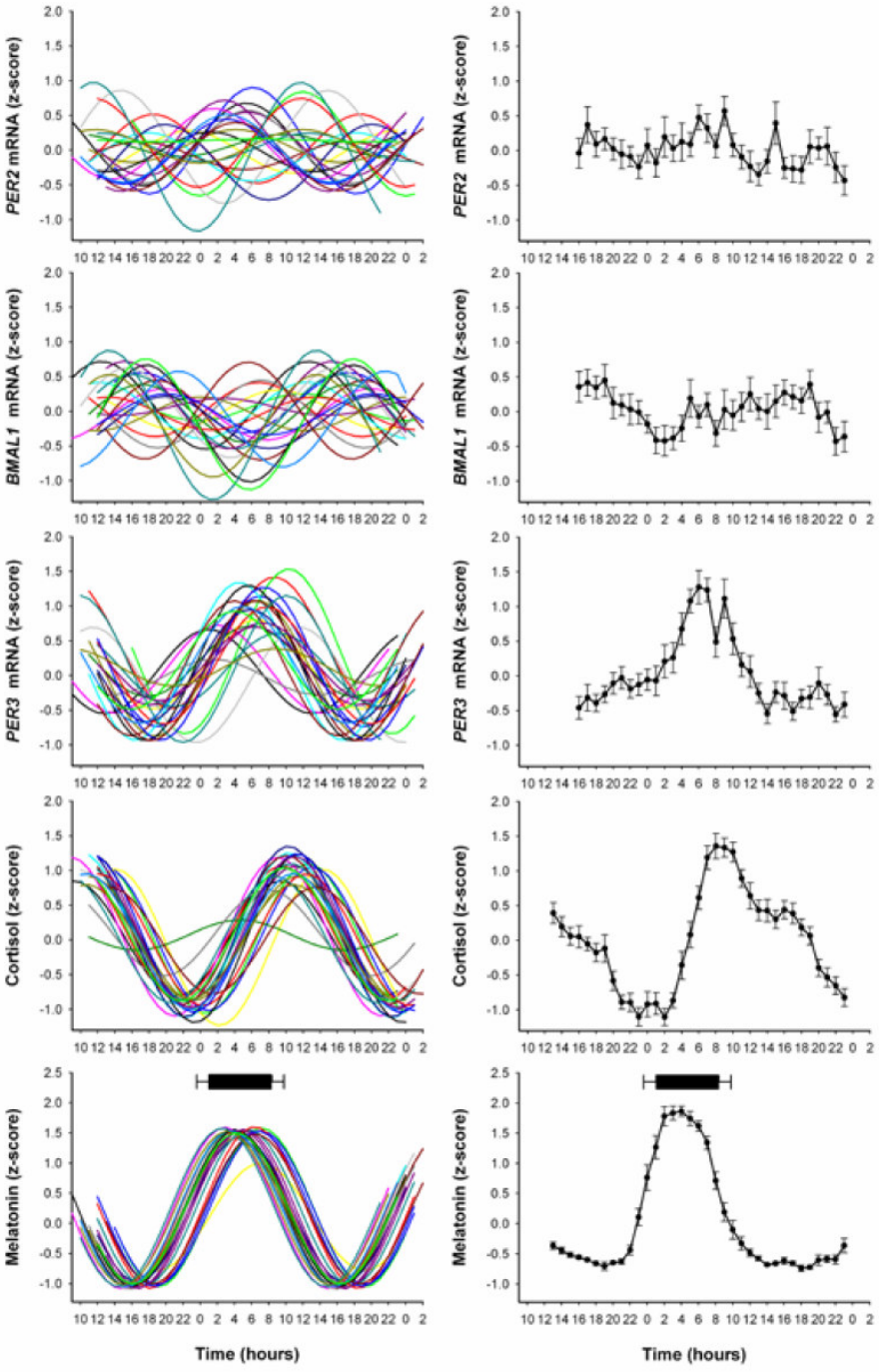

***FIGURE 2** : Oscillations individuelles et moyennes des marqueurs circadiens. Droite: Normalisation modélisée (z-score) des variations individuelles de PER2, BMAL1, PER3, mélatonine et du cortisol. Gauche: Moyenne des marqueurs (± SD). Moyenne (± SD) de l'heure d'endormissement et d'éveil illustré par les barres noires au niveau de la courbe de mélatonine (Archer, Viola et al. 2008).*

Les rythmes ainsi générés et contrôlés par le SCN résultent de cette boucle inhibitrice impliquant ces gènes dit de l'horloge et leurs protéines respectives (Schibler and Naef 2005).

L'un des gènes de l'horloge, le gène *Period 3* (*PER3*) semble au niveau moléculaire fortement impliqué dans cette boucle d'autorégulation. Une étude avec trois volontaires sains a caractérisé pour la première fois un rythme circadien de l'expression de l'ARNm du gène *PER3* (Boivin, James et al. 2003).

Nous avons confirmé ce résultat sur 24 sujets incluant une modélisation mathématique caractérisant un rythme circadien (Archer, Viola et al. 2008). L'expression des gènes *PER2* et *PER3* impliqués dans la boucle d'autorégulation négative ainsi que celle du gène *BMAL1*, connu pour présenter une expression en opposition de phase avec les deux autres,

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ont ainsi été décrites (Archer, Viola et al. 2008). Comparé à *PER2* et *BMAL1*, *PER3* présente une expression circadienne notable, une amplitude significativement plus importante et une faible variabilité de sa phase d'expression. De plus, l'expression de l'ARNm du gène *PER3* est fortement associée avec les rythmes circadiens de la mélatonine et du cortisol. Ceci nous a permis de caractériser l'expression de *PER3* comme un nouveau marqueur du rythme circadien chez l'homme (Figure 2) (Archer, Viola et al. 2008).

Cette étude a ainsi montré que la phase circadienne de l'expression des gènes de l'horloge, évaluée en absence de l'influence de cycle jour-nuit ainsi que de rythme veille-sommeil, est corrélée aux heures de sommeil. Pour la première fois, une étude sur un groupe de 24 jeunes hommes et femmes sains avec une cinétique de 40 h incluant des prélèvements sanguin toutes les heures dans des conditions dites de « routine constante » a permis de démontrer l'association du temps habituel d'endormissement avec l'expression des gènes de l'horloge. L'expression circadienne de *PER3* a également montré une corrélation avec les marqueurs circadiens habituels tels que la mélatonine, le cortisol et le rythme veille-sommeil. Par contre l'expression de *PER2* et *BMAL1* ne présente aucune corrélation significative avec les marqueurs circadiens.

De plus, *PER3* présente un nombre variable de séquences répétées caractérisées par des sites de phosphorylation et déterminant ainsi un polymorphisme (Ebisawa, Uchiyama et al. 2001). Ces séquences sont formées de 18 acides aminés pouvant être répétées 4 fois (*PER3-4*) ou bien 5 fois (*PER3-5*). Trois types de populations peuvent ainsi être caractérisés : les homozygotes *PER3-4/4*, les homozygotes *PER3-5/5* et les hétérozygotes *PER3-4/5*. Le polymorphisme *PER3-5/5*, le moins représenté dans la population (11% des caucasiens), a été associé avec la préférence diurnale dite du matin (se couche tôt et se lève tôt). A l'opposé, le polymorphisme *PER3-4/4* (42% des caucasiens) a été associé avec le syndrome de retard de phase. Cette association pourrait être expliquée par les différences de phosphorylation de la protéine PER3 (Archer, Robilliard et al. 2003). Ainsi, une expression différente au niveau hypothalamique et dans d'autres régions cérébrales serait impliquée dans la régulation du sommeil (Takumi, Taguchi et al. 1998; Uschakov, Gong et al. 2006) et caractériserait cette protéine comme un potentiel candidat dans la régulation des rythmes circadiens et de l'homéostasie du sommeil.

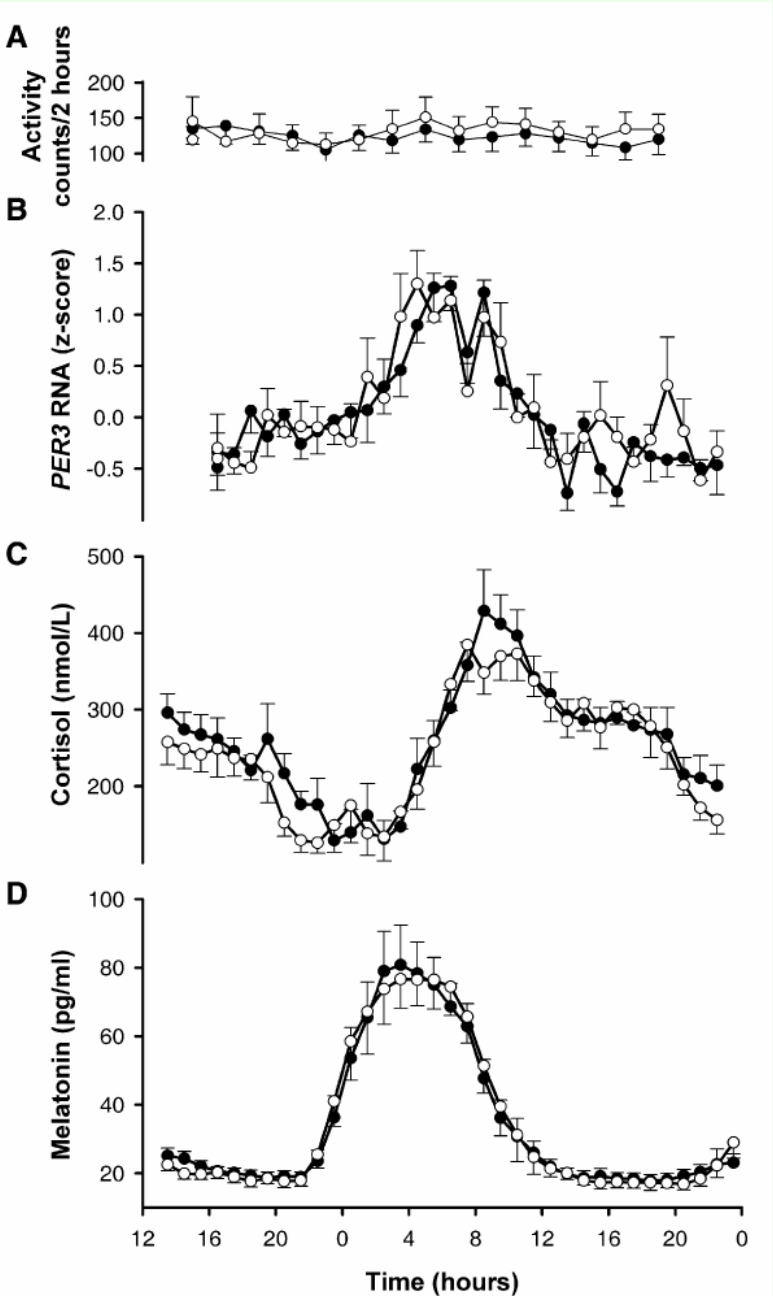

FIGURE 3 *: Les rythmes circadiens ne se différencient pas entre les Homozygotes PER3-5/5 et PER3-4/4 (Viola, Archer et al. 2007).*

(A) Activité au cours de la « routine constante ». Evolution temporelle, (B) du niveau d'ARNm PER3, (C) de la concentration de cortisol plasmatique, et (D) de la concentration de la mélatonine plasmatique. Ces données illustrent les moyennes (± SE) de 10 sujets PER3-5/5 (symboles ouverts) et 14 sujets PER3-4/4 (symboles fermés).

Afin de déterminer l'implication de *PER3*, l'étude réalisée par Viola et al. (Viola, Archer et al. 2007) a

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pour particularité d'avoir sélectionné des volontaires sains uniquement sur la base de leur polymorphisme sans aucune connaissance de leur préférence diurnale et de la durée de leur sommeil. L'étude implique deux groupes appariés de 10 participants ayant le polymorphisme *PER3-5/5* (4 femmes; age ± SE 25.2 ± 1.1 ans) et 14 participants avec le polymorphisme *PER3-4/4* (6 femmes; 24.8 ± 1.0 ans). Des analyses médicales approfondies ainsi qu'une évaluation de leur sommeil (agenda de sommeil et actigraphie pendant environ 3 semaines précédant l'étude en laboratoire) permettent de montrer la régularité de leur sommeil. Ces mesures ne montrent aucune différence significative concernant la préférence diurnale, ainsi que l'heure de coucher (*PER3-5/5* 01:03 ± 0:32 h; *PER3-4/4* 01:03 ± 0:21 h; *P*>0.05), de réveil (*PER3-5/5* 07:57 ± 0:28 h; *PER3-4/4* 08:41 ± 0:21 h; *P*>0.05) ou la durée de sommeil (*PER3-5/5* 06:51 ± 0:14 h; *PER3-4/4* 07:19 ± 0:12 h; *P*>0.05). L'étude en laboratoire se compose d'une nuit contrôle suivie d'une « routine constante » d'environ 40 heures et d'une période de sommeil de récupération. La « routine constante » (Duffy and Dijk 2002) permet de mesurer les variations circadiennes endogènes, en maintenant les sujets éveillés en position semi-allongée et en lumière contrôlée (<5 lux). L'analyse des marqueurs circadiens (i.e. mélatonine, cortisol, ARNm *PER3*) montre de fortes variations circadiennes. Toutefois, aucune différence significative tant pour l'amplitude que pour la phase n'est observée entre les 2 populations de polymorphismes

Malgré une forte similitude des variations circadiennes entre les deux polymorphismes homozygotes de *PER3*, on observe d'importantes différences au niveau de l'homéostasie du sommeil, de l'électroencéphalogramme de veille et de sommeil et des performances ainsi qu'un déséquilibre de la balance sympatho-vagale.

L'analyse de la structure du sommeil montre une modification notable entre les deux polymorphismes (Viola, Archer et al. 2007). Aucune différence significative n'a cependant été observée sur sa durée totale ainsi que sur les durées de sommeil paradoxal et des stades 1 et 2. Cependant, les sujets *PER3-5/5* montrent un endormissement nettement plus rapide que les sujets *PER3-4/4* (*PER3-5/5* 8.6±1.3 min; *PER3-4/4* 18.1±2.6 min; P<0.005). De plus, les *PER3-5/5* passent beaucoup plus de temps en sommeil à ondes lentes (SOL) (*PER3-5/5* 22.7±1.6%; *PER3-4/4* 15.7±1.6% du temps de sommeil; P=0.006). L'analyse spectrale de l'électrœncéphalogramme au cours des stades du sommeil et de l'éveil, montre que le polymorphisme *PER3* n'affecte pas l'EEG de manière globale, mais modifie les diverses bandes de fréquence relatives aux divers états de vigilance.

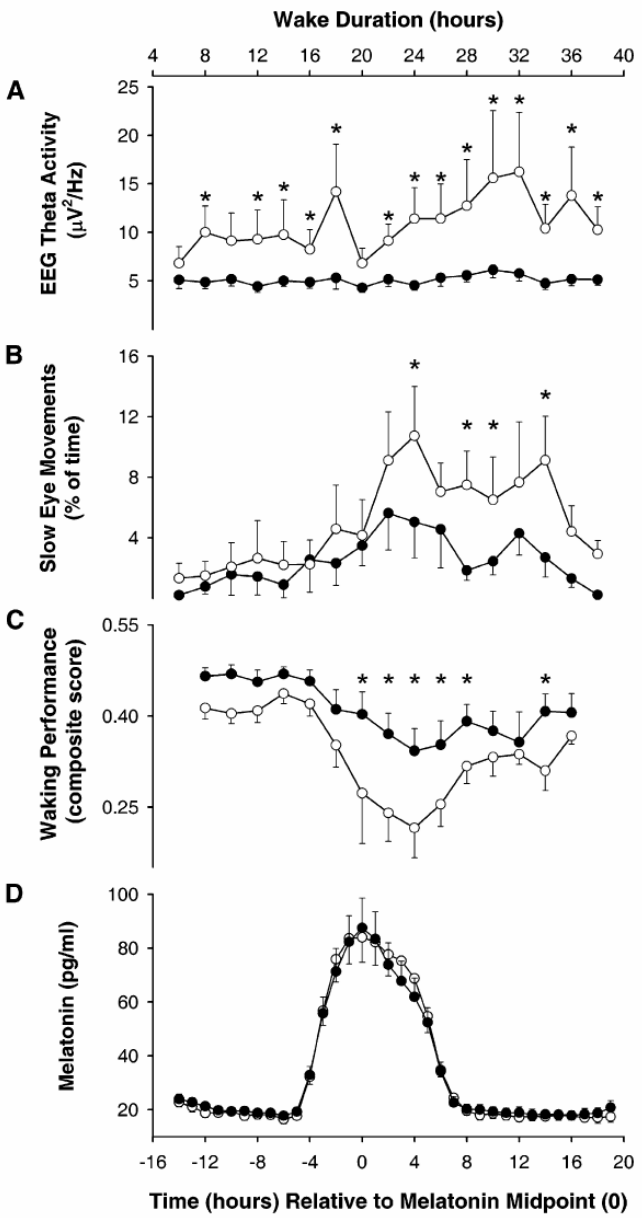

FIGURE 4 : *Diminution des performances à l'éveil et augmentation de l'activité thêta et du nombre de mouvements oculaires lents au cours de la privation de manière plus importante chez PER3-5/5 que chez les PER3-4/4. Evolution temporelle de (A) l'activité EEG thêta (5–8 Hz) au cours de l'éveil, (B) mouvements oculaires lents (MOL) (pourcentage du nombre de période de 30 s contenant au moins 1 MOL), et (C) performance cognitive (composante résultante de 12 tests) ajustée au rythme de la mélatonine plasmatique. 10 sujets PER3-5/5 (symboles ouverts) et 14 sujets PER3-4/4 (symboles fermés). (* p < 0.05) (Viola, Archer et al. 2007).*

Au cours du sommeil non paradoxal (non-SP) la différence se caractérise uniquement dans la bande de fréquence des ondes delta, alors qu'au cours du sommeil paradoxal (SP) et au cours de l'éveil, la différence s'exprime dans la bande fréquence des ondes alpha et théta.

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Enfin, l'EEG révèle des variations topographiques. Les variations de l'activité delta au cours du SOL se présentent particulièrement dans la zone frontale alors que les ondes alpha et thêta sont nettement plus importantes dans la zone centrale, voire pariétale du scalp au cours du sommeil paradoxal et de l'éveil.

Ceci montre donc clairement qu'une augmentation des ondes lentes en sommeil non-SP ainsi que des ondes alpha/thêta en SP et en phase d'éveil sont caractéristiques d'une forte pression de sommeil et de somnolence. Ces modifications de l'EEG montrent que le polymorphisme *PER3* affecte l'homéostasie du sommeil dans ses trois états de vigilance.

La privation de sommeil a permis de montrer que des différences persistent au cours du SOL et du SP de la nuit dite de récupération en fonction de la prédisposition génétique. Au cours de la nuit de récupération qui fait suite aux 40 heures d'éveil, l'activité des ondes lentes au cours du non-SP de début de nuit, ainsi que l'activité alpha au cours du SP demeurent élevées chez les sujets homozygotes *PER3-5/5*.

La privation de sommeil en condition dite de « routine constante » nous a ainsi permis de mettre en évidence une augmentation notable de l'EEG d'éveil spécifiquement dans la bande de fréquence thêta/alpha chez les sujets ayant le polymorphisme *PER3-5/5*. De plus, le nombre de mouvements oculaires lents (MOL), marqueurs de l'inattention, au cours de l'éveil est également plus important chez les *PER3-5/5*. On note ainsi une différence majeure suite à la privation de sommeil lors du matin du deuxième jour de privation. Face à une privation de sommeil, les différences observées entre ces deux polymorphismes, grâce aux marqueurs de somnolence et d'inattention, laissent supposer une vulnérabilité différente entre les individus.

Afin d'évaluer les variations cognitives affectées par le manque de sommeil, des tests de performances ont été réalisés au cours des 40 heures d'éveil (Viola, Archer et al. 2007; Groeger, Viola et al. 2008).

Durant la première partie des 40 heures, quand la privation de sommeil n'est pas encore avérée, les deux polymorphismes réagissent de manière similaire à la batterie de tests. Les différences se creusent avec l'extension de la période d'éveil au cours de la nuit. Les performances des *PER3-5/5* sont nettement plus dégradées après le pic de mélatonine. Une analyse plus détaillée des performances montre que l'effet du polymorphisme *PER3* combiné à la privation de sommeil affecte de manière prononcée les performances sollicitant particulièrement les fonctions exécutives. La baisse de performance est ainsi accentuée en fonction de la difficulté du test. En début de nuit, les performances cognitives ne

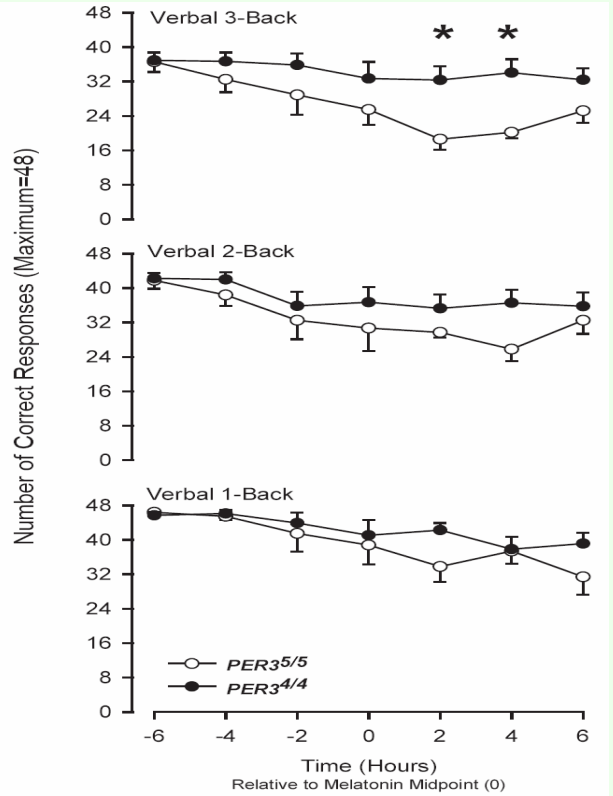

FIGURE 5 *: Performance évaluée par le test du "Verbal N-Back". Moyenne du nombre de réponses correctes (± SE) ajustée en fonction du rythme de mélatonine plasmatique.* P < 0.05 (Groeger, Viola et al. 2008).*

présentent pas de différence entre les deux polymorphismes. C'est avec la pression du sommeil que l'on observe une détérioration significativement plus importante chez les *PER3-5/5* entre 2 et 4 h après le pic de mélatonine (soit entre 06 :00 et 08 :00 h du matin).

Notons au passage que c'est également à ce même moment des 24 h que le nombre d'accidents dus au manque de sommeil est le plus important (Rajaratnam and Arendt 2001). Ces variations cognitives existent autant sur les fonctions exécutives que non exécutives. Elles apparaissent sous l'influence de la pression homéostatique du sommeil qui semble être modifiée selon le polymorphisme *PER3*.

La pression homéostatique affecte non seulement notre sommeil mais également notre vigilance et nos performances. Cependant, même si une association a été décrite entre le SOL et notre activité cardiaque évaluée par la variabilité cardiaque, aucune étude n'a encore décrit comment la pression homéostatique, particulièrement accentuée par une privation de sommeil, affecte le système nerveux autonome au cours de la nuit de récupération. Du fait des différences interindividuelles face à la pression homéostatique du sommeil, le polymorphisme *PER3* semble être un bon modèle d'évaluation de

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l'interaction entre l'activité sympatho-vagale et le sommeil (Viola, James et al. 2008).

L'analyse de la variabilité cardiaque et de ses indices dans l'évaluation de la balance sympatho-vagale montre que les homozygotes *PER3-5/5* présentent une variabilité cardiaque amoindrie par rapport aux homozygotes *PER3-4/4*. L'analyse spectrale et temporelle du signal cardiaque montre une prédominance de l'activité sympathique sur la balance sympatho-vagale. Cette prédominance sympathique se révèle au cours de l'éveil et du sommeil avec une différence plus prononcée au cours du non-SP. La réduction de la variabilité cardiaque et l'augmentation de la prédominance sympathique sur le système nerveux autonome sont fortement associées aux accidents cardio-vasculaires (Malliani, Lombardi et al. 1994; Kamen and Tonkin 1995). Cette association permet de caractériser le polymorphisme *PER3* comme un marqueur génétique de la vulnérabilité cardiaque associée au manque de sommeil.

Le sommeil et l'alternance de ses stades affectent de manière considérable la fréquence cardiaque et la variabilité cardiaque (Baharav, Kotagal et al. 1995; Otzenberger, Gronfier et al. 1998). Il est clairement établi qu'au cours du SOL l'activité parasympathique est à son maximum avant de laisser place à l'activité sympathique et ce, particulièrement au cours du SP. Il a ainsi été montré qu'au cours de la nuit l'activité parasympathique est associée à des ondes lentes delta (Viola, James et al. 2008). Cependant, l'étude de cette association en comparant les polymorphismes *PER3-5/5* et *PER3-4/4* montre une dissociation entre l'activité du système nerveux autonome et les ondes delta. L'activité delta, au cours du non-SP de début de nuit, des *PER3-5/5*, est particulièrement plus élevée que celle des *PER3-4/4*. Toutefois, au cours du non-SP, les participants *PER3-5/5* montrent une activité parasympathique nettement amoindrie par rapport à celle des *PER3-4/4*. Chez les sujets présentant le polymorphysme *PER3-5/5*, l'analyse de la variabilité cardiaque au cours de la nuit montre également une perte de la rythmicité ultradienne de l'activité sympatho-vagale d'où une dissociation avec les stades de sommeil. Ce nouveau modèle génétique permet de mettre en avant une dissociation entre le contrôle du sommeil et le système nerveux autonome et suggère que, bien que présentant une rythmicité ultradienne synchrone, les deux systèmes ne sont pas causal l'un l'autre.

L'ensemble de ces données, confirmé par des études en cours de publication et d'autres en cours d'analyse, conforte l'idée d'une pression homéostatique constante ainsi que d'une vulnérabilité certaine à la privation de sommeil chez les personnes présentant un polymorphisme *PER3-5/5*. Ce polymorphisme *PER3* est associé à divers phénotypes, tels que la préférence diurnale et le syndrome d'avance de phase (Archer, Robilliard et al. 2003; Pereira, Tufik et al. 2005), l'augmentation du risque de cancer du sein (Zhu, Brown et al. 2005), les désordres bipolaires (Nievergelt, Kripke et al. 2006), la dépendance aux drogues (Zou, Liao et al. 2008), la structure du sommeil (Viola, Archer et al. 2007), le système nerveux autonome (Viola, James et al. 2008), les performances cognitives (Groeger, Viola et al. 2008; Vandewalle, Archer et al. 2009). Il est certain qu'il faut être extrêmement prudent en affirmant l'existence d'un lien direct entre l'unique protéine *PER3* et ces diverses variations physiologiques. Même si le lien moléculaire et ses effets physiologiques reste à établir, nous ne pouvons négliger le fait que le nombre variable de séquences répétées caractérisant ce polymorphisme *PER3* peut être considéré comme un marqueur génétique lié à de nombreux autres loci responsables de ces phénotypes. Cependant, le polymorphisme *PER3* n'est pas un polymorphisme

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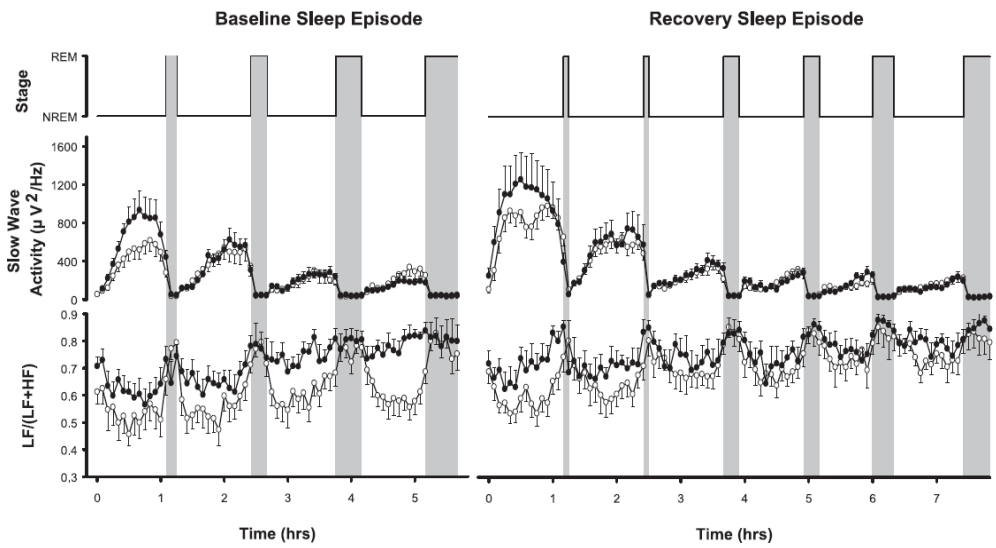

FIGURE 6 : *Effet du polymorphisme PER3 sur l'activité à ondes lentes (SWA) de l'EEG et sur la balance sympatho-vagale [LF/(LF + HF) ratio] au cours des 4 premiers cycles de sommeil de la nuit contrôle et des 6 premiers cycles de la nuit de récupération faisant suite à environ 40 h de privation de sommeil. Moyennes (± SE) de 9 sujets PER3-5/5 (symboles fermés) et 13 sujets PER3-4/4 (symboles ouverts) (Viola, James et al. 2008).*

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variant à partir d'un unique nucléotide, puisqu'il présente une variation de quatre ou cinq séquences répétées incluant des nucléotides. Ceci crée une différence de 18 aminoacides au niveau de la protéine. Ces séquences répétées contiennent des « clusters » de site de phosphorylation connus pour modifier la période circadienne (Blau 2008). Il est ainsi permis de penser que ces variations de séquences répétées affectent la phosphorylation de *PER3*, et permettent l'expression des différences qui en résultent au niveau de la structure tertiaire. Ces différences structurales affectent donc l'action de la fonction de *PER3* qui modifie directement ou indirectement le phénotype observé. Le rôle et les mécanismes impliquant les gènes de l'horloge restent encore à établir. De nouveaux gènes semblent également impliqués dans le contrôle homéostatique du sommeil, comme par exemple le gène contrôlant l'adénosine désaminase (A2A) (Rétey, Adam et al. 2005).

L'ensemble de ces résultats permet d'émettre l'hypothèse que le polymorphisme *PER3* jouerait un rôle important dans la vulnérabilité face à la pression homéostatique et pourrait représenter un marqueur important tant au niveau de la recherche qu'au niveau clinique et social.

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Comité scientifique

Xavier Bonnefont, Howard Cooper, Olivier Coste, Franck Delaunay, Elisabeth Filipski, Claude Gronfier, Jean-Christophe Leloup, Valérie Simonneaux, Michèle Teboul

Parc National du Mercantour

Site web : http://www.sf-chronobiologie.org/

Programme préliminaire

Mercredi 8 septembre 2010

12:30-14:00 : Accueil des participants

Symposium 1 : Photoréception et synchronisation

Modérateur : Howard Cooper

14:00-14:30 : Rob Lucas
14:30-15:30 : Communications orales (3 x20 min)
15:30-16:00 : Pause café
16:00-17:00 : Posters

Symposium 2 : Modélisation des rythmes biologiques

Modérateur : Jean-Christophe Leloup

17:00-17:30 : Felix Neaf
17:30-18:30 : Communications orales (3 x20 min)
18:30-19:30 : Assemblée générale de la SFC
20:00 : Diner
22:00 : table ronde sous les oliviers

Jeudi 9 septembre 2010

Symposium 3 : Neuroendocrinologie des rythmes biologiques

Modérateur : Xavier Bonnefont

8:30-9:00 : Garreth Leng
9:00-9:30 : Jacques Epelbaum
9:30-10:30 : Communications orales (3 x20 min)
10:30-11:00 : Pause café

Symposium 4 : Système circadien et métabolisme

Modérateur : Michèle Teboul

11:00-11:30 : Hélène Duez
11:30-12:30 : Communications orales (3 x20 min)
12:30-14:00 : Pause déjeuner

Symposium 5 : Rythmes saisonniers

Modérateur : Valérie Simonneaux

14:00-14:30 : Martine Migaud
14:30-15:00 : Annika Herwig
15:00-16:00 : Communications orales (3 x20 min)
16:00-16:30 : Pause café
16:30-17:30 : Posters
17:30-18:00 : Conférence plénière
20:00 : Banquet

Vendredi 10 septembre 2010

Symposium 6 : Aspects chronobiologiques en médecine du travail

Modérateur : Olivier Coste

9:00-9:30 : Till Ronnenberg (à confirmer)
9:30-10:30 : Communications orales (3 x20 min)
10:30-11:00 : Pause café

Symposium 7 : Chronobiologie du sommeil (en partenariat avec la SFRMS)

Modérateur : Claude Gronfier

11:00-11:30 : Damien Léger
11:30-12:30 : Communications orales (3 x20 min)
12:30-14:00 : Pause déjeuner

Symposium 8: Rythmes biologiques, santé et pathologie (en partenariat avec l'ARTBC)

Modérateur : Elisabeth Filipski

14:00-14:30 : Bert Van der Horst
14:30-15:30 : Communications orales (3 x20 min)
15:30-16:00 : Pause café

Workshop on cancer chronotherapeutics

Francis Lévi

16:00-18:00 : programme to be announced
20:00 : Diner
22:00 : Table ronde sous les oliviers

Samedi 11 septembre 2010

Symposium 9: Hot topics

Modérateur : Jorge Mendoza

9:00-10:00 : Communications orales (3 x20 min)
10:30-11:00 : Pause café
11:00-12:00 : Communications orales (3 x20 min)
12:30-14:00 : Pause déjeuner

Workshop on cancer chronotherapeutics

Francis Lévi

9:00-12:00 : programme to be announced
12:30-14:00 : Pause déjeuner
14:00-18:00 : programme to be announced

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Les échelles de détermination du chronotype

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Les sujets du matin et les sujets du soir (chronotype) présentent des caractéristiques circadienne et homéostatique du cycle veille/sommeil bien spécifiques qui expliquent leurs horaires préférentiels d'activité et de repos. L'estimation du chronotype par questionnaire va permettre au clinicien de mieux appréhender les besoins de sommeil du patient, les troubles du sommeil liés aux chronotypes extrêmes. Elle va éclairer les données obtenues sur l'hygiène du cycle veille/sommeil, l'addiction aux substances éveillantes, ainsi que sur la co-morbidité ressentie, en particulier l'humeur. Il existe de nombreux questionnaires qui permettent d'estimer le chronotype des sujets. Ce travail va présenter succinctement les caractéristiques des chronotypes et ensuite présenter les questionnaires de matinalité/vespéralité les plus utilisés et leurs limites.

Caractéristiques de sujets du matin et du soir

Les sujets du matin sont fatigués le soir, se couchent et se lèvent tôt, se réveillent en forme et alertes, et trouvent qu'il est difficile de rester éveillé la nuit. Les sujets du soir ont leurs performances au maximum le soir, se couchent et se lèvent relativement tard, se réveillent fatigués et trouvent qu'il est difficile de rester éveillé le matin. L'estimation du chronotype évolue au cours de l'âge : les enfants sont généralement du matin, progressivement ils deviennent du soir pour atteindre un maximum de vespéralité autour de l'âge de 20 ans et ensuite progressivement, ils redeviennent du matin avec l'âge (Roenneberg, Curr Biol, 2004). Dans une population d'adultes (17-80 ans), on observe 40 % de sujets du matin et 11 % de sujets du soir (Taillard, J Sleep Res, 1999). Après correction de l'effet de l'âge sur le chronotype, on observe 25% de sujets du matin et 26 % de sujets du soir dans une population âgée de 30 à 49 ans (Paine, J Biol Rhythms, 2006) ; et 28 % de sujets du matin et 20 % de sujets du soir dans une population de 44 à 58 ans (Taillard, J Biol Rhythms, 2004).

Certaines études ont montré que les chronotypes extrêmes seraient associés à un ou plusieurs polymorphismes au niveau de gènes impliqués dans l'horlogerie circadienne (Clock et Per3) (Katzenberg, Sleep, 1998, Sack, Sleep, 2007, Archer, Sleep, 2003, Jones, J Sleep Res, 2007) mais d'autres études ne l'ont pas confirmées (Robilliard, J Sleep Res, 2002, Groeger, Sleep, 2008). Cette association n'implique pas que tous les sujets du matin ou du soir sont identifiables par leur génotype. Toutefois il est relativement acceptable de penser que les préférences d'horaires du sommeil soient sous contrôle génétique. La phase circadienne de nombreuses variables comportementales et physiologiques apparaît en moyenne 2 heures plus tôt chez les sujets du matin comparativement aux sujets du soir (Baehr, J Sleep Res, 2000, Duffy, J Investig Med, 1999, Kerkhof, Neurosci Lett, 1996, Taillard, J Sleep Res, 2003). La régulation homéostatique du sommeil serait aussi différente en fonction du chronotype. Les sujets du matin accumuleraient plus rapidement la pression homéostatique pendant l'éveil et la dissiperaient plus rapidement au cours du sommeil que les sujets du soir (Taillard, J Sleep Res, 2003, Mongrain, J Sleep Res, 2006). L'angle de phase (durée entre le minimum thermique et l'heure de lever), qui renseigne généra-

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lement de la relation de phase entre rythme veille/sommeil et rythme circadien de la température et qui s'avère le reflet fidèle de la longueur de la période endogène, est différent entre les sujets du matin et du soir. Les sujets du matin ayant un angle de phase plus grand que les sujets du soir, (Kerkhof, Electroencephalogr Clin Neurophysiol, 1991, Duffy, J Investig Med, 1999, Baehr, J Sleep Res, 2000, Liu, Neurosci Lett, 2000, Taillard, J Sleep Res, 2003, Mongrain, J Biol Rhythms, 2004) ceci démontrerait que les sujets du matin et du soir ne se réveillent pas au même moment circadien. Cette différence d'angle de phase confirmerait aussi que la période circadienne serait différente entre les sujets du matin et du soir (plus courte chez les sujets du matin) (Duffy, Behav Neurosci, 2001). 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 (i.e. sujet du soir). De plus les sujets du soir exprimeraient des besoins de sommeil plus grands que les sujets du matin (Taillard, J Sleep Res, 1999, Taillard, J Biol Rhythms, 2004).

Les durées de sommeil et les heures de lever et de coucher sont beaucoup plus stables chez les sujets du matin que chez les sujets du soir (Taillard, J Sleep Res, 1999, Ishihara, J Hum Ergol (Tokyo), 1988). Ainsi, les sujets du matin présenteraient une rigidité du sommeil et des habitudes de vie, tandis que les sujets du soir présenteraient une flexibilité du sommeil et des habitudes de vie (Baehr, J Sleep Res, 2000). Cette mauvaise hygiène du sommeil, additionnée à des besoins plus grands et des horaires préférentiels de sommeil différents de ceux imposés par notre société, font que les sujets du soir se trouvent en dette de sommeil pendant les jours de travail et tentent de compenser cette dette de sommeil en allongeant leur durée de sommeil pendant les jours de repos (Taillard, J Sleep Res, 1999, Taillard, J Biol Rhythms, 2004, Roenneberg, J Biol Rhythms, 2003). Ceci peut expliquer pourquoi les sujets du soir ont un plus grand penchant pour la consommation de substances éveillantes (Taillard, J Sleep Res, 1999, Adan, Addiction, 1994). Les sujets du soir auraient aussi une plus grande facilité à s'adapter au travail de nuit ou posté (Hilliker, Sleep, 1992).

Concernant les traits psychologiques, il ressort de différentes études que les sujets du soir ont tendance à être des personnes extraverties, impulsives, neurotiques et avides de sensations (Caci, Eur Psychiatry, 2004, Tankova, Pers Individ Dif, 1994). Nous avons aussi montré que le chronotype est lié à des plaintes du sommeil spécifiques, la tendance à être du matin serait liée à des difficultés de maintien de sommeil et l'impossibilité de se rendormir dans le petit matin, et la tendance à être du soir serait liée à des difficultés d'endormissement et une somnolence matinale (Taillard, J Biol Rhythms, 2001, Carrier, J Sleep Res, 1997). Nous avions aussi montré que la tendance à être du soir serait aussi liée au trouble de l'humeur (Taillard, J Biol Rhythms, 2001). Cette association a été confirmée par 2 équipes (Gaspar-Barba, J Affect Disord, 2009, Hidalgo, Psychiatry Clin Neurosci, 2009).

Estimation du chronotype : Les questionnaires de matinalité/vespéralité

L'estimation du chronotype se fait à l'aide de questionnaire. Actuellement, quatre questionnaires sont fréquemment utilisés. Chacun présentent des avantages et des inconvénients. Généralement ces questionnaires se basent sur les heures de lever et de coucher des sujets pour déterminer des scores qui permettront d'estimer leur chronotype. Ceci pose un problème car avec l'âge, on se couche et on se lève de plus en plus tôt. Ainsi, dans des populations de sujets matures et/ou âgés, ces questionnaires identifient très peu de sujets du soir.

Pour contrecarrer ce problème nous avons proposé, pour un questionnaire, de nouvelles bornes capables d'estimer le chronotype chez des sujets matures (Taillard, J Biol Rhythms, 2004). Caci et al. (Caci, Sleep Med, 2009) suggèrent que la solution la mieux adaptée serait de déterminer des scores T du score total pour chaque catégorie d'âge dans de larges échantillons représentatifs.

Tous ces questionnaires sont des auto-questionnaires. Les procédures d'autoévaluation impliquent que le sujet évalué comprenne les consignes qui lui sont données, les questions qui lui sont posées. De plus, certains sujets ne sont pas capables de déterminer leurs horaires préférentiels de vie. L'activité professionnelle ou certaines pathologies vont influencer la perception de ces horaires préférentiels comme par exemple les troubles de l'humeur, les troubles du sommeil…

Dans tous les cas, ces questionnaires ne sont pas adaptés aux personnes qui travaillent suivant des horaires inhabituels.

Questionnaire de Horne et Ostberg (MEQ)

Le questionnaire de matinalité/vespéralité élaboré par Horne et Ostberg (Horne, Int J Chronobiol, 1976) est actuellement le plus utilisé pour estimer le chronotype chez l'adulte. Généralement ce questionnaire est considéré comme le gold standard et c'est celui qui est proposé par l'AASM (American Academy of Sleep Medicine) pour estimer le chronotype. Il est composé de 19 questions portant sur les préférences de vie (activité, cycle veille-sommeil, repas) et l'état de fatigue et de somnolence à certains moments de la journée.

Les propriétés psychométriques du questionnaire anglo-saxon sont très satisfaisantes (Smith, J Appl

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Psychol, 1989) mais elles n'ont pas été vérifiées pour la version française. Toutefois, nous avons réalisé une validation externe du questionnaire français en enregistrant la température corporelle chez 9 sujets du matin et 9 sujets du soir dans des conditions très contrôlées (constant routine) (Taillard, J Sleep Res, 2003).

Le score total du questionnaire peut varier entre 16 et 86. Un score inférieur à 42 identifie les sujets du soir et un score supérieur à 58 identifie les sujets du matin. Les deux chronotypes extrêmes sont identifiés lorsque le score est inférieur à 31 (sujet nettement du soir) et supérieur à 69 (sujet nettement du matin). Comme ce questionnaire est très sensible à l'âge nous avons proposé une classification adaptée aux sujets matures : de 44 à 58 ans, un score inférieur à 53 identifie les sujets du soir et un score supérieur à 64 identifie les sujets du matin. Les deux chronotypes extrêmes sont identifiés lorsque le score est inférieur à 47 (sujet nettement du soir) et supérieur à 69 (sujet nettement du matin). Aucune validation externe de ces scores n'a été effectuée.

La principale critique de ce questionnaire est sa longueur.

Questionnaire réduit de Horne et Ostberg (rMEQ)

Ce questionnaire a été développé par Adan (Adan, Person Individ Diff, 1991). Il utilise les 5 questions les plus pertinentes du questionnaire de Horne et Ostberg (questions 1,7, 10, 18 et 19). Les auteurs gardent la même cotation que le questionnaire initial. Les propriétés psychométriques du questionnaire anglo-saxon sont satisfaisantes (Chelminski, Pers Individ Dif, 2000), mais elles n'ont pas été vérifiées pour la version française. Nous avons effectué une validation externe de ce questionnaire chez plus de 2000 sujets en prenant en compte les heures de lever et de coucher pendant les jours de travail et les jours de repos.

Le score total du questionnaire peut varier entre 4 et 25. Un score inférieur à 12 identifie les sujets du soir et un score supérieur à 17 identifie les sujets du matin. Les deux chronotypes extrêmes sont identifiés lorsque le score est inférieur à 8 (sujet nettement du soir) et supérieur à 21 (sujet nettement du matin). Ce questionnaire est sensible à l'âge (Caci, Sleep Med, 2009). Il n'existe à notre connaissance aucune correction des bornes en fonction de l'âge pour ce questionnaire.

L'échelle composite de matinalité (Smith 1989)

Ce questionnaire a été développé par Carla Smith. Il est composé de 13 questions (9 du MEQ et 4 du questionnaire de Torsvall et Akerstedt (Torsvall, Scand J Work Environ Health, 1980)). Ses propriétés psychométriques sont légèrement supérieures à celles du MEQ. (Caci, Eur Psychiatry, 1999, Caci, Eur Psychiatry, 2000) ont démontré que les propriétés psychométriques du questionnaire français étaient satisfaisantes et ont effectué une validation externe en utilisant les heures de lever et de coucher pendant les jours de travail et les jours de repos.

Le score total peut varier de 13 à 55. Initialement, il n'existe pas de bornes pour déterminer les chronotypes. Les bornes sont calculées en utilisant les 10èmes et 90èmes percentiles dans chaque population étudiée. Ce questionnaire n'est donc pas utilisable dans sa version initiale pour déterminer immédiatement le chronotype des sujets ou patients. De plus, ce questionnaire est sensible aussi à l'âge (Caci, Sleep Med, 2009). Pour supprimer ces 2 limites d'utilisations, Caci et al. ont déterminé des scores T du score total dans différentes catégories d'âges en fonction du sexe (visualisable sur http://pagesperso-orange.fr/herve.caci/Chronobio/page10/page10.html). Pour chaque catégorie le nombre de sujet est faible, surtout pour les classes d'âges supérieures à 35 ans (effectif <50), et surtout les sujets inclus ne semblent pas provenir d'échantillons vraiment représentatifs (essentiellement des étudiants).

Le questionnaire de chronotype de Munich développé par Roenneberg et al. (Roenneberg, J Biol Rhythms, 2003)

Ce questionnaire est beaucoup plus facile à utiliser que tous les autres questionnaires. Les sujets doivent donner l'heure à laquelle ils se couchent, l'heure à laquelle ils s'endorment, l'heure à laquelle ils se réveillent et l'heure à laquelle ils se lèvent pendant les jours de travail et les jours de repos. Ils notent aussi la perception de leur chronotype sur une échelle de 0 (type matinal extrême) à 6 (type tardif extrême).

Ce questionnaire permet de déterminer le chronotype sur la base de la localisation du milieu du sommeil (exprimé en hh : mm calculé entre l'endormissement et le réveil). Le milieu du sommeil calculé pendant les jours de repos permettrait de déterminer le chronotype. Actuellement, une formule permet de calculer le milieu du sommeil corrigé en fonction de l'éventuelle dette de sommeil occasionnée par le travail (MSFSc) et de déterminer le chronotype. Ainsi :

$$\mathbf{MSFSc = MSF - 0.5 \times (SLDF - SLD\Phi)}$$

où :

- $SLD\Phi = (5 \times SLDW + 5 \times SLDF)/7$
- MSF : Milieu du sommeil pendant les jours de repos
- SLDW : Durée du sommeil pendant les jours de travail

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- SLDF : Durée du sommeil pendant les jours de repos
- SLD Φ: Durée moyenne de sommeil

Les sujets du matin ont un MSFSc ≤ 2.17 et les sujets du soir ont un MSFSc > 7.25.

Ce questionnaire a été largement utilisé (plus de 55.000 personnes âgées de 10 à 90 ans) et pourrait être une alternative à tous les questionnaires permettant d'estimer le chronotype, mais il semble que l'auteur fasse évoluer au fil du temps le calcul du score (Milieu du sommeil pendant les jours de repos, ensuite milieu du sommeil en fonction des besoins de sommeil et, pour terminer, milieu du sommeil en fonction de la dette de sommeil...). De plus, ce questionnaire est aussi sensible à l'âge et au sexe.

Remarques sur l'estimation du chronotype

Roenneberg (Roenneberg, Sleep Med Rev, 2007) montre qu'une courte description des 2 chronotypes [Par exemple : si vous aimez (ou programmez de) dormir un peu plus les jours de repos que les jours de travail, ou si vous ne pouvez généralement pas sortir du lit le lundi matin, alors probablement vous êtes un sujet du soir. Si, toutefois, vous vous réveillez à heure fixe et vous vous sentez en forme dès l'instant où vous sautez du lit et si vous préférez vous couchez tôt que d'aller à une soirée, alors vous êtes un sujet du matin.], suivie de la perception du chronotype sur une échelle de 0 (type matinal extrême) à 6 (type tardif extrême), permet d'obtenir le même résultat que celui obtenu après les 19 questions du questionnaire de Horne et Ostberg. Nous avions déjà bien démontré (Taillard, J Biol Rhythms, 2004) que la question 19 du questionnaire de Horne et Ostberg (estimation du chronotype) corrèle fortement avec le chronotype calculé en fonction du score total. En d'autres termes, nous estimons très bien notre chronotype.

Conclusion

Pour l'instant, il est difficile de conseiller l'utilisation de tel ou tel questionnaire de matinalité/vespéralité. Chacun présente des limites d'utilisation. Le questionnaire de Horne et Ostberg a souvent été critiqué, mais la plupart des caractéristiques des composantes circadiennes et homéostatiques des sujets du matin ou du soir ont été démontrées grâce à l'utilisation de ce questionnaire. Ces caractéristiques ont été confirmées par les études utilisant les questionnaires plus récents et soit disant plus fiables. De plus, l'intérêt clinique d'utiliser ces nouveaux questionnaires n'a pas été démontré.

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Prix 2010 "Jeune Chercheur / Jeune Chercheuse" de la Société Francophone de Chronobiologie

La Société Francophone de Chronobiologie attribue chaque année un prix "Jeune Chercheur / Jeune Chercheuse" d'un montant de 1500 €. Ce prix est accordé sur la base de travaux scientifiques de haut niveau dans le domaine des rythmes biologiques.

Nouvelles conditions d'attribution

Le prix sera attribué à un chercheur ou une chercheuse de moins de 35 ans révolus, d'expression française et le ou la lauréat(e) s'engage à rédiger un article dans sa spécialité pour le journal RYTHMES et à venir présenter ses travaux au 42e congrès de la Société Francophone de Chronobiologie qui se tiendra du 08 au 11 septembre 2010 à La Colle sur Loup (Alpes Maritimes).

Afin de s'assurer de la présence du ou de la lauréat (e) lors du congrès annuel, l'attribution du prix inclut désormais une bourse de voyage.

Composition du dossier

Chaque dossier de candidature sera fourni en 6 exemplaires et comprendra :

- un *curriculum vitae* avec photo;
- une page résumant les travaux principaux;
- une description des résultats et perspectives en un maximum de 10 pages, références comprises;
- une liste des publications scientifiques;
- éventuellement, une lettre de présentation du Directeur du laboratoire.

Le dossier de candidature sera adressé au plus tard **le 31 avril 2010** à:

Etienne CHALLET, Secrétaire Général de la SFC
Dépt Neurobiologie des Rythme, INCI
CNRS PR3212,
5 rue Blaise Pascal,
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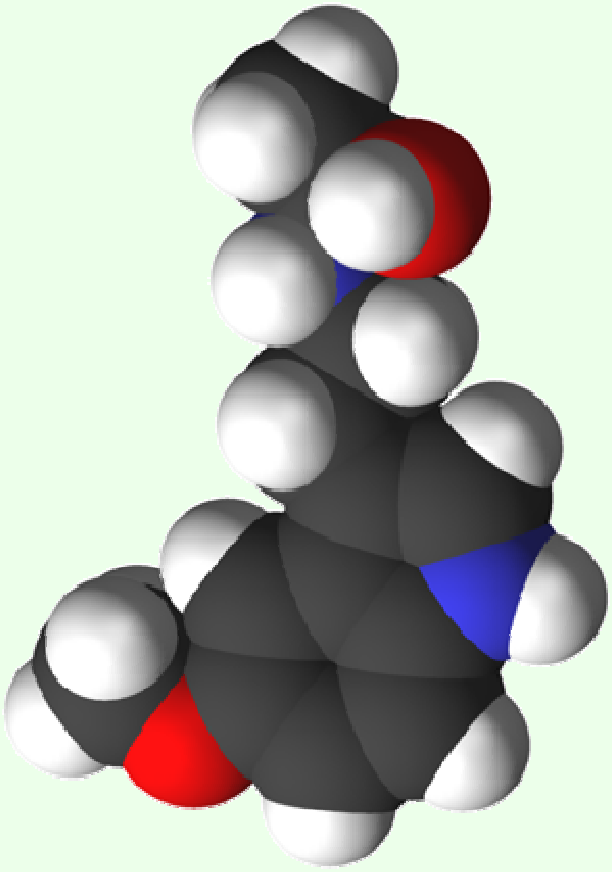
***Nota**: la commission d'évaluation se réserve le droit de ne pas attribuer le prix si aucun dossier n'atteint le niveau escompté.*

11ème Congrès de l'European Biological Rhythms Society 22-28 août 2009, Strasbourg, France.

Résumés des communications (suite)

Communications affichées : seconde partie



8. Melatonin: receptors and actions

Effects of melatonin on the hypothalamus-pituitary-interenal axis in goldfish (Carassius auratus): stress gene expression

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Purpose: The objective of the present study was to investigate the possible effects of melatonin (MEL) on the expression of certain genes related to the activation of the hypothalamus-pituitary-interenal axis (HPI) in goldfish. Methods: Fish maintained at 12L:12D photoperiod were distributed into 4 groups: group 1, control (intraperitoneally (IP) injected with teleost saline during 4 days); group 2, stress (IP injected with saline during 4 days, and stressed by 2 minutes of handling and air exposure on day 4); group 3 and 4: stress and daily MEL injection (20 µg/g bw) three hours before lights off (group 3: IP injected with teleost saline at day 4; group 4: IP injected with MEL at day 4). Two hours after final treatment, blood and tissues were obtained and gene expression of CRH, CRH-R1, POMC, MC2R and StAR protein were analyzed by real time RT-q-PCR in hypothalamus, pituitary and interenal tissues. Results: Plasma glucose and lactate levels significantly increased in the stressed fish compared to the control group, and MEL treatment (groups 3 and 4) reduced the circulating lactate increase. All target genes showed higher mRNA expression in stressed fish compared to controls, and MEL injections for 4 days (group 4) reduced slightly such increase and counteracted significantly the expression of POMC gene. This MEL effect was not observed in fish that did not receive MEL injection on the last day, coincident with the stress exposure (group 3). Conclusions: The stress applied on the model organism in the present study demonstrates the genetic mechanisms underlying the stress responses in fish. Our results suggest that MEL may be involved in the regulation of such responses via modulation of gene expression at different levels of the HPI axis.

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Pharmacological characterization of the MT2 melatonin receptor

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Purpose: To compare the pharmacological properties of 2 compounds, considered as antagonists of MT2 receptors in 3 different species. Methods: CHO-K1 cells (Chinese Hamster Ovary) were transfected with human, rat and ovine recombinant MT2 melatonin (MLT) receptor (hMT2, rMT2 and oMT2). The agonist or antagonist activities of MLT and 2 compounds (4-phenyl-2-propionamidotetralin, 4-P-PDOT and luzindole) were tested by 3 methods: cAMP assay, [35S]-GTP?S binding assay and cellular dielectric spectroscopy (CDS). Results: Assessed by cAMP production, MLT showed an agonist effect on hMT2, rMT2 and oMT2 with comparable potency (EC50=0.4nM). 4-P-PDOT showed a partial agonist effect on hMT2 and oMT2 (EC50=1.4nM and 35nM, respectively), while it did not stimulate rMT2, suggesting a potential antagonist property. Luzindole showed a partial agonist effect on hMT2 (EC50=7.2nM) and an antagonist effect on rMT2 and oMT2. These results contrasted with those obtained with [35S]-GTP?S binding which showed an antagonist activity of 4-P-PDOT and luzindole on the 3 MT2 receptors.(CDS), a technology based on the change of intercellular impedance of a monolayer of cells, was used to assess agonist or antagonist activity of the compounds. 4-P-PDOT showed a partial agonist effect on the 3 MT2 receptors. Luzindole showed a partial agonist effect on hMT2 and rMT2, and did not show any agonist effect on oMT2. Conclusions: The same compound showed different agonist and/or antagonist activities on MT2 receptors of different species. Moreover, the differences observed using pathway specific (cAMP) or mode global functional responses (GTP?S, CDS), showed different functional responses, suggesting additional indirect coupling pathways on MT2 receptors.

Cloning and developmental expression profile of two different arylalkylamine N-acetyltranferase 1 genes in the flatfish Solea senegalensis.

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Purpose: The purpose of this work was to investigate whether two AANAT1 (1a and 1b) genes are present in the retina of the Senegalese sole, and to investigate their pattern of expression, focusing on the early development and metamorphosis stages. Methods: A cloning strategy was used to amplify the Aanat genes from sole retinal extracts. Their developmental expression patterns were investigated using Real Time quantitative PCR. The corresponding mRNAs where localized by in situ hybridization in retinas of sole larvae at different developmental stages. Results: The Senegalese sole expresses three Aanat genes, Aanat1a, Aanat1b and Aanat2. Aanat1a and Aanat1b exhibited inverse and stage-specific expression patterns in the sole. During the early larval stages, Aanat1a expression was higher than Aanat1b expression; and, only Aanat1a exhibited a daily pattern with higher levels of expression at night. At metamorphosis, Aanat1a expression decreased and the day-night variations where lost. In contrast, Aanat1b expression increased along the metamorphic process and exhibited higher nocturnal levels. In adults, retinal Aanat1a mRNA levels displayed no day-night variation, while significant daily differences were found in Aanat1b expression. Both genes were mainly expressed in the inner nuclear and outer photoreceptor layers of the retina. Conclusion: Aanat1a probably plays a more important role than Aanat1b during early developmental stages in the Senegalese sole. It is possible that besides melatonin synthesis the AANAT1 enzymes play other roles, such as dopamine acetylation. A possible functional antagonism between the AANAT and the thyroid hormone systems during flatfish metamorphosis is proposed.

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Aryl-alkylamine-N acetyl transferase (Aa-nat) expression in the cones of Arvicanthis ansorgei retina

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Purpose: Melatonin (mel), for which Aa-nat is the limiting enzyme of synthesis, is thought to play roles in photoreceptor phagocytosis, critical for photoreceptor survival. As a first step to investigate the role of mel in mammalian retinal physiology, we studied mel RNA and protein production in diurnal rodent retinas. Methods: Retinas of adult Arvicanthis ansorgei a diurnal rodent, kept in a 12-hour light-dark cycle (LD) were processed for non-radioactive in situ hybridization (ISH) using an Aa-nat riboprobe. Also, circadian Aa-nat gene expression was studied by real time PCR analysis on retinas from 3 groups housed under different lighting conditions (LD, constant dark (DD), and constant light (LL) cycles). Finally, retinas of adult Arvicanthis maintained under DD cycles for 72h were processed for western-blotting using a specific anti-AA-NAT antibody in order to investigate circadian AA-NAT expression. Results: Aa-nat gene expression revealed by ISH reached a maximum at night (ZT19) and a trough during the day (ZT6). Most importantly, Aa-nat expression was restricted to cone photoreceptors in a two row cell layer. Complete circadian analysis of Arvicanthis retinal Aa-nat expression confirmed robust circadian expression of Aa-nat, maximum at ZT19 in a 12h LD cycle and conserved in DD conditions albeit at reduced amplitude. The 36h LL exposure altered the slope and time of maximum Aa-nat mRNA expression. Finally, AA-NAT protein expression in Arvicanthis retinas in DD rose at night but was maximal at CT7 in the middle of the subjective day. Conclusions: LD and DD circadian Aa-nat expression occurs uniquely in cones of Arvicanthis retina. However, LL conditions induce

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a marked alteration in the expression profile, and protein presence in the subjective daytime of DD conditions appears paradoxical. Use of diurnal cone-rich mammalian species may help understand mel function in retina.

Indications for melatonin in synaptic plasticity and memory formation in the mouse

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Purpose: Little is known so far about the putative role of rhythmically released pineal hormone melatonin and memory formation. The goal of this study was therefore to explore the impact of melatonin on the well-known day-night dependence of hippocampal.synaptic plasticity, learning, and gene expression. Methods: The presence of the melatonin receptor 1 (MT1) and MT2 and major clock genes (Per1, Per2, Cry2, Clock, Bmal1) in the mouse hippocampus were demonstrated by PCR, immunoblot and immunohistochemistry. We compared in a food-rewarded, hippocampal-dependent behavioural test (radial arm maze) C3H wildtype (WT), with MT1-/-, MT2-/-, MT1-/-/MT2-/-, and with low melatonin secretor C57Bl mice. Behavioural experiments were also performed with C57BL mice that received melatonin in physiological concentration with the drinking water. In addition, melatonin was tested in primary hippocampal cell cultures of WT and MT-/- mice for its efficacy to affect signaling events. Results: MT mRNA and clock gene mRNA was detected in hippocampal extracts from WT mice. Rhythmic clock gene mRNA and protein levels in WT animals were greatly altered in MT-/- mice. MT1-/-/MT2-/- mice showed a significantly decreased performance in the radial arm maze test as compared to WT animals. Conclusion: The presence of MTs in the mouse hippocampus provides the basis for a temporally gated influence of the pineal hormone melatonin on signalling events involved in memory processing. This assumption is supported by the observed differential expression profiles in diurnal clock genes between WT and MT-/- mice, and the impaired memory consolidation in MT-/- animals as compared to C3H mice. We conclude that melatonin modulates memory acquisition, either by directly inflicting on genes, relevant for synaptic plasticity, or indirectly, via phase-shifting hippocampal clock gene expression.

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Molecular organization and dynamics of the MT1 melatonin receptor/RGSZ1/Gai protein complex

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Purpose: Regulators of G protein signaling (RGS) proteins are signaling modulators that accelerate GTP hydrolysis of G? subunits and facilitate termination of signaling initiated by G protein-coupled receptors (GPCRs). By searching for protein complexes associated with the carboxy-terminal (Cter) domains of the two Gai protein-coupled human MT1 and MT2 melatonin receptors using an original proteomic approach (Maurice et al, Mol. Cell. Proteomics. 2008), we identified RGSZ1 as a specific partner of MT1. Methods and Results: Using biochemical, fluorescence imaging, and functional approaches, we showed that MT1 interacts with RGSZ1 in the ovine pituitary pars tuberalis and demonstrated that RGSZ1 and Gai bind directly and independently to the third intracellular loop and the Cter of MT1 to form a functional pre-associated complex. Agonist activation of MT1 rearranged this complex as monitored by bioluminescence resonance energy transfer (BRET) between probes inserted at multiples sites in the MT1/Gai1/RGSZ1 complex. We propose a 3D model of the evolution of the complex and show that it is compatible with the BRET data. We used crystal structures of Gai1 under basal and activated conditions and demonstrate the molecular changes inside the structure of Gai1 and the mechanism of interaction with RGSZ1. Conclusions: Collectively, our data support the concept of pre-associated RGS protein/GPCR complexes that rearrange upon agonist-promoted receptor activation and identify RGSZ1 as a new regulator of melatonin signaling.

Melatonin production in gastrointestinal tissue of rainbow trout: immunochemical and biochemical localization, effect of pinealectomy and daily variations

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Purpose: Although the pineal organ is known to be the main source of blood melatonin in vertebrates, there is evidence that the gastrointestinal tissue (GIT) also could contribute to local and systemic levels of the hormone. We have recently shown the expression of AANAT2 gene in trout GIT, thus suggesting the local synthesis of melatonin. Therefore, the purpose of the present study was to characterize the production and distribution of melatonin in different segments and tissues of trout GIT. Methods: In a first experiment, the presence of melatonin in different segments of rainbow trout GIT was studied by using immunochemical techniques and HPLC measures. In second experiment, groups of trout were either pinealectomized or sham-operated to evidence changes in melatonin levels in the intestinal tissue and plasma. In a third experiment, groups of trout were sacrificed during the day-night cycle in order to outline the daily variations in the levels of melatonin in the middle and posterior intestine. Results: Melatonin-containing cells were detected in all segments of the digestive tract, particularly in stomach and middle intestine. The tissue localization showed that melatonin was present in the mucosa and the muscular layer, probably in relation with serotonin-containing cells. Measurement of melatonin levels agreed with the presence of the hormone in all GIT segments, with a relatively higher amount in the muscular wall than in the mucosa. Pinealectomy, which abolished daily rhythm of melatonin in blood, did not affect melatonin levels in intestine. By last, we have not found significant daily changes in melatonin levels in trout intestine, but a tendency to peak was evident during the afternoon and late night. Conclusions: The results support the existence of an endogenous production of melatonin in trout GIT, and suggest that melatonin could have a role in the coordination of digestive processes.

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Crosstalk in melatoninergic and catecholaminergic system regulating human T regulatory and T effector cells

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Human blood is constituted of different sub-populations of lymphocytes. To understand the changes and significance of the endogenous melatonin and catecholamine in the immune system, we explored their synthesis by CD4+CD25- T effector and CD4+CD25+ regulatory T lymphocytes in activated states. We found that CD4+CD25- T effecter lymphocyte contains high endogenous melatonin whereas CD4+CD25+ T regulatory lymphocyte contains high catecholamine. Catecholamine treatment (1µM) to CD4+CD25- T effecter lymphocyte completely diminished the level of melatonin in vitro. Presence of Mel1a and Mel1b receptor was noted in both CD4+CD25- T effecter as well as in CD4+ CD25+ T regulatory lymphocytes. Melatonin treatment (10-7) was able to activate CD4+CD25- lymphocyte by increasing the production of IL-2 in vitro. Melatonin receptor antagonist luzindole treatment decreased IL-2 production and IL-2 expression by T effector lymphocytes thus confirming the role of melatonin in cytokine production. Melatonin treatment also increased Fox P3, TGF ß expression significantly. Increase in Fox P3, TGF ?, TH and IL-10 following melatonin treatment in T regulatory cells represented some internal regulation between T effecter and T regulatory cells. Therefore, we may suggest a tonic control of immune system by those two subsets of lymphocytes mediated via specific membrane/ nuclear receptors. Such a control may be explained in terms of a trade-off mechanism/ regulation between the endogenous melatonin of T effecter cells and catecholamine of the T regulatory cells in maintaining the homeostasis in the immune system depending upon the physiological status or required status of the cells.

Chronic maternal melatonin suppression during pregnancy induces morphological and functional changes in newborn sheep brown adipose tissue

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In human and sheep, brown adipose tissue (BAT), accrued during pregnancy is used for newborn thermogenesis whereas fetal BAT thermogenic function is inhibited by factors produced or transported by the placenta. We hypothesize that one of these factors is maternal melatonin since functional melatonin receptors are present in sheep fetal BAT Purpose: To assess the role of maternal melatonin on newborn perirrenal BAT weight, morphology, triglyceride (TAG) content and lipolytic response (glycerol) to norepinephrine. Methods: Maternal melatonin was suppressed by exposing 9 pregnant sheep to constant light from 63% until delivery (term 145 days). Four sheep received daily oral melatonin replacement (12 mg). Controls were 5 newborns from mothers maintained in 12h light:12h dark. At 4-6 days of age, newborns were euthanized and perirrenal BAT was dissected. A piece of BAT was preserved in TRIzol for UCP1 mRNA measurement, another piece was criopreserved for histology and portions of BAT were used fresh in culture. BAT explants (~25 mg) were pre-incubated in triplicate for 6-h at 37ºC in culture medium followed by 12 hours in 2 ml medium alone (basal) or containing 0.1 µM of norepinephrine. Glycerol production was measured in the supernatants and TAG content in the explants. Results: The absence of maternal melatonin during gestation selectively decreased BAT weight/kg body weight and the size (area) of unilocular cells in conjunction with increasing BAT UCP1 expression. BAT explants showed decreased basal TAG content, increased basal lipolysis and a blunted lipolitic response to norepinephrine. These effects were reversed in BAT from newborns whose mothers received melatonin during pregnancy. Conclusion: Exposure to maternal melatonin during gestation is important in determining amount and functionality of BAT in the newborn.

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Defining the function of GPR50 through its interaction partners

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Purpose: GPCRs (G protein coupled receptors) respond to a variety of stimuli from peptides to hormones. They share a general seven-transmembrane topology and signal through heterotrimeric G proteins. Because of their fundamental role in physiological and pathological processes, they are widely studied. However, numerous GPCRs and putative ligands are still unpaired. Several deorphanization strategies are purposely explored to fill this gap. GPR50, an orphan GPCR, was shown to be involved in psychiatric disorders and energy homeostasis. In addition, GPR50 knockdown mice are resistant to high fat diet-induced obesity. However, apart from the fact that GPR50 heterodimerise with MT1 and MT2 melatonin receptors; very little is known about the function of GPR50 on the molecular level. As GPR50 does not bind any known ligand, a way to investigate its function is to identify its interacting partners. Methods: A yeast two-hybrid screen and a tandem affinity purification assay coupled to mass spectrometry were performed in our laboratory to identify GPR50 associated protein complexes. Results: Protein associations were confirmed with complementary methods in exogenous and endogenous systems. GPR50 impact on signaling pathways of most interesting partners were dissected. Conclusion: Dissection of those new protein networks aims at understanding GPR50 physiology and at developing therapeutic opportunities.

Effects of melatonin on neuronal activity in the rat suprachiasmatic nuclei (SCN) in vitro

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Purpose: To investigate the acute effects of exogenous melatonin on Per1-expressing and non Per1- expressing SCN neurons in vitro. Methods: Hypothalamic slices con-

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taining the SCN were prepared from Per1::GFP-expressing transgenic mice and GFP expression visualised using fluorescence microscopy to identify Per1+ve and Per1-ve SCN neurons. Cells were recorded from under either current- or voltage-clamp mode and melatonin (1nM) applied via the perfusion line for ~1.5 min. In current clamp recordings, melatonin was also applied to some cells in the presence of the fast sodium channel blocker, tetrodotoxin (TTX), to determine whether responses were pre- or postsynaptically mediated. Results: In current clamp mode, exogenous melatonin, at near-physiological concentrations, hyperpolarised the majority of SCN neurons tested at all times of the projected light:dark cycle. In addition, 1 nM melatonin depolarised a small proportion of cells. No differences were observed in melatonin's effects between Per1+ve or Per1-ve cells, however neurons in the dorsal SCN were more likely to be hyperpolarised by melatonin than those tested in the ventral region. The majority of melatonin's effects on resting membrane potential were blocked by TTX and in voltage clamp mode, 1nM melatonin increased the frequency of GABA-mediated postsynaptic events in SCN neurons. Conclusions: This study provides the first report of melatonin-induced hyperpolarisations in Per1-expressing SCN neurons, a subset of cells containing one of the key components of the molecular clock. In contrast to previous investigations, our results suggest that the majority of melatonin-induced hyperpolarisations are mediated presynaptically and also that melatonin is likely to exert its effects by modulating inhibitory GABAergic transmission.

Detection of GPR50 in rodent and human hypothalamus

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Purpose: There is accumulating evidence for the expression of the orphan G-protein-coupled receptor, GPR50, in the hypothalamus. However, due to the absence of a ligand or an antibody, precise in vivo localization of the receptor is still lacking. The aim of the present study is to confirm the expression of this receptor. Methods: Using antibodies generated and characterized by our group, we investigated the distribution of GPR50 by immunocytochemistry. Results: GPR50 expression has previously been detected in rodent and ovine hypothalamus on the mRNA level. Also, the recent generation of mice lacking functional GPR50 through insertion of a lacZ gene into the coding sequence of GPR50, suggested the expression of GPR50 in mouse hypothalamus. Here, we confirm for the first time using a sensitive and specific approach, that GPR50 is expressed in rodent and human hypothalamus. Furthermore, cell-type specific markers have been used to characterize the GPR50-expressing cells. Conclusions: This study offers new pharmacological perspectives for targeting GPR50, a receptor previously associated to bipolar and depression disorders and energy homeostasis regulation.

Melatonin protects oocyte and granulosa cells from reactive oxygen species (ROS) during the ovulatory process

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Purpose: ROS are produced within the follicle especially during the ovulatory process. We examined whether melatonin acts as an anti-oxidant within follicles and protects oocytes and granulosa cells from oxidative stress. Methods: 1) Follicular fluid was sampled at oocyte retrieval during in vitro fertilization and embryo transfer (IVF-ET) and intrafollicular concentrations of melatonin, 8-OHdG (8-hydroxy-2'-deoxyguanosine) and progesterone (P) were measured. 2) Oocytes recovered from immature ICR mice were incubated in medium with hydrogen peroxide (H2O2) and melatonin. 3) Infertility women who failed to become pregnant in the previous IVF-ET cycle with a low fertilization rate (<50%) were recruited to IVF-ET with melatonin treatment (3mg), and fertilization rate and pregnancy rates were compared to the previous IVF-ET cycle. 4) Luteinized granulosa cells collected from follicular fluids of IVF-ET patients were incubated with H2O2 and melatonin, and P concentrations in the medium were measured. Results: 1) Melatonin levels in follicles increased depending on follicular growth. Melatonin showed a negative correlation with 8-OHdG and a positive correlation with P, and there was a negative correlation between P and 8-OHdG. 2) Reduced percentages of the oocyte with first polar body caused by H2O2 were recovered by melatonin addition. 3) Melatonin levels in follicles were significantly increased and 8-OHdG levels were significantly decreased after melatonin treatment. The fertilization rate and pregnancy rate were improved by melatonin treatment. 4) P production was inhibited by H2O2 in a dose-dependent manner, and the inhibitory effect was blocked by melatonin. Conclusions: Melatonin plays an important role as an anti-oxidant in the follicle. Melatonin protects oocytes and granulosa cells from ROS during the ovulatory process, and contributes to oocyte maturation and luteinization of granulosa cells.

cDNA microarray analysis of melatonin receptor1 (MT1)-dependent gene expression in the mouse pars tuberalis

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Purpose: Melatonin is an important rhythmic signal within the circadian system of mammals. The hypophyseal pars tuberalis (PT) expresses a high density of MT1 receptors (von Gall et al. 2002a) and is believed to regulate seasonal changes in physiology and behavior (Korf and Stehle 2002). Although laboratory mice are relatively insensitive to photoperiod, a deficiency in MT1 signaling in the PT affects not only prolactin secretion (von Gall et al. 2002b) but also abolishes expression of clock genes which encode for transcriptional regulators of rhythmic gene expression (Jilg et al. 2005). In addition, the expression of the negative regulators Per1 and Cry1 are acute inhibited and activated by melatonin, respectively (Dardente et al. 2003). In this study, the effect of melatonin acting through the MT1 receptor on gene expression in the PT was analyzed by comparing wild-type (WT) and MT1-/- mice at two different time points: CT06 (low melatonin levels) and CT18 (high melatonin levels) by cDNA microarray analysis. In situ hybridization was used to con-

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firm observed day/night differences and to study the acute effect of daytime-melatonin application on gene expression in WT PT. Expression of Mt1 and Tim was higher during the day as compared to night in PT of WT mice, suggesting an inhibitory role of melatonin. In contrast, expression of Cry1, Neurod1 and Npas4 was lower during the day as compared to night in PT of WT mice, suggesting an activatory role of melatonin. Day night differences in expression of these genes were not observed in PT of MT1-/- mice, demonstrating the importance of the MT1 receptor for gene expression in this tissue. However, acute effects of day-time melatonin application could be observed on the expression of Mt1, Tim, and Cry1 but not on the expression of Neurod1 and Npas4. We conclude that melatonin regulates gene expression primarily through the MT1 receptor by either acute or long term mechanisms.

A new perspective for melatonin action in the human adrenal gland: melatonin inhibits ACTH-stimulated cortisol production by reducing BMAL1 and StAR and 3ß-hydroxysteroid dehydrogenase (3ß-HSD) expression

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Recent evidence shows that clock genes are required for primate adrenal response to ACTH. In addition, the clock gene protein BMAL1 is important for ACTH stimulation of the key steroidogenic proteins StAR and the enzyme 3ß-HSD. Melatonin inhibits clock gene expression in the primate adrenal gland and ACTH-stimulated cortisol and corticosterone production in primates and rats. The melatonin receptor MT1 is expressed in the human adrenal gland. Purpose: To assess whether melatonin effects on ACTH-stimulated cortisol production involves suppression of BMAL1 protein and steroidogenic proteins. Methods: Normal adrenal tissue was obtained from 5 patients undergoing laparoscopic unilateral nephrectomy-adrenalectomy. Explants were prepared from 3 adrenal glands, preincubated at 37°C for 6 h and starting at 2000 h, incubated in triplicate for 12 hours in 2 ml medium alone (basal) or containing 100 nM of 1-24 ACTH in the presence or absence of 100 nM melatonin and in the presence or absence of 1 µM Mt1/Mt2 antgonist luzindole. Clock gene expression of Per2, Bmal1, Cry2 and Clock was investigated in the other 2 adrenals. Results: Clock genes were expressed in the human adrenal gland. ACTH increased cortisol production, StAR and 3ß-HSD content and BMAL1 content over 2 fold. These responses were blocked by coincubation with melatonin and reversed by the antagonist luzindole. Conclusion: Our study demonstrates that melatonin acts directly upon the human adrenal gland, as a local inhibitor of ACTH-induced cortisol production by down-regulation of the clock protein BMAL1, required for StAR expression.

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Melatonin is released in the third ventricle in humans. A study in movement disorders

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Purpose: To determine sources and metabolism of melatonin in human cerebrospinal fluid (CSF). Methods: melatonin and 6-sulfatoxymelatonin (aMT6S) concentrations were measured by GCMS and RIA respectively in cerebrospinal fluid (CSF) sampled during the day (09:00-15:00) in both lateral and third ventricles in patients displaying movement disorders (Parkinson's disease, essential tremor, dystonia or dyskinesia) during neurosurgery and compared with their plasma levels. Previous determinations in nocturnal urine had showed that, despite drug administration of drugs, the patients displayed melatonin excretion in the normal range, compared with healthy controls matched according to age. Results: A significant difference in melatonin concentration was observed between lateral and third ventricles, with the highest levels in the third ventricle (8.69±2.75 and 3.20±0.33 pg/ml respectively, $p<0.01$). CSF aMT6s levels were similar in both ventricles (2.6±0.22 and 2.7±0.26 $p=0.21$) and of low magnitude, less than 5pg/ml. They were not correlated with melatonin levels. Melatonin levels were significantly higher in third ventricle than in the plasma (4.38±1.32 pg/ml, $p<0.05$), whereas there was no difference between plasma and lateral ventricle levels. Conclusions: These results, involving a specific assay (GCMS) for CSF melatonin determination, show that melatonin may enter directly the CSF through the pineal recess in humans, even during the day. The physiological meaning of these data remains to be elucidated.

Melatonin effect on differentiated functions of mouse osteoblast

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The physiological and neuroendocrine functions of melatonin and its therapeutic potential critically depend on the understanding of its target sites and mechanism of action. It was reported that melatonin promoted osteoblast differentiation and mineralization in MC3T3-E1 (mouse preosteoblast) and rat osteoblast-like osteosarcoma 17/2.8 cells. Purpose: the aim of this study was to assess the effect of melatonin treatment on mouse osteoblast in culture by studying the Cbfa1 gene expression, the major regulator of the osteoblast phenotype, and induction of mineralization as expression of differentiated osteoblasts. Material and methods: mouse osteoblasts from different passages were seeded for RNA extraction or von Kossa specific staining for mineralization and treated with melatonin (concentrations ranged between 10-11-10-6 M). Cells from passage 1 and 2 have been treated with melatonin for 2 weeks. After incubation period cells were harvested and RNA extracted. 1µg RNA was reverse transcribed to single stranded cDNA and then specifically amplified for Cbfa1. Cells were also stained specifically for alkaline phosphatase and mineralization. Results: We used an experimental model of mouse osteoblast culture from newborn calvaria set up by our group. As a specific marker for osteoblast differentiation we used Cbfa1 gene expression. Melatonin reduced Cbfa1 expression in both experiments. Groups treated with melatonin showed high

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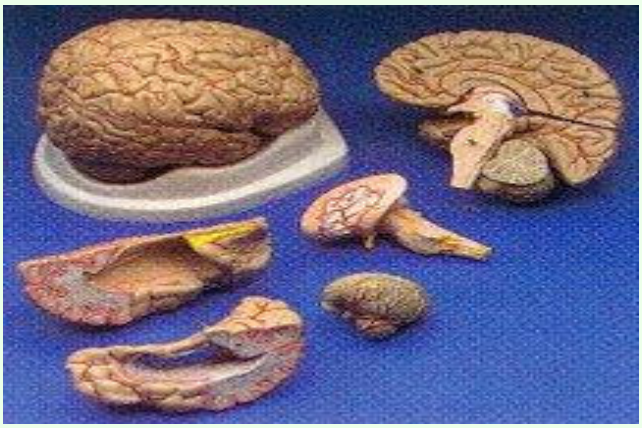
mineralization in an early development stage, in the second week of culture, when normally mineralization does not occurs (as could be seen in control sample). Von Kossa positive staining showed that melatonin promotes mineralization in osteoblasts in early development stages. Conclusion: The slight inhibition in Cbfa1 expression is correlated with the precocious fully differentiation of osteoblasts (mineralizing cells). As the human melatonin receptors are very similar to mouse melatonin receptors, the mouse osteoblast culture is a good model for human osteoblasts.



9. Brain, energy metabolism and rhythms

Variation in orexin activation in CD-1 mice

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Purpose: To determine the rhythmic profile of orexinergic neuron activation in the CD-1 mouse. Methods: Adult male CD-1 mice were maintained under a 12h:12h light-dark cycle and sacrificed at varying points across the daily cycle: ZT6, ZT11, ZT13 and ZT18 (where ZT12=lights-off). Brains were processed for double-labelling immunohistochemistry using c-Fos and orexin specific antibodies as follows: the suprachiasmatic nuclei (SCN) were processed for c-Fos and the lateral hypothalamus (LH) was processed for both c-Fos and orexin. Results: The profile of SCN activation, as measured by the number of c-Fos immunoresponsive neurons, revealed an increased level of c-Fos-ir at ZT6, ZT11 and ZT13 compared with ZT18. Double-labelling immunohistochemistry of the LH for c-Fos and orexin revealed a higher percentage of double-labelled neurons at the points ZT11 and ZT18 but not ZT13 compared to ZT6. Conclusions: The SCN in CD-1 mice display rhythmic c-Fos expression across the sleep-wake cycle similar to that seen in other mouse strains, that is, with higher levels during the animal's inactive period. In the LH, levels of orexinergic neuronal activation varied across the sleep-wake cycle with higher levels of c-Fos:orexin co-localization during the middle of the animal's active period (ZT18) than during the middle of the animal's inactive period (ZT6); again demonstrating similarities with other rodent species. Further, there was an increase in c-Fos/orexin co-localisation at ZT11 compared to ZT6. This suggests that these neurons become active prior to the onset of activity during the nocturnal period.

Confinement and extended wakefulness effects on propensity to take risks

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The consequence of confinement and extended wakefulness on risk-taking propensity is a key issue in crew management. We investigated both confinement (partial social isolation and "day light" equal at 300 lux in living areas) and gender effects on risk propensity and performance during up to 36 h of extended wakefulness. We studied 4 groups of 3 men and 3 women [N = 24, mean age (+/- SD) = 32.9 +/- 5.8 yr] for 10 consecutive days: a 7 days period of confinement or a 7 day baseline condition preceding one control night of normal sleep, one night of extended wakefulness, and one recovery night in the laboratory. Risk propensity (EVAR scale) and simple reaction time task performances were monitored every 2.25 h (09H30-19H45) during confinement and every 2.11 h (09H30-07H45) during the extended wakefulness condition. Overall risk propensity during extended wakefulness showed a variation in both conditions with two diurnal peaks separated by a nocturnal minima. After the confinement period, no second peak was found. Number of lapses (reaction time > 500 ms) on the SRTT varied daily in both conditions. During the night of extended wakefulness, risk-taking propensity decreases and remains stable the following day in the confinement condition while it increases after the baseline period. Low light level habituation in confinement condition could be responsible in smoothing risk propensity level when subjects were sleep deprived.

The effect of water deprivation on supraoptic nucleus and subfornical organ of a desert rodent Meriones shawi: GFAP immunohistochimical study

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Purpose: Supraoptic nucleus is part of the hypothalamus-neurohypophysial system, which constitutes an obvious example of activity-dependent neuroglial plasticity, in which certain physiological conditions, such as dehydration, are accompanied by a structural remodeling of the neurones, their synaptic inputs and their surrounding glia. The subfornical organ is a brain structure involved in osmotic stress and water balance. In the current work, adult Meriones shawi (rodent adapted to desert life) are used as an animal model. Methods: Using GFAP expression as an indicator of astrocytes activation, the effect of prolonged episode of water deprivation on the supraoptic nucleus of the hypothalamus (SON) and subfornical organ (SFO) were examined. We studied the immunoreactivity of GFAP in various hydration states (total deprivation of drinking water for 3 and 6 months compared to hydrated animals). Results: Our immunohistochemical study demonstrates that the dehydration produces an important decrease of GFAP immunoreactivity in both SON and SFO after 3 and 6 months of water restriction. Conclusions: This finding with others, may explain a real involvement of SFO and SON astrocytes of Meriones shawi in this osmotic stress situation. Furthermore, these data could open further investigations concerning the possible involvement of SFO and SON astrocytes in the seasonal regulation of the hydrous balance and resistance to dehy-

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dration of this desert rodent.

Evaluation of the effect of aluminium chloride exposure on daily activity of rat brain

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Aluminium is the most abundant metal on our ground. In nature, the aluminium does not exist in a free state. Combined with oxygen, fluorine and silicon, it constitutes approximately 8% of the Earth's crust. Aluminium incorporates into the body either through respiratory, digestive or cutaneous ways. We intend to evaluate the neurocomportemental and immunohistochemical parameters induced by chronic exposure to the aluminium chloride (AlCl3) in the adult rat. For this study, we used rats wistar exposed to 0.3% of aluminium chloride in drinking water during two months. Behavioural studies were performed using the “Open field” and the “Dark/light box” tests. The animals were used thereafter for the immunohistochemical study by using antibody anti GFAP (glial fibrillary acidic protein). This study enables us to evaluate the reaction of the system glial to the aluminium chronic intoxication on the level of the cerebral cortex, the hippocampus and the cerebellum cortex. The results obtained showed a reduction respectively, in locomotor activity and an increase in anxiety in the rats treated compared to controls. The immunohistochemical study of the GFAP revealed a significant increase of GFAP-immunolabelling compared to controls. These results show that aluminium affects the daily behavior of rats. Reduced glial expression could induce a dysfunction of the neuronal system. The present study brings a support for the role of the aluminium in brain dysfunction.

The Krüppel Like Factor KLF10 is a molecular link between the circadian clock and metabolism in liver

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Purpose: Epidemiological and experimental evidence suggest that the circadian clock system is intimately linked to metabolism in mammals. However, the genetic circuitry that link clock genes to metabolic outputs remains poorly defined. In the present study we investigated the role of the transcriptional regulator KLF10 in the circadian regulation of metabolism in liver. Methods: The clock regulation of Klf10 was determined using reporter gene assays and Bmal1 deficient mice. The physiological role of KLF10 was analysed using microarrays and qPCR mRNA profiling, metabolic phenotyping in wild-type and Klf10 deficient mice. Results: The circadian expression of Klf10 is driven by the liver clock through a conserved E-box element. Genome-wide analysis of the liver transcriptome in Klf10-/- mice identified 158 target genes with a significant enrichment for genes involved in lipid and carbohydrate metabolism. Male Klf10-/- mice exhibited elevated postprandial and fasting glycemia. Consistently, the gluconeogenic gene Pepck was upregulated and hepatic glucose production was increased in Klf10-/- mice. Females Klf10-/- mice were normoglycemic but displayed higher plasma triglycerides and altered circadian expression profiles of lipogenic genes including Fas, Elovl6 and Srebp1c. Furthermore, the response to PPAR??ligands was altered in Klf10-/- primary hepatocytes. Conclusions: Collectively, these data establish KLF10 as a circadian transcriptional regulator that links the molecular clock to energy metabolism in liver.

The biological clock modulates the sensitivity of the arcuate nucleus to metabolic changes

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Purpose: Blood glucose levels in the blood follow a circadian rhythm which is controlled by the suprachiasmatic nucleus (SCN). The arcuate nucleus (ARC) is considered as a brain area that senses the concentration of circulating metabolites and hormones. The ARC has reciprocal connections with the SCN indicating that the SCN may play a role in changing the sensitivity of the ARC to circulating hormones and metabolites. Our objective is to investigate the influence that the SCN exerts on the ARC under negative metabolic conditions, such as fasting, and hypoglycemia. Methods and Results: Wistar rats received a jugular vein and icv canula for injection of 2-deoxyglucose (2-DG) centrally or into the general circulation at two different daytime points. Two hours after the 2-DG challenge, the ARC responds differently to hypoglycemia at the two different time points, with a major expression of c-Fos at the end of day. This activation is exclusive to the ventromedial area of the ARC where mainly NPY containing neurons are activated by the hypoglycemic challenge. To elucidate the mechanisms by which the SCN is controlling the sensitivity of the ARC, Wistar rats received a SCN unilateral lesion, after recovery they were fasted for 2 days. We observed an increment of c-Fos activity at the lesioned side, indicating inhibitory projections from the SCN to the ARC during the light period. To define the mechanisms of this putative SCN inhibition of the ARC to hypoglycemic conditions, Wistar rats were canulated in the jugular vein and received a unilateral lesion of the SCN. After a period of recovery, animals were injected intravenously with 2-DG and blood samples were taken for analizing the circulating levels of glucose. Two hours after the injection the brains were perfused and analyzed for c-Fos and NPY. Conclusions: Our results indicate that the SCN inhibits the ARC glucosensitive NPY neurons during the light period. These results allow us to propose that the SCN gates the transmission of metabolic information of the ARC to the rest of the brain, involving it in the daily homeostatic control.

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Balanced nutrient foods with high glycemic index but not sugar alone is suitable entraining signals of mouse liver clock

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Background: Mouse peripheral circadian clock is known to be entrained not only by light-dark cycle but also daily restricted feeding schedule. Behavioral and cell culture experiments have suggested the increase of glucose may be one of factors for such feeding-induced entrainment. When the evidence of feeding-induced entrainment is applied for human life, nutrient content and variation of foods should be considered. Principal finding: In order to elucidate the composition of food in this entrainment, we examined whether complete or partial substitution of nutrient affects phase-shifting the liver clock. Nutrient composition of carbon hydrate were selected by according to low glycemic index (GI) or high GI value. When standard mouse diet, AIN-93M formula was given for 2 days at middle day, liver bioluminescence rhythm of Per2::luciferase knock-in mouse caused 3-4 hrs advances in comparison to fasting or ad lib feeding mouse. One hundred percent of cornstarch or soybean oil moderately but not of casein produced phase advance, while 100% of glucose or sucrose failed to do it. When cornstarch was substituted by glucose, sucrose, fructose, or polydextrose caused phase advance in parallel with the order of GI values. When cornstarch was substituted by high amylose cornstarch (low GI) or gelatinized cornstarch (high GI), high amylose cornstarch caused a weak phase advance than cornstarch or gelatinized cornstarch. Corn flour with high GI also caused large phase advance than that with low GI. Conclusions: The present results strongly suggest that balanced nutrient foods containing at least carbon hydrate are good nutrition for restricted feeding-induced entrainment of peripheral circadian clock, and that food.

Positive circadian transcription factor complex CLOCK/BMAL1 and HES family members, HES1 and HES6, regulate human LDLR promoter activity via distinct promoter elements

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Purpose: We recently observed that HES6 oscillates in the mouse liver in an E-box- and functional circadian clock-dependent manner (The 20th Annual Meeting of KSMCB, Poster No. P29-27). Here, we explored possible roles of CLOCK/BMAL1, HES1 and HES6 in the regulation of low density lipoprotein receptor (LDLR) promoter. Methods: Human LDLR promoter reporter constructs were transiently transfected into HepG2 cells and the effects of CLOCK/BMAL1, HES1, and HES6 expression were analyzed by dual luciferase reporter assays. Serial deletion and point mutation constructs were used to delineate responsible promoter elements. Results: We found that positive circadian transcription factor complex CLOCK/BMAL1 upregulates human LDLR promoter activity in a serum-independent manner, while transcriptional repressor HES1 downregulates it only under serum-depleted conditions. Both effects were mapped to proximal promoter region of human LDLR, where mutation or deletion of well-known serum response element (SRE) abolishes only the repressive effect of HES1. Surprisingly, expression of HES6, well-known repressor of HES1 repressor, also inhibits human LDLR promoter activity in a dose-dependent manner and involves distinct promoter element. Conclusions: These results suggest that transcriptional regulation of LDLR is very complicated and subject to circadian control involving CLOCK/BMAL1 and HES family transcription factors.

Disruption of circadian rhythms by constant light does not inhibit cell proliferation in the hippocampus of adult rats

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Purpose: REM sleep deprivation (RSD) has been shown to inhibit hippocampal neurogenesis in the adult rat independent of elevated adrenal stress hormones (Mueller et al. 2008). Attenuated circadian rhythms of locomotion, waking, and drinking are side effects of RSD procedures. This study was conducted to identify the role of disrupted circadian organization as a potential mediator of the anti-neurogenic effect of RSD. In line with this idea, various reports have suggested the importance of circadian regulation in neurogenesis as well as in hippocampal dependent learning. Methods: In order to abolish circadian rhythms of behavior, male Sprague Dawley rats were subjected to constant bright light (LL) for either 4-days or 10-weeks. Arrhythmicity of locomotion was validated with overhead motion sensors and sleep stages were verified via EEG recordings. Newly proliferating cells in the granular cell layer (GCL) of the dentate gyrus (DG) were labeled with a single IP injection of 5-bromo-2-deoxyuridine (BrdU), and animals were sacrificed 2h later. To confirm reports of daily variation of hippocampal cell proliferation (Guzman-Marin et al. 2007), we additionally assessed the number of new cells in the DG early and late in the light period under LD 12:12. Results: Neither short term LL (4-days), nor long term LL (10-weeks) altered cell proliferation in the GCL of the DG despite circadian disorganization of behavior. Furthermore, we did not detect significant daily variation of cell proliferation at two times points previously reported to vary by a factor of 2. Conclusions: Our results suggest that disrupted circadian rhythm by constant light does not alter basal level of hippocampal cell proliferation. Therefore, the reported reduction of hippocampal cell proliferation after 4 days of RSD can be seen as a primary result of sleep deprivation and not as a secondary effect of circadian disorganization.

Daily rhythm in morphine sensitization and conditioned-place preference

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Purpose: We aimed at measuring the daily rhythm of morphine-induced behaviour sensitization and conditioned place preference (CPP), two behaviours reflecting the development of dependence to this drug. Methods: Therefore, we assessed the development and the expression of these two behaviours at 4 different time-points (ZT4, ZT10, ZT16 and ZT22) in C57Bl6/J male mice. During 8 conditioning daily sessions of 30 min, mice received, alternatively, saline or morphine (10 mg/kg; i.p.) injections while exposed to one of two different floor types (CS- and CS+, respectively). During the CPP test session (15min), mice were put in the box with both types of floor available; time spent on each floor was recorded. Behavioural sensitization was assessed measuring the locomotor activity of

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the animals during the 4 CS+ conditioning sessions of the CPP procedure. In order to verify that the rhythmicty observed was not only occuring during the expression of the CPP but well due to daily differences in the acquisition, we performed, two days later, a second session of CPP test with a delay of 12h for each time-point. Results: Significant daily patterns could be observed in the development of sensitization and CPP when measured at different time-points. However, although the peak of sensitization was observed at ZT22, the one for CPP was measured at ZT10. Moreover, the group of mice conditioned at ZT10 was still showing the highest CPP score when mice were tested 12h apart from their conditioning time, thereby confirming the involvement of the internal state during the acquisition of the conditioning. Conclusions: The present study reveals the existence of a daily rhythm in the propensity to develop morphine-induced sensitization, as well as in the conditioning towards the reinforcement properties of morphine. Interestingly, both patterns are opposite to the one observed previously in cocaine-induced sensitization and CPP.

The intergeniculate leaflet may provide information about the feeding condition to the suprachiasmatic nucleus

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Purpose: To investigate whether the Intergeniculate leaflet may provide the suprachiasmatic nucleus (SCN) information about the metabolic state of the animal. The hypothesis of the study was that the Intergeniculate leaflet (IGL) may use its transmitter NPY to signal to the SCN not only locomotor but also metabolic information. Methods: Six groups (n=5) male Wistar rats were used for immunohistochemical analysis of NPY under the following conditions: fed ad libitum; fasted for 48 hours; MSG-lesioned in the arcuate nucleus (ARC) and fed ad libitum; MSG-lesioned and 48h-fasted; electrolitic lesioned in the IGL; lesioned in the IGL and 48h-fasted. Results were quantified by optical densitometry. Results: 48h-fasting increases NPY optical density in the ARC, IGL and SCN, especially in the anterior part of the nucleus. Since part of the NPY innervation may originate from the ARC, the effect of a lesion of one of the two nuclei was analyzed under ad libitum and fasted conditions. Results confirm that most of the NPY fibers in the SCN descend from the IGL, but also indicate that the increase seen after fasting originates from the IGL. In fact, after IGL lesion, only few NPY fibers were left in the SCN, and their number was not increased after fasting. Arc lesion, did not affect NPY presence in the SCN, both in animals with free access to food and fasted, since optical densitometry did not show any significant difference between the two conditions. Conclusions: It is well established that the IGL transmits non-photic information to the SCN using NPY. Since the IGL receives innervations from the ARC, our data suggest a new role for the IGL, which is to transmit to the SCN information that is connected, directly or indirectly, with the feeding conditions.

Scheduled feeding prevents internal desynchrony and obesity in a model of night work in rats

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Purpose: Understand the mechanisms underlying the internal desynchrony for night work and propose strategies to prevent this disturbance. Methods: we have developed an experimental model of night-work in rats based on schedules of forced activity. From Monday to Friday rats are placed for 8 hours in slow rotating wheels during their inactivity phase (from 9 AM to 5 PM). During the remaining hours of the day and during weekends rats are returned to their individual home cages placed in a monitoring system in order to register their activity, and their feeding patterns, besides monitoring the metabolic, and temperature rhythms and body weigh. Results: After 4 weeks under this "working" schedule rats diminished their nocturnal activity and voluntarily shifted their food ingestion towards "working" hours. Metabolic rhythms were dampened or uncoupled from the suprachiasmatic nucleus (SCN) activity, which remained fixed to the LD cycle and promotes increased body weight and abdominal fat accumulation. Since feeding schedules are a strong entraining signal for metabolism and behavior, we explored whether the changed feeding patterns developed by working rats could have promoted the internal desynchrony. Feeding restricted to the dark phase (the normal activity phase) reverted all metabolic disturbances to the normal range and prevented body weight increase and abdominal fat accumulation, demonstrating the important contribution of feeding habits to prevent internal desynchrony and obesity in the night worker. Conclusions: Our study demonstrates the relevance of feeding schedules to prevent circadian misalignment in the night worker and may well explain metabolic disturbances arising from disturbed feeding patterns

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Critical role of dorsomedial hypothalamus (DMH) in light-induced but not feeding-induced entrainment of liver circadian rhythm in Per2::luc knock-in mouse

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Purpose: The role of the DMH - the brain area responsible for restricted feeding-induced anticipatory activity rhythm - in the entrainment of the peripheral circadian clock was examined. Methods: Mice were thermally lesioned on the hypothalamus including DMH or sham operated, and then were restricted their feeding time in the daytime or exposed from a LD to the dark-light (DL) cycle. After sacrifice we examined the liver Per2 rhythm by using Per2::luciferase knock-in mice. Results: A lesion on the DMH significantly reduced the restricted-feeding-induced anticipatory activity rhythm, but did not affect the entrainment of the liver Per2 rhythm. In sham-operated mice, change from a LD to the DL cycle reversed the peak of the liver Per2 rhythm as well as the locomotor activity, and

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feeding rhythms within two weeks following changes in LD condition. On the other hand, lesioned animals sustained a delay in the re-entrainment of the liver Per2 rhythm phase as well as in activity and feeding rhythms. However, within four weeks, DMH-lesioned mice showed a new phase identical with sham-operated mouse. Simultaneous changes to lighting conditions (i.e., from LD to DL cycle) and feeding conditions (i.e., from ad lib feeding to early light period) caused a rapid entrainment of liver Per2 rhythm within two weeks. Conclusions: The present results clearly demonstrate that the hypothalamus including DMH is an important brain area vis-à-vis light signal-induced entrainment of the liver clock, but not for restricted feeding-induced entrainment therein. Thus, DMH areas may relay LD signals to the liver from the suprachiasmatic nucleus.

Synchronization of the locomotor activity rhythm to the light-dark cycle is impaired by additive exposures to calorie restriction and thermal stresses in Microcebus murinus.

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Purpose: Calorie restriction (CR) is today well known to interfere with the entrainment of the biological clock. Moreover, cold and warm exposures recently appeared to potentially disturb the daily organization of locomotor activity (LA). We thus decided to investigate whether CR interfered with the effects of cold and warm exposures on the daily rhythmicity in LA in the mouse lemur (Microcebus murinus). We also investigated whether these responses differed according to the season, since this primate exhibits thermoregulatory responses particularly well synchronized on photoperiod. Methods: Adult mouse lemurs acclimated to LD10/14 or LD14/10 were exposed to 10–day periods at 25, 12°C and 34°C. LA rhythms were recorded by telemetry in animals fed either ad libitum (control diet: CTL) or exposed to chronic calorie restriction (CR: 70% of the CTL diet). Results: No diet effect on the synchronization of the LA rhythm to lights off was observed at 25°C whatever the photoperiod. Synchronization was also preserved in CTL animals under thermal exposures, except for cold-exposed animals under LD 14/10 which expressed a phase advance of the rhythm. By contrast, CR induced significant phase advances in all conditions of thermal and photoperiod exposures, with a clear anticipation of lights off in all experimental conditions. Conclusions: In chronically calorie-restricted animals, perturbation of the synchronization of the LA rhythm on the LD cycle can be revealed by exposing animals to changes in ambient temperature. This suggests that additive energetic pressure of calorie restriction and of thermoregulatory response to thermal stress can lead to a desynchronization of circadian rhythms. Since aging is well known to impair the circadian rhythmicity, it could be interesting to investigate the effects of age on such thermoregulatory responses.

Automated physio-behavioral intra-home-cage phenotyping in SCA17 transgenic rats facilitates multidimensional recording of various activity pattern

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Purpose: To automatically screen transgenic rats in a home-cage-like environment for several parameters at a high temporal and spatial resolution, allowing experimenter independent monitoring of laboratory rodents and avoidance of stress-artifacts, higher throughput, higher sensitivity of measures (online, circadian). Methods: The PhenoMaster system represents a modular set-up that measures indirect calorimetric parameters, activity, drinking and feeding behavior, learning (operant wall) or wheel running and is based on conventional type IV Thermoplast cages, with each cage being equipped with the technical requirements to individually monitor one rat per cage at a time. The present system is set-up to measure 12 animals in parallel with high resolution for locomotor activity, O2-consumption, CO2-production, respiratory exchange rate (RER) and food and water consumption. A transgenic rat model for Spinocerebellar Ataxia Type 17 (tgSCA17) is monitored repeatedly over an experimental time of 72h on a monthly basis across 12 months. Results: Transgenic animals show a reduced rearing behavior and an increase in the respiratory exchange rate. These differences are mainly seen in the dark phase of the day. Conclusion: The system allows for the first time a comprehensive behavioral/ physiological activity pattern suitable for multivariate statistics and detection of novel multidimensional behavioral output.

10. Photoperiodism; Circannual rhythms; Hibernation and torpor

Seasonal variation of the locomotor activity rhythm of Talitrus saltator from a beach in the gulf of Gabes (South of Tunisia)

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Purpose: to characterize the seasonal variation of the locomotor activity of the supra-littoral Amphipoda Talitrus saltator collected from the gulf of Gabes (N 3° 52' 34.0"; E 10° 07' 38.8") where tides amplitude is the most important in the Mediterranean basin. Methods: Adult individuals were collected with hand, in May and October 2008. These individuals were transferred individually in actographs, equipped with an infra-red recording system. These actographs are placed during 14 days (7 days LD and 7 days DD) in a controlled environment cabinet able to control photoperiod and temperature (maintained con-

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stant at 18±0.5°C). Results: The analysis of the double-plotted actograms, as well as the waveform per hour per day curves showed various types of patterns of locomotor activity: unimodal, bimodal and plurimodal. However, the unimodal pattern is the most observed during the spring season whatever the photoperiodic regimen imposed. Moreover, the Periodogramm analysis revealed an endogenous circadian period (˜24h) with a circatidal component (˜12h). In addition, the analysis of the SNR (Signal Noise to Ratio) of both circadian and circatidal components showed they were better defined in spring than in autumn. A highly significant differences, between the two rhythmicities circatidal and circadian, were revealed whatever the season of recording. Conclusions: specimens of Talitrus saltator from a beach of Gabes's gulf exhibit an endogenous circadian activity rhythm with circatidal component. Moreover, this locomotor rhythm is more stable during the spring season.

The role of RFRP peptides in the seasonal regulation of reproduction

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Purpose. RFamide-related peptide (RFRP) is the mammalian orthologue of avian Gonadotropin Inhibitory Hormone. In rodents, it encodes two peptides, RFRP-1 and RFRP-3, expressed in the dorsomedial hypothalamus. In rats and sheep, RFRP-3 seems to play an inhibitory role on the reproductive system. However, in Syrian hamsters, the RFRP gene is strongly downregulated by melatonin in short photoperiod (SD), when the animals are sexually inactive. This data is not coherent with an inhibitory function of RFRPs. Therefore, our work aims to determine the role of RFRP in the seasonal regulation of reproduction in the Syrian hamster. Methods. To determine the physiological effects of the peptides on the reproductive axis, testicular activity of SD hamsters was analysed after several weeks of central administration of RFRP-1 and RFRP-3, and plasma LH and FSH concentrations were measured after an acute central injection of RFRP-3 in LD animals. Furthermore, the effect of chronic peripheral injections of RF9, a selective NPFF receptor antagonist, was tested on hamsters transferred from LD to SD. Results. The chronic central administration of RFRP-3, but not RFRP-1, increased testicular weight and plasma testosterone concentrations, and significantly increased the number of Kiss1 neurons in the arcuate nucleus. Furthermore, the acute central administration of RFRP-3 induced a significant increase in LH and FSH plasma concentrations. Daily intraperitoneal injections of RF9 significantly reduced paired testes weight. Conclusions. These data show a stimulatory action of RFRP-3 on the reproductive function in the Syrian hamster, via the Kiss1 neurons of the arcuate nucleus promoting LH secretion. Since RFRP expression is modulated by melatonin, our study points to RFRP as a central player in the seasonal control of reproduction.

Melatonin and testosterone dependent variations in Kiss1 expression drive seasonal reproduction in the Syrian hamster

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Purpose: To investigate how photoperiod, melatonin and testosterone regulates Kiss1 expression and determine a protocol of peripheral kisspeptin (Kp) administration to restore gonadal activity in photoinhibited hamsters. Methods: Kiss1 expression was examined by in situ hybridisation in the arcuate nucleus (Arc) and anteroventral periventricular nucleus (AVPV) from Syrian hamsters kept in various experimental conditions: 1) kept either in long days (LD) or short days (SD), 2) intact or castrated and kept in LD with a daily injection of melatonin, 3) equipped with testosterone implants and kept in SD. Finally, the effects of various protocols of long term administration of Kp to SD hamsters were tested. Results: Kiss1 expression in Arc and AVPV was markedly reduced in SD hamsters as compared to LD hamsters. In intact hamsters, melatonin decreased Kiss1 mRNA level in both AVPV and Arc, whereas in castrated animals, melatonin inhibited Kiss1 expression in Arc only. Testosterone increased Kiss1 expression in AVPV but decreased it in Arc. Finally, long term (4-5 weeks) administration of Kp increased testes weight when given as two intraperitoneal injections per day but not as a constant release by osmotic minipumps. Conclusions: Kiss1 expression in downregulated in SD conditions with different mechanisms. In Arc, melatonin inhibits Kiss1 by a direct effect on the hypothalamus whereas in AVPV, the decrease in Kiss1 expression depends on melatonin-driven reduction of testosterone level. Remarkably, SD-induced gonadal regression can be reversed by long term repeated peripheral administration of Kp. Taken together, our data support the hypothesis that Kiss1 mediates melatonin effect on the gonadotropic axis of the Syrian hamster.

Matrix metalloproteinases (MMP-3, MMP-7) immunoexpression in prostatic lobes of Libyan jird (Meriones libycus) during seasonal reproductive cycle and after castration

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Purpose: An immunohistochemical study of matrix metalloproteinases (MMP-3, MMP-7) was undertaken on the prostatic lobes (ventral prostate and coagulating gland) of the Libyan jird (Meriones libycus) to verify their implication in cellular process of reproduction and in seasonal tissue remodeling observed in this gland during the seasonal reproductive cycle. Methods: Meriones libycus were collected in breeding period (spring and early summer), in resting phase (late summer, autumn, winter) and from castrated animals during one month in the spring. The work was done using immunohistochemistry. Results: During breeding period MMP-3 (stromelysin-1) and MMP-7 (matrilysin) were strongly immunoexpressed by epithelial

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cells of ventral prostate, with a slight immunoexpression in smooth muscle cells (SMC) and without any immunolabelling in the extracellular matrix (ECM) and secretion. The secretion produced by ventral prostate showed a granular texture and was dug by empty circular areas of variable size. A small amount of an immunolabelled secretion was surrounding or mixed to the secretion. This phenomenon reflected a detachment of secretion allowing its movement in the tubules. In non-breeding phase and castrated animals, the ventral prostate was extremely regressed and both MMPs immunoreactivities were maintained with a similar value in the epithelial cells and SMC. During breeding period, MMP-3 and MMP-7 were immunodetected in epithelial cells and SMC of coagulating gland but without any immunostaining in the ECM. The secretion showed a fluid and smooth aspect and was immunostained. This immunoreactive pattern was similar in both sexual quiescent and castrated animals. Conclusions: The MMP-3 and MMP-7 are essential for cellular mechanisms of reproduction and seasonal tissue remodeling of the prostate. These enzymes are also useful to the flow of secretion in the ventral prostate. The steady presence of these MMPs during the seasonal reproductive cycle would ensure a rapid transition to the recrudescence of organs in active phase or to their regression in resting period.

Testosterone regulates vasopressin and galanin expression in the bed nucleus of stria terminalis' neurons and their target areas in the Syrian hamster.

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Purpose: We investigated whether all strains of Syrian hamster lack vasopressin in the Bed Nucleus of Stria Terminalis (BNST). Since vasopressin BNST neurons usually colocalize vasopressin and galanin, and both peptides are usually regulated by sex steroids, we also analysed the effect of sex steroids on the production of galanin in BNST. Methods: We compared vasopressin and galanin expression in BNST neurons of adults' male Syrian hamsters from two breeding stocks: wild animals and standard type. Furthermore, we investigated the effect of sex steroids on galanin expression in the BNST of standard male Syrian hamsters. For this, four groups of hamsters were used: long photoperiod (LP), short photoperiod (SP), LP castrated animals and SP implanted with testosterone capsules. mRNA levels were quantified by non radioactive in situ hybridization. Protein expression was analyzed by immunohistochemistry. Results: No vasopressin-expressing neurones were found in BNST of wild hamsters and hamsters bred in our facilities. Castration and exposure to SP induced a two-fold reduction of galanin immunoreactivity and mRNA, as compared to LP hamsters. Conclusions: We confirmed that the absence of vasopressin neurones in BNST Syrian hamsters' males is not restricted to animals from laboratory breeding colonies but also exists in wild animals. The fact that galanin appears regulated by sex steroids as vasopressin and galanin are in other rodent species, this reinforces the hypothesis that the subpopulation of galanin neurons that usually co-expresses vasopressin has not been programmed to do so during development.

5-methoxytryptophol induced c-Fos expression dependently on season in the SCN and thalamic structures of the jerboas (Jaculus orientalis)

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Purpose: To determine the season during which 5-Methoxytryptophol (5-ML) exerts a control in the brain in relation with its control upon the reproductive function in a seasonal breeder the jerboa (Jaculus orientalis). Methods: For each season, 10 adult female jerboas captured in the field and maintained in natural photoperiod conditions received each a unique subcutaneous injection of 25 µg of 5-ML or vehicle solution 2 h after sunrise and then were perfused with a fixative solution 1h30 later. Brains were processed for c-Fos immunocytochemistry. Results: 5-ML induced c-Fos expression in the paraventricular thalamic nucleus (PVT) and lateral habenula specifically in spring and summer period when the photoperiod was long. In the suprachiasmatic nucleus of the hypothalamus (SCN), 5-ML induced a significant c-Fos expression in autumn. Conclusions: 5-ML induced differential effects on the central structures according to seasons. 5-ML modulates SCN activity during the season of the maximal expression of its rhythm in the pineal gland when animals are sexually inactive, whereas in the PVT and habenula it induced the maximal c-Fos expression during the period of sexual activity. Reproductive function of the jerboa by 5-ML would thus involve these structures alternatively.

LHRH system of Jerboa (Jaculus orientalis) and its modulation by photoperiod, sex hormones and thyroid hormones

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Purpose: In this work, we studied in the male Jerboa a hibernating and seasonally reproductive desert rodent species of Morocco: the regulation of LHRH system by the photoperiod and its modulation by feedback from testosterone and thyroxin.

Methods: Male Jerboas captured in their biotope were divided into 8 groups and then subjected to either a long photoperiod or a short photoperiod. Animals in LP: Controls, Castrated, Thyroidectomized, Thyroidectomized and castrated; animals in SP: Controls, Castrated with Testosterone implants, Thyroxin T4 with Testosterone implants, Thyroxin T4. Three cerebral regions containing LHRH neurons were analyzed: the diagonal band of broca, the preoptic area, the arcuate nucleus. Results: Castration in long photoperiod increases the number of immunoreactives cells in the preoptic area, while thyroidectomy in the same photoperiod enhances the number of cells in the preoptic area and the arcuate nucleus. Conclusions: The LHRH cells are under the control of the photoperiod, whose the action can be either direct by acting on the

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frequency of LHRH cell hormone release or indirect by challenging sexual hormones such as testosterone. Also the thyroid hormones act on the LHRH system and are involved in the suppression of reproductive functions. These two hormonal systems are involved in the control of reproduction in the Jerboa, but always in relation to the photoperiod.

Overwinter body temperature patterns in captive jerboas (Jaculus orientalis)

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Purpose: The jerboa (Jaculus orientalis) a desert rodent of the family of North African Dipodidae was described previously as a true hibernator but no clear reliable data exists in this species during winter on the daily rhythmicity of body temperature (Tb) and on the occurrence of torpor bouts. In this study, Tb patterns were determined in isolated or grouped jerboas maintained in captivity under natural variations of light and ambient temperature (Ta). Methods: Tb and Ta variations were recorded (at 30-min intervals) with temperature loggers (iButtons). After implantation of loggers in the abdominal cavity of jerboas, Tb was recorded during 2 consecutive years (December 2006 -April 2007 and October 2007-April 2008).Tb patterns were determined in isolated males and females and in a group (3males + 3 females). Results: During the 2 years study, 6/18 female jerboas showed numerous torpor bouts (Tb < 33°C) alternating with short euthermia from November to February. Torpor bouts had longer durations (mean: 4-5 days) than periods of euthermia (1-4 days) and occurred at low ambient temperature (Tas: 10.2-11.6°C). In the same time, only 1/12 male showed some torpors (max.: 2 days) occurring later (February-March) and at higher Tas (15.1°C). When jerboas were grouped (3 males + 3 females), 2 females and all males exhibited concomitant torpor bouts. If females showed longer bout durations and lower Tb, the longest bouts were observed in the males during the second half of hibernation. Conclusions: Females jerboa show clearly characteristics of seasonal hibernators when males are more reluctant to enter daily torpor. Much more work needs to be done to understand a complex regulation of hibernation in relation to sex, behaviour and specific environment (Ta, food availability, energy strategy).

Energy-responsive expression of the melatonin-related receptor, GPR50

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Purpose: To detail the expression of GPR50 in mouse tissues under normal conditions and during altered energy status. Methods: Genotype and feeding-state dependent changes in gene expression were assayed using in situ hybridisation, Q-PCR and immunohistochemistry. Acute modulation of GPR50 expression was also examined following peripheral administration of energy-related signals (e.g. leptin). GPR50 signalling pathways were examined in vitro through transient transfection in HEK293 cells. Results: Mice lacking GPR50 were found to be highly prone to torpor in response to fasting. In wild-type mice, GPR50 mRNA and protein expression was localised to neurons in the dorsomedial nucleus of the hypothalamus (DMN) and tanycytes lining the 3rd ventricle. Expression in both regions was down-regulated by fasting and following 5 weeks of high-fat diet. Furthermore, Gpr50 expression is reduced in obese ob/ob mice, but can be elevated to levels observed in WT mice following 5 days of leptin treatment. GPR50 is also expressed in important tissues including the liver, adipose and pancreas. In vitro studies reveal that, like the melatonin receptors, GPR50 engages an inhibitory pathway, signalling through Gi proteins to reduce cAMP. Conclusions: GPR50 is expressed in key metabolic tissues (including the hypothalamus), and mice lacking this receptor exhibit altered metabolism and are highly prone to torpor. Therefore, GPR50 signalling is likely to be important in regulatory responses to altered energy status.

Regulation of behavioral onset and offset by SCN neuronal activity levels

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Purpose: We determined the level of neuronal activity in the suprachiasmatic nuclei that corresponds to the transition between behavioral activity and rest. We investigated whether this level is affected by exposure to short or long day photoperiods. Methods: We performed long-term recordings of SCN multiple unit activity (MUA) with the aid of implanted micro-electrodes in parallel with the drinking activity in freely moving mice. The animals were kept in a 12h:12h light-dark cycle (LD 12:12) and in short (LD 8:16) and long day photoperiods (LD 16:8). Results: Onsets and offsets of behavioral activity occurred when SCN discharge was around half-maximum values. Of the onsets 80% and of the offsets 62% occurred when SCN electrical activity differed less than 15% from the half-maximum electrical activity levels. Transitions between rest and activity could be described by a sigmoid shaped probability curve with Hill coefficients of 7.0 for onsets and 5.7 for offsets. Exposure to short or long day photoperiods induced significant alterations in the waveform of electrical activity but did not affect SCN electrical activity levels at which behavioral transitions occurred. In all photoperiods, the SCN signal was skewed with more rapid discharge changes during onsets (19% per hour) than offsets (11% per hour). The precision of the circadian system appears optimized, as transitions between behavioral activity and rest occur when the change in SCN electrical activity is maximal, both during the declining and inclining phase. Conclusions: The control of behavioral activity by the SCN can be described by a probability function around half maximum electrical activity levels. This function is remarkably insensitive to environmental conditions. Coding for photoperiod relies on waveform changes in the SCN, but not on changes in the threshold levels.

Localization of BMAL1-like immunoreactivity in Japanese quail brain

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Purpose: It is well established that the circadian clock is involved in the photoperiodic time measurement, but the location of the "photoperiodic clock" remains elusive. Using Japanese quail, an excellent model animal for the investigation of the photoperiodic time measurement, we have examined localization of BMAL1 like immunoreactivity (lir) in brain. Methods: Brains were collected every 4 h for semi-quantitative analysis and processed for immunohistochemistry using anti-chicken BMAL1 polyclonal anitibody. The temporal changes in the BMAL1-lir protein in the pineal gland, the suprachiasmatic nucleus (SCN), the ependymal cells (ECs), and the pars tuberalis (PT) were quantified with image-J (NIH) software. Results: Strong BMAL1-lir was observed in critical circadian pacemakers, the pineal gland and the medial SCN (mSCN). In the visual SCN (vSCN), very weak BMAL1-lir was confirmed. We also observed strong immunoreactivity in the mediobasal hypothalamus (MBH), including the ECs, the infundibular nucleus (IN), the median eminence (ME), and the adjacent PT, which are involved in the regulation of photoperiodism. Semi-quantitative analysis suggested that BMAL1-lir show daily fluctuation in the pineal gland, the SCN, the ECs, and the PT. Conclusions: Clock proteins locate not only in the circadian pacemakers, pineal gland and the SCN, but also in the key machinery regulating the photoperiodism, the ECs and the PT. The circadian clock in the PT and ECs can regulate or modulate the photoperiodic signal transduction cascade and may be involved in photoperiodic time measurement.

Kisspeptin in the brain of a desert hibernator, the jerboa: Effect of sex and seasons

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Purpose: The jerboa (Jaculus orientalis) is a seasonal rodent in which reproductive activity depends on seasons, being sexually active in spring-summer. Because hypothalamic kisspeptin (Kp) has recently been demonstrated to be crucial for the regulation of reproduction, the aim of this study was to determine the distribution of Kp neurons in the brain of adult jerboa at different seasons with a special attention to sex differences. Methods: Expression of Kp was examined by immunohistochemistry using a highly specific polyclonal antibody (JVL-1) in male and female jerboas captured in the field of the Middle Atlas mountain (Morocco), either in early spring or autumn. Results: In animals captured in April, cell bodies and fibres displaying Kp immunoreactivity (Kp-ir) were observed within the mediobasal hypothalamus along its rostrocaudal extent. In the anteroventral periventricular nucleus, the number of Kp-ir neurons was notably higher in female than in male jerboas, while in the arcuate nucleus, a large distribution of kisspeptin immunoreactivity was noted in both sexes. The internal layer of the median eminence, site of neurohormone release, exhibited a much higher density of Kp-ir fibers in the female as compared to male jerboas. Seasonal variations in this kisspeptinergic pattern is currently under investigation. Conclusions: The general pattern of Kp-ir cells and fibers in the jerboa hypothalamus correlates well with data obtained in other rodents. Strikingly, the number of Kp neurons in the AVPV and fibers in the median eminence is much higher in female as compared to male Jerboas indicating that this network may be important for the ovary activity. By contrast Kp neurons of the ARC do not display sex differences.

Comparison of melatonin binding sites between seasonal and non seasonal breeders

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Purpose: To compare, between seasonally and non-seasonally breeding species, the density of melatonin binding in the premammillary hypothalamus (PMH), a target of melatonin for the control of reproduction. Methods: The density of melatonin (MLT) binding was studied by autoradiography using 2-[125I]-MLT in 5 regions (pars tuberalis (PT), PMH, suprachiasmatic nucleus (SCN), hippocampus and cortex; 8 brain slices by region) in sheep and goats (seasonal breeders) and sows, rats and cows (low and non-seasonal breeders), using young sexually mature animals (4 in each species). The same measurements were made in calf and lamb brains (new-born animals, sexually immature, 4 in each group). Brain slices were incubated with 130pM 2-[125I]-MLT; non-specific binding was measured on adjacent slice in presence of 10µM of MLT, and 2-[125I]-MLT binding was performed either in PBS or Tris buffer. Results: Higher binding was seen in Tris buffer for rats and sows, but in PBS for sheep and goats, and equal binding in both buffers for cows. All animals showed MLT binding in the PT (7 to 38fmol/mgproteins) and in the hippocampus. Binding in the SCN was observed only in sows and rats. Goats and ewes showed the highest density of binding sites in the PMH and cows the lowest one (10.43±1.73, 5.57±1.58 and 1.46±0.28fmol/mgproteins respectively, $p<0.05$). In the sow and rat, density of binding site is intermediate between that of the ewe and cow (3.44±0.44 and 2.80±2.97 fmol/mgproteins, NS). For lambs and calves, similar results to adult animals were observed. Conclusions: Although the results for the sow lie between the ewe and the cow, these results suggests a relationship between seasonality and the density of melatonin binding sites in the PMH, in contrast to the PT. These results need to be extended to more species and to the wild homologues with seasonal reproduction.

Hibernation: when a clock slows down?

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Purpose: To elucidate the control of the torpor-arousal cycle of hibernating mammals. Methods: Analysis of published data. Results: Throughout winter, hibernating mammals alternate between long phases of torpor and short phases of euthermia (arousals). Torpor bout duration varies with body temperature Tb and within the season. Its control has so far been ascribed to some sandglass-type

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mechanism. However, the temporal organization within the cycle with esp. anticipatory processes hints at a control by a clock. That in three distinct experiments a single factor acts simultaneously on the durations of both phases also favours a common mechanism. The maximal torpor bout durations of 39 species (Geiser and Ruf 1995) have been reassessed: they are independent of body mass over at least 3 orders of magnitude. This is the signature of a clock: among all metabolic processes, only circadian and circannual clocks have no allometric dependence on body mass. Let us assume that a circadian clock (e.g. the food-entrainable clock) has lost its temperature compensation in hibernators, permanently or through epigenetic seasonal control. Pure temperature effects on enzymatic reactions such as those of the clock machinery follow Arrhenius' law. The data of Twente et al.(1977) on bout duration vs. Tb obtained over 11 years in 3 sp. of Spermophilus fit precisely to 3 parallel Arrhenius plots, not distinguishable from that of enzymes from hibernators in vitro (Malan, 1983). Using the parameters calculated from these data, one predicts that the tau of a non-compensated circadian clock will expand from 24 h at 37° C to 330 h at the hibernation Tb of 5°C (mean log 2.54 ± 0.04). This does not significantly differ from the mean torpor bout duration of the 39 hibernating species (2.46 ± 0.11). Conclusions: The torpor-arousal cycle of hibernating mammals seems to be controlled by a non-temperature-compensated circadian clock. A torpor bout would stop when the circadian time reaches one circadian day, corresponding at hibernation Tb to about 12 zeitgeber days.

Seasonal differences in biological rhythms of Brazilian native indians

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Purpose: Compare summer and winter data of wrist temperature and activity/rest pattern of adult indians who live in a settlement without electricity. Methods: Participants were 22 Guarani adult indians aged 18 to 80. The settlement "Boa Vista" is near the Atlantic coast in southeast Brazil (23o21'S; 44o51'W). Photophases during data collections were approximately 13h in summer and 11h in winter. Wrist temperature (WT) were collected every 30min along three consecutive days with thermistors (Thermochron®, iButton type DS 1291H). Rest/activity (RA) was registered with actimeters (MicroMini Motionlogger® - Ambulatory Monitoring, Inc.) for 7 consecutives days, in bins of 1minute. The data were analyzed with the COSINOR method and the parameters acrophase, amplitude, MESOR were compared in two moments. The Pearson moment correlation test was applied to analyze the changes in COSINOR parameters according to age. The onset, offset and duration of rest between seasons were compared with one-way ANOVA. Results: WT: All subjects showed a significant circadian rhythmicity (COSINOR: $p<0.0001$). The amplitude values were higher and MESOR values were lower in winter than in summer. Although submitted to distinct photophases, acrophase values did not differ according to season. RA: the indians showed a phase advance in winter compared to summer values (onset winter: 21h23 and onset summer: 22h42; $F=18.69$, $p<0.00004$) (offset winter: 06h12 and offset summer: 07h18; $F=23.98$, $p<0.00001$) without difference for rest duration. The phase relation between WT and RA varies according to the season: ??winter = 11h13 and ?? summer = 12h46. Conclusions: The adult indians showed WT rhythms comparable to urban populations in similar latitude and longitude. The RA seems linked to specific characteristics of Guarani routine. In winter the indians' activities began near the sunrise and during summer they used to phase-delay their rhythms probably due to social demands involving nighttime activities during summer.

Hibernation in the Algerian Hedgehog (Atelerix algirus) during autumn and winter

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Purpose: The Algerian Hedgehog (Atelerix algirus) of North Africa seems to have a reduced locomotor activity during winter, but no data of body temperature (Tb) changes have been available. Methods: Tb changes in hedgehogs were recorded continuously with temperature data loggers (iButton) implanted in the abdominal cavity. In autumn and winter, during several years (from 2005), Tb was registered (every 20 min) in animals kept under (a) semi-natural (room with open windows) or (b) external environmental conditions. Results: During autumn and winter, the hedgehogs under semi-natural conditions showed numerous daily torpor bouts (Tb < 33°C) alternating with euthermia (Tb > 33°C). From mid-November to mid-December, torpor bouts of short amplitude occurred daily (duration < 12 hr). From mid-December to beginning of March, torpor bouts showed longer durations (mean: 4-5 days; max. 6-7 days) in relation with short periods of euthermia (< 24 hr) and low ambient temperatures (Tas: 12.0-9.7°C). In March, daily torpor bouts occurred in relation with lower Ta levels. No sex difference was evident in Tb patterns; although it seems that females hibernate more than males. In external conditions, similar patterns were observed with lower Tbmin (< 5°C). Conclusions: According to the torpor bouts observed in autumn and winter, Atelerix algirus is able to reduce its metabolism and energy expenditure when Ta decreases sharply and presents all the characteristics of a deep hibernator.

Molecular mechanism of photoperiodic response of gonad in mice

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Purpose: Recent analysis on birds (Nakao et al., Nature 2008) has revealed that long-day induced thyrotropin (TSH) in the pars tuberalis (PT) of the pituitary gland triggers DIO2 expression in the ependymal cells (EC) of the hypothalamus, the process of which induces seasonal reproduction. In mammals, nocturnal melatonin secretion provides an endocrine signal of the photoperiod to the PT,

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but the interface between melatonin signal and the TSH levels remains unclear. Thus, we analyzed this process using mice. Methods: Effect of changing daylength and melatonin was examined in melatonin-proficient CBA/N mice and melatonin-deficient C57BL/6J (B6) mice. For each experiment, the expression levels of TSHB (TSH beta subunit), CGA (TSH alpha subunit), DIO2, DIO3 were analyzed by in situ hybridization. TSH was injected into the third ventricle of short-day B6 mice and expression of DIO2 was analyzed. Results: Induced expression of TSHB, CGA, and DIO2, and reduced expression of DIO3 was observed in melatonin-proficient CBA/N mice. These responses were not found in melatonin-deficient B6 mice, but treatment of B6 mice with exogenous melatonin showed similar effects on the expression of the genes. ICV injection of TSH induced DIO2 expression dose dependently. Finally, we showed that melatonin administration did not affect the expression of abovementioned genes in TSHR-KO mice. Conclusions: Although most mouse strains are considered to be nonseasonal, a robust photoperiodic response comprising induced expression of the genes participating in photoperiodic signal transduction were detected. Our study has shown that mice can be a new model animal for studying molecular mechanism of photoperiodic response.

Strain dependent influence of photoperiod on emotional state in mice

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Purpose: To examine whether photoperiod affected anxious and/or depressive-like behaviors in two mouse strains. Methods: Three weeks old C57BL/6j and CBA/j mice were grouped-housed under a 14:10 light-dark cycle with light on at 7h and light off at 21h (long photoperiod; LP). Three weeks after arrival, half of the mice were randomly chosen for transfer in another room under a 10:14 light-dark cycle with light on at 7h and light off at 17h (short-photoperiod; SP). Animals were then tested after a delay of 8 weeks to assess anxiety-related behavior in the dark/light, plus maze and novelty induced hypophagia test and depressive-like behavior in the forced swim test. Results: While day-light length did not affect behavioral performances in C57BL/6j mice, SP conditions elicited anxiogenic-like effects in most of these tests in CBA/j mice. Swim performance was not significantly affected by the photoperiod regimen in none of these mouse strains. Conclusions: These findings support the idea that emotional states are dependent of the photoperiodic environment. These responses are strain selective in mice possibly in relation with the presence or absence of melatonin.

Voluntary exercise and photoperiodism in Phodopus sungorus

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Purpose: To study the mechanism by which voluntary exercise overrides photoperiod induced physiological responses. Methods: Expt 1. The effect of photoperiod on the activity pattern of P. sungorus with and without access to a running wheel (RW) was recorded by infrared motion detectors under natural photoperiod and ambient temperature. Expt 2. Hamsters were housed in artificial SD for 8 weeks with or without a RW. At the end of the experiment food intake was measured before the hamsters were killed. Liver glucose and lipids were determined by a colorimetric assay. VGF, DIO3 and SRIF mRNA levels in the dmpArc, ependymal layer of the third ventricle and ARC, respectively, were quantified by in situ hybridization. The effect of photoperiod and activity on growth hormone (GH) was determined by Northern blot analysis, carried out on RNA extracted from pituitary glands. Results: Expt 1. In exercising hamsters the annual activity pattern showed an entrained and well-defined rhythm throughout the year. However, hamsters without a RW showed a tendency towards an indistinct rhythm during times of decreasing photoperiod in autumn and winter months. Expt 2. With access to a RW, the typical SD decrease in testes and body mass was attenuated and food intake was nearly doubled. SRIF gene expression was decreased and VGF gene expression was further increased by RW activity in SD, whereas DIO3 gene expression was not affected. Liver glucose levels were increased by RW activity and GH expression in the pituitary was increased in SD. Conclusions: The annual activity pattern and DIO3 gene expression of the RW hamsters demonstrates that the SD signal is perceived, but the hamsters show an altered physiological response. Expressions of VGF and SRIF are affected by RW activity. The latter may contribute to an increased release of GH to increase body mass, and an increase in GnRH might cause the delay of testes regression.

Global timing – latitude dependent pigment production in Neurospora crassa *wild-type strains*

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Light is not only the fundamental energy source for life on Earth but is also one of the most important environmental signals. As most other organisms, fungi also use light to synchronize their physiology and behavior to daily and seasonal changes in their environment. The former refers to entrainment of the circadian clock and the latter to photoperiodism, a biological mechanism by which organisms can "tell" time-of-year and can, therefore, anticipate seasonal changes.

To investigate photoperiodism on the physiological, molecular and genetic level we chose eight wild type strains of Neurospora crassa, a filamentous fungus, collected at different geographical locations: in Scotland (55°N), Italy (44°N), Spain (41°N), Louisiana (29°N), India (12°N), Costa Rica (10°N), Venezuela (5°N) and Congo (4°S). We developed a low-budget, automatic pigmentation assay that allows fast analysis, based on computerized images of fungal mats.

All eight strains show a typical photoperiodic response curve in their pigment production, i.e., a distinct profile and

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not a simple linear increase with increasing light exposure. Strains collected neat the equator – unaccustomed to larger changes in photoperiod – show strong responses in long photoperiods. In general, stronger responses are found outside range of photoperiods, the respective strains are used to. Neurospora strains, collected at different latitudes, apparently "know where they come from" even after being in the laboratory for many years when tested under artificial conditions. This result indicates a genetic basis for the observed "photoperiodic memory".

Implication of Harderian glands in nycthemeral rhythm in two desert rodents: the diurnal Psammomys obesus and the nocturnal Gerbillus tarabulis

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Purpose: in order to determine the implication of the Harderian glands in circadian and seasonal rhythms in two desert rodents; diurnal species Psammomys obesus and nocturnal species Gerbillus tarabulis. Methods: Harderian glands (HG) of the two species were removed and fixed for histology and cytology, in the middle of each phase of both photoperiods long (LD 14:10) and short (LD 10:14). Results: Morphological variations according to light/dark rhythm are shown. The HG of diurnal species has a dark color du to the presence of melanin; its histology show a uniform epithelium with plasmatic cellular type, the lumen of the gland often contains porphyrins and cellular debris. This glandular morphology suffers a drastic change along dark phase during long photoperiod in this species, since a new cellular type appear; It is characterized by numerous and large lipid droplets. The HG of nocturnal species has a yellowish pale color; the glandular epithelium presents two cellular types; prismatic cells similar to those described in Psammomys and pyramidal basal cells without any contact with the glandular lumen. The lumen of the gland sometimes contains porphyrins and cellular debris. Interestingly the same change described above for diurnal species along dark phase during long photoperiod is observed in this nocturnal species along light phase during short photoperiod being. This new cellular type with numerous and large lipid droplets as before mentioned. Conclusion: The morphological variations of HG in both species show that is light dependant; indeed, it appears more active in LD 14:10 for Psammomys and in LD 10:14 for Gerbil; that could be related to the reproductive activity of this animals. Melanin, porphyrin and mast cells are abundant in diurnal species this is related to their habits

The hypothalamic thyroid-axis during torpor and hibernation in hamsters

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Purpose: To examine seasonal and acute changes in thyroid-hormone feedback in the hypothalamus during torpor and hibernation in Siberian (Phodopus sungorus) and European (Cricetus cricetus) hamsters respectively. Methods: Brains from hamsters housed under long (LP) or short photoperiod (SP) were instantly frozen and stored at -80°C until cryosectioning. In situ hybridisation experiments were undertaken using 33P radiolabelled riboprobes for thyrotropin releasing hormone (TRH), deiodinase type II and type III (Dio2, Dio3) and monocarboxylate transporter 8 (MCT8). Results: The key components involved in thyroid hormone signalling in the hypothalamus were highly localised to the ependymal layer of the 3rd ventricle, and showed seasonal modification in P. sungorus. Specifically, Dio2 was down-regulated under SP; this was in contrast to Dio3 and MCT8 which were up-regulated during SP. TRH, a major output of this pathway was up-regulated in the paraventricular nucleus (PVN) under SP and elevated further during torpor. Hypothalamic thyroid hormone modulation also appears to play a role during hibernation of C. cricetus, for example MCT8 is down-regulated during hibernation bouts in comparison to SP housed normothermic hamsters. Conclusions: Our data implicates an important role for thyroid hormone modulation in seasonal models of hypometabolism.

Delayed response of thyrotropin-beta subunit gene to melatonin in the pars tuberalis Syrian hamsters

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Purpose: In birds, long photoperiod induces the expression of thyrotropin-beta subunit (TSHb) in the pars tuberalis (PT) to trigger the expression of type 2 deiodinase (Dio2) in the ependymal cell layer (EC) of the infundibular recess of the third ventricle and thereby stimulate gonadal axis. To address the involvement of this molecular cascade in mammals, we examined the temporal expression of Dio2 and TSHb after melatonin injection in Syrian hamsters. Methods: Male 8-9 weeks old hamsters were kept under long-day condition (16L:8D) for 3 weeks, and then animals were injected with melatonin at ZT14 for 10 days. Animals were sacrificed at ZT9 on 1, 2, 4, 10 day after the onset of injection. Short-term effect was also examined every 6 hours during 25 hours after the first injection. Expression of TSHb and Dio2 and protein levels of TSHb were examined by in situ hybridization and immunohistochemistry. Results: Dio2 was already suppressed 1 day after the first melatonin injection, whereas the expression of TSHb was not suppressed until 10 days after the onset of injection. Expression and protein levels of TSHb were not affected during 25 hours after the first injection, a period which is sufficient to suppress Dio2 expression. Conclusion: Our results demonstrate that the melatonin-dependent downregulation of the Dio2 expression in the EC occurs before the downregulation of TSHb expression. This suggests that Dio2 in the EC is regulated by melatonin via changes in the secretion of TSHb which are independent of transcriptional/translational regulation or via additional factors.

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Food restriction reverses thyroid hormone system components that drive short daylength induced body weight loss in the Siberian hamster

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Purpose: To investigate how starvation interacts with photoperiod on the hypothalamic thyroid hormone system, in a photoperiodic animal model, the Siberian hamster (Phodopus sungorus). Methods: Adult Siberian hamsters were kept in long days (LD) or short days (SD) for 8 weeks before being starved for 48 hours. After starvation brains were processed for in situ hybridisation using probes for genes involved in a) the hypothalamic thyroid hormone system (deiodinase 2 and 3 (D2, D3), monocarboxylate transporter 8 (MCT8)), b) homeostatic response of the hypothalamus to starvation (neuropeptide Y (NPY), thyroid releasing hormone (TRH)) and c) hypothalamic response to photoperiod (VGF). Serum was collected to determine peripheral thyroid hormones. Results: Eight weeks in SD induced weight loss in the hamsters. In the hypothalamus of the animals, anabolic D2 expression was decreased, catabolic D3 was induced and also thyroid hormone transporter MCT8 expression was increased. 48h starvation reversed the direction of gene expression change for D2, D3 and MCT8 induced by SD. Serum levels of T3 and T4 increased in SD and were decreased upon starvation. NPY in the arcuate nucleus and TRH in the paraventricular nucleus did not respond to photoperiod, but increased and decreased upon starvation respectively. VGF in the dmpARC increased in SD but did not respond to starvation. Conclusions: Thyroid hormone (T3) availability to the hypothalamus is an important driver of photoperiodically induced body weight loss in the Siberian hamster as well as the central response to food restriction in rats. Here we show that energy deprivation interacts with SD photoperiod on hypothalamic tanycytes to control components of the thyroid hormone system. The data point to a mechanism in which the thyroid hormone system may act as an integrator of long term and short term energy challenges.

11. Clock in real life; Sleep; Shift work

Blue light, but not green, phase shifts human PER3 and melatonin rhythms in young and older subjects

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Purpose: The timing of PER3 clock gene expression in human leukocytes was investigated both before and after a phase-advancing light stimulus to determine whether the response is wavelength- and/or age-dependent. Methods: Eleven young (23.0 ± 2.9 years) and 15 older (65.8 ± 5.0 years) healthy males participated in two laboratory sessions including a 2 h monochromatic light pulse (~6x1013 photons/cm2/sec), individually timed to begin 8.5 h after their melatonin onset determined in a prior visit. Subjects were exposed to blue light (?max 456 nm) in one session and green light (?max 548 nm) in another. Blood samples (30–60 min) were taken on the pre-stimulus and post-stimulus nights for measurement of plasma melatonin and the clock gene PER3. RNA was extracted from whole blood cells and reverse transcribed for semi-quantitative real-time PCR. Results: We demonstrate for the first time that PER3 gene expression in human leukocytes is phase-shifted following monochromatic light exposure. Subjects exhibited significant phase advances following blue, but not green, light for both PER3 gene expression and plasma melatonin independent of age. A significant age-dependent decline in PER3 amplitude was also observed. Conclusion: These findings confirm the previously reported blue light sensitivity of the circadian timing system. PER3 expression in leukocytes may be a reliable phase marker of the human circadian system, since it has a robust rhythm that can be phase-shifted by light. Simultaneous measurement of clock gene expression and melatonin from blood samples would allow responses in both the central and a peripheral oscillator to be assessed.

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Melatonin phase, chronotype and PER3 in relation to night shift adaptation in Antarctica

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Purpose: Halley (750S) personnel carry out a week of nightshift (2100-0800h) in rotation. Most adapt to nights, and rate of adaptation may depend on diurnal preference and the related variable tandem repeat polymorphism, 4/4, 4/5 or 5/5 in PER3. This project evaluated circadian phenotype and genotype in this population. Methods: Base personnel (N=47, 11F, 36M, 30.6?6.9 years, X?SD), took part in the study during 2000, 2001, 2003 & 2006. They completed Horne-Ostberg (HO) questionnaires and collected sequential urine samples for 10-12 days preceding and during nightshift, to determine phase by 6-

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sulphatoxymelatonin (aMT6s, cosinor analysis). Rate of phase shift was derived by subtracting average acrophase for the days preceding nightshift from that on day 4 or 5 of nightshift. PER3 genotyping was according to Archer et al., 2003. Analysis was by linear regression. During 2003 and 2006 extra light (Philips Lighting) was provided from March to October as alternating periods of 4-5 weeks standard white (4000K) or blue enriched (10,000K, 2003, 17,000K, ActiViva, 2006). Results: Genotyping gave 5 x 5/5, 24 x 4/5 and 18 x 4/4 distribution of PER3. There was a trend (p=0.09, excluding one outlier p=0.04) to correlate with chronotype (morningness with 5/5 and eveningness with 4/4) as expected. Using winter data only (May-August, to avoid seasonal effects), 22 participants provided suitable data for evaluation of entrained phase and rate of adaptation to nightshift. HO score was strongly related to rate of adaptation (p=0.013) but not entrained phase, PER3 did not provide significant relationships. Restricting data to periods of extra white light in winter (n=17, 2 x 5/5, 10 x 4/5, 5 x 4/4), 2003 and 2006 gave similar results. Conclusions: In this small population HO score, but not PER3 genotype predicted rate of circadian adaptation to nightshift.

Effects of partial chronic sleep deprivation on the mouse blood coagulation cascade

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Purpose: Our previous work has demonstrated the presence of a circadian rhythm in FVII activity in the mouse and established that FVII is a clock controlled gene since its rhythmic transcription is directly controlled by the circadian clock via the BMAL1:CLOCK and BMAL1:NPAS2 complexes. In the present investigation we studied the influence of partial chronic sleep deprivation (PSD) on factor VII (FVII) and thrombin generation. Methods: We measured FVII and thrombin generation (intrinsic and extrinsic pathways) levels in the blood of mice (C57BL/6J strain) subjected to PSD using fluorogenic substrates. In the PSD protocols mice were only allowed to sleep for 4-hours a day in the first part of the light phase (ZT0-4). Mice were subjected to 3 or 7 days of PSD and to 7 days of PSD followed by a recovery in normal condition for 3 days. Blood was collected at ZT1. Results: After 3 days of PSD thrombin generation levels upon either extrinsic or intrinsic activation of the coagulation cascade were not significantly different with respect to the control mice. Conversely, after 7 days of PSD thrombin generation levels upon extrinsic activation were significantly reduced (about 30%) in PSD mice. These observations suggest that PSD conditions mainly affect levels of coagulation factors involved in the extrinsic pathway. Therefore, we tested FVII activity in mouse plasma. After 3 or 7 days of PSD FVII activity levels were significantly reduced in PSD mice (about 30% and 50%, respectively) as compared to controls. Thrombin generation and FVII activity levels in mice subjected to 7 days of PSD followed by 3 days of recovery were not significantly different with respect to controls. Conclusions: Our finding indicates that PSD might affect the regulation of haemostatic balance.

Effects of circadian and awakening components of cortisol production upon sleepiness, activation and cognitive performance: A forced desynchrony study

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Purpose: Determining the role of the circadian and awakening component of cortisol production in sleepiness, activation and cognitive performance immediately after awakening (sleep inertia) and during the day. Methods: 9 healthy adults (22.7 y ± 2.1) were subjected to a forced desynchrony protocol, composed of 6 sleep/wake cycles of 20 hours. Free salivary cortisol samples were taken 1, 15, 30, 45, 60 and 90 minutes after waking up and from here on hourly for the remaining part of the day. At the same time points melatonin samples were taken to estimate circadian phase. Cognitive performance tests (addition task and reaction time task) and subjective ratings of sleepiness (KSS), and activation (Thayer) were obtained every 15 minutes during the first 90 minutes after awakening and 2-hourly during the day. Results: Preliminary results indicate that subjective ratings of sleepiness, and activation immediately after waking up did not differ under different circadian phases of cortisol production. During the day however, subjective sleepiness increased and subjective activation decreased as the circadian peak in cortisol production shifted to the subjective evening (anti-phase). Conclusions: The difference in the relationship between cortisol and alertness ratings at waking up, as compared to measurements during sustained wakefulness suggests a complex relationship between cortisol and alertness.

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Can PER3 length polymorphism predict sleep duration in older individuals?

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Purpose: To investigate whether sleep-wake behaviour patterns measured by wrist actigraphy can differ between older subjects homozygous for the longer allele of the gene PERIOD3 (PER35/5) and heterozygous (PER34/5) individuals. Methods: Healthy volunteers were selected on the basis of their genotype, irrespective of diurnal preference or other sleep-related characteristics. Approximately, 150 healthy older men and women (age 55-75) have been genotyped for the PER3 polymorphism in an ongoing study, thus comprising individuals homozygous for the longer (PER35/5) and shorter allele (PER34/4) as well as heterozygous individuals (PER34/5). In parallel, subjective assessment of sleep and chronotype was conducted using Pittsburgh sleep quality index (PSQI), Horne-Östberg and Munich Chronotype Questionnaire, Insomnia severity index and Epworth sleepiness scale. So far, 23 healthy older subjects completed sleep analysis by actigraphy and sleep diaries to characterize sleep timing during three con-

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secutive weeks. Here, we present data of 12 older participants, whose genotypes have already been characterized. Since only two PER34/4 subjects completed this sleep analysis so far, they were not included in these results. Results: Preliminary results on 12 healthy older subject, six PER35/5 subjects and six PER34/5 subjects, indicate no differences between the two genotypes with respect to age, gender, body mass index and to any of the sleepiness, sleep quality, insomnia index and chronotype questionnaires. On the other hand, actiwatch analysis indicated highly significant differences between the two genotypes: PER35/5 subjects exhibited an earlier sleep time (p=0.0001) and shorter sleep duration (p=0.007) in comparison to PER34/5 subjects. Conclusions: These preliminary results indicate an association between the length of the PER3 polymorphism and sleep duration in older subjects.

Dream recall during a multiple nap paradigm: are there biological day and night differences?

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Purpose: To investigate the biological day and night differences and age-related changes in dream recall, number of dreams and emotional domain characteristics of dreaming. Methods: Dream recall was investigated in 17 young (20–31y) and 15 older (57–74y) healthy volunteers Analysis of dream recall and sleep EEG (NREM/REM sleep) was performed during a 40-hour multiple nap protocol (150 minutes of wakefulness and 75 minutes of sleep, thus comprising 10 naps) under constant routine conditions. Dream recall was assessed at the end of each nap trial with the Sleep Mentation Questionnaire, which addresses recall, number and the emotional domain of dreaming. For the classification of biological day and night, a nap was classified as a night nap (biological night) if the melatonin concentration of the last saliva sample prior to the nap was above the individual mean; otherwise, it was classified as a day nap (biological day). Results: Comparisons of dream recall and number of dreams for both age-groups during the biological day and night indicated that dream recall [rANOVA: main effect 'age'; $F(1, 60)=11.51$, $p < 0.05$] and number of dreams [rANOVA: main effect 'age'; $F(1, 60)= 8.21$, $p < 0.05$] were significantly higher in young individuals during subjective day in detriment to older subjects. Regarding the emotional composite score, there were no significant subjective day/night differences between young and older individuals. When considering the time course of these dream variables, the significant age-related differences were mostly observed in naps 1, 2, 3 and 9, thus occurring exclusively during the biological day, when saliva melatonin was at the lowest levels. Conclusions: Our data suggests that dream recall and number of dreams varies significantly across the circadian cycle and between age groups, with older subjects exhibiting fewer dreams after naps scheduled during the biological day.

Hypoxia-induced changes in recovery sleep and core body temperature after simulated long-duration flights

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Purpose: Fatigue and sleep disorders often occur after long-haul flights even when no time zones are crossed. Mild hypobaric hypoxia, resulting from cabin pressurization, may contribute to this phenomenon through effects on circadian time structure and on recovery sleep. Methods: In this study, the effects of two levels of hypoxia, corresponding to cabin altitudes of 8000 and 12,000 ft, were assessed. Recovery sleep and core body temperature (CBT), which is a classical circadian marker, were studied in parallel in twenty young healthy male volunteers exposed for 8 h (08:00-16:00 h) in a hypobaric chamber to a simulated cabin altitude of 8000 ft and, 4 weeks later, 12,000 ft. Each subject served as his own control. Sleep was recorded by polysomnography for three consecutive nights for each exposure. CBT was continuously monitored by telemetry (Vitalsense) during the three corresponding 24-h cycles (control, hypoxic exposure and recovery). Results: Our results showed significant changes in circadian patterns of CBT at both altitudes, suggesting a phase delay, and also some changes in recovery sleep but only at the simulated altitude of 12,000 ft. We observed an increase in sleep onset latency which was positively correlated with the increase in CBT levels, during the first recovery night, and a decrease in the amount of N2 sleep, which was negatively correlated with the mid range crossing time, a reliable phase marker of CBT rhythm. Conclusions: This study confirms the impact of mild hypobaric hypoxia on circadian time structure during air flights. Mild hypoxia leads to a phase delay of CBT, independent of jet lag, with moderate, but significant consequences on sleep during recovery.

Study of sleep-wake cycle and chronotype in nursing students

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Purpose: The aim of this research was to analyze the sleep pattern and chronotype of the freshman students of the nursing undergraduate course at the University of Campinas – Unicamp (n=40) with a mean age of 20.58 years. Individual patient information was obtained by using questionnaires and sleep diaries during 15 days. The results were as follows: indifferent chronotype (50%), moderately morning type (26.47%), moderately evening type (20.59%) and definitely evening type (2.94%). The sleep quality showed regular due to frequent sleep interruptions – significant data for the locution 'how you feel when you wake up' (Anova, p=0.0067) –, and there was variability regarding the sleep time during weekdays. Conclusion: Most subjects demonstrated indifferent chronotype, which allows flexibility in sleep habits, collaborating in a certain way with students having different time periods to study and rest.

Phase delaying the sleep-wake cycle reduces sleep onset latency in women with vascular

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dysregulation and difficulties initiating sleep

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Purpose: Women with cold extremities (vasospastic dysregulation, WVD) exhibit a deranged phase angle between sleep-wake cycle (SWC) and circadian system which could be the cause of prolonged sleep onset latency (SOL). Aim of the study was to reduce SOL by phase delaying SWC by 1h changing thereby phase of entrainment. Methods: Subjects were 10 healthy WVD (mean±SEM; age: 23.7±0.8 y; BMI: 20.7±5.1) with difficulties initiating sleep (SOL: 26.1±1.5 min). Participants carried out a mixed ambulatory-/laboratory protocol (AMB/LAB) consisting of a baseline (BL) and a SWC-shifted (SH) week administered in a balanced order. Each week started with 5 AMB days under real life situation followed by a controlled 35-h LAB part (16-h CR-protocol before and after an 8-h sleep episode. Measurements: PSG; body temperatures; salivary melatonin concentration, MEL etc.). The only difference between BL and SH was that in the latter participants had prescribed 1-h delayed bedtimes. MEL was measured in LAB throughout the protocol and at home for one evening under dim light conditions. Results: Sleep duration showed no significant differences between BL and SH, however, sleep midpoint was significantly phase delayed in SH (SH: 4.13±0.13 vs. BL: 3.37±0.12 h; $p<0.0001$). SOL was significantly reduced in SH compared with BL (AMB: 19.0±2.2 vs. 35.6±4.6 min, $p<0.009$; LAB: 9.1±1.4 vs. 16.9±2.6 min, $p<0.005$). Dim light melatonin onset did not significantly differ between SH and BL under both, AMB and LAB conditions. Taken together, we could demonstrate that phase delaying the SWC in relation to the circadian system by about one hour significantly reduces SOL in WVD. Conclusions: These findings indicate that an optimal phase of entrainment between SWC and the circadian system is crucial for a short SOL.

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Assessing internal time in shift-workers

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Purpose: Establishing an algorithm that allows a reliable assessment of individual internal time (chronotype) in shift-workers on the basis of self-reported sleep and wake-behaviour. Methods: A total of 992 shift-workers filled out a shift-work adapted version of the MCTQ (Munich ChronoType Questionnaire). The following variables were assessed for estimating chronotype on the basis of mid-sleep corrected for a prior accumulation of sleep deficit (MSFsc): mid-sleep (the midpoint between sleep onset and sleep end) on the respective work shifts (MSWM, MSWE, MSWN); mid-sleep for the respective succeeding free days (MSFM, MSFE, MSFN); shift-specific sleep durations on work-days and on the succeeding free days. Results from the MCTQ were compared to a large database of day-workers (n>56,000), sleep-logs (n=78) as well as actimetry (n=19). Results: MSFsc is very stable across shifts and closely matches that of day-workers, in particular MSFsc following an evening shift (MSFEsc).This suggests that shift-work schedules have little impact on the timing of sleep on free days and that MSFsc predicts chronotype in shift-workers as it does in day-workers. MSFEsc correlates very well with results from daily sleep logs and actimetry phase markers. Once categorized into chronotypes, shift-workers show strong chronotype-specific sleep behaviour (timing and duration of sleep) in the distinct shifts. Conclusion: Like in day-workers, MCTQ-assessed MSFsc is a reliable marker for chronotype in shift workers. Internal time by means of MSFEsc can be easily assessed on the basis of a few questions, without any need for external interventions and environmental controls.

The effect of 'blue-enriched' or control white light on sleep, mood and alertness in older people

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Purpose: With age sleep problems increase and light exposure is reduced. The current study aimed to assess the effect of control light (colour temperature 4000 K) and 'blue-enriched' light (17000 K) on sleep, alertness and mood in older people (? 60 years) with self-reported sleep problems (Pittsburgh Sleep Quality Index > 5). Methods: Healthy volunteers (n=12; 65.3 ± 4.0 years; 7F, 5M) participated in an 11-week at-home study (randomised, cross-over design) and were exposed daily to the light condition (~ 400 lux) for 2 h in the morning and 2 h in the evening for 3 weeks followed by 2 weeks of washout. They completed sleep diaries, daily mood and alertness scales, and wore an activity monitor (Actiwatch-L) continuously. Results: Proc GLM was used to test for carry-over effects (t-test) and to compare light conditions (corrected for baseline). Weekly means were compared using RM one-way ANOVA with post-hoc Bonferroni comparison. No carry-over effect was observed for any parameter. There were few statistically significant differences between the two light conditions, except control light produced higher actigraphic sleep efficiency and improved cheerfulness at lunchtime while 'blue-enriched' light significantly decreased sleepiness in the morning. Compared to baseline control light significantly improved subjective sleep efficiency, reduced sleep latency and advanced subjective and actigraphic wake time. During washout cheerfulness significantly increased in the morning compared to during the 3 weeks of control light; and calmness improved in the morning and at lunchtime compared to the second week of control light. Conclusions: Timed light exposure, especially the control light, showed some beneficial effects on sleep, mood and alertness in older people with sleep problems.

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Does the chronotype classification need to be updated? An analysis of individuals with a bimodal pattern of answers to Morningness-Eveningness questionnaire

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Purpose: Traditionally, chronotype classification is based on a morningness-eveningness questionnaire (MEQ). It is implicit in the classification that indifferent individuals attribute intermediate punctuations to most of the MEQ questions. However, a small group of intermediate individuals has a different pattern of answers. Sometimes they are "morning-types"(higher scores), sometimes "evening-types"(lower scores), resulting in an intermediate final score. We postulate that these individuals belong to a fourth class of chronotype, called bimodal. Methods: MEQ was applied for 1628 undergraduate students. An algorithm to classify bimodal individuals according to the number of morning-type/evening-type answers was developed. Morning, evening, intermediate and bimodal-types (M, E, I and B-types, respectively) were selected for sleep/wake data collection, one week during school term and another week during vacation (n=8 for each group). Data from weekdays were compared by means Mann-Whitney U test and Wilcoxon Matched pair test. DNA samples were collected in order to analyze PER3 4/5 length polymorphism (11 B-types). Results: During vacation, B-types woke up earlier ($p<0.05$) and showed a tendency to an earlier sleep onset time ($p=0;06$) when compared to I-types. Also, B-types and E-types had a shorter sleep duration when compared to M-types, in school term and vacation ($p<0.05$). This difference was not found when I-types were compared to M-types. B-types showed a tendency to have a higher heterozygous frequency compared to a Brazilian random population ($p=0.052$; ?2=5.93). Conclusions: These preliminary results suggest that bimodal individuals show different responses to temporal challenges when compared to intermediate individuals, which could be related to molecular and/or neuroanatomical properties of the circadian timing system.

A day in the life – a project of an exhibition

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Circadian rhythms are present throughout living matter, from procaryotes to the most complex organisms. Although well known in the scientific community, facts and concepts of biological rhythms are poorly understood and frequently subject to mistifications such as the so-called "biorhythms". In this communication we will present a project of an exhibition on circadian rhythms in the human aimed at students and teachers of basic education. The general idea of the exhibition is to offer interactive information of changes in behaviour and physiology of humans at different times of a 24h day. Life-size models of a family will be displayed along a circular path covering the 24 hours - at each point in time models will display typical behaviours (such as sleep, exercise, study, work, leisure, etc.) and information on the underlying physiology (brain activity, heart beats, hormones, etc.) will be available through animations. A virtual exhibition will be available in the internet. The aim of the exhibition is to provoke self-observations in the students and help them build a dynamic concept of life as a necessary complement to the classical view present in biology teaching - where a cell, for instance, is displayed immobile, devoid of life, under the microscope or in a picture. The project has been approved by a Brazilian science funding agency and is currently under development aiming at implementation in late 2010, early 2011 at the science museum of the Universidade de São Paulo in the city of São Paulo, Brazil.

Human chronobiology at a tropical zone in South America: chronotype latitudinal cline

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Purpose: To test the hypothesis that latitude affects circadian rhythms regulation in humans. Methods: We applied an on-line version of the Horne-Östberg (HO) questionnaire in a sample of the population along all latitudinal cline of Brazilian territory, which ranges from the equator line, 0°, until about 33° south. Insolation levels per degree of latitude were used as marker of latitudinal cline. Results: We have analyzed HO scores and demographic questions from 12.884 people living in the same time zone. The population was relatively young; being that 58.6% was up to 30 years old. The mean age was 31.3 ± 10.5 yo, ranging from 18 to 75 and 69.9% were females. When we analyse the HO score trough the latitudinal cline, taking in consideration the mean insolation level, we can observe a clear pattern to eveningness towards higher latitudes or to smaller insolation levels. Conclusion: Our data indicates that humans are sensitive to different sunligth signaling given by latitude which result in a chronotype latitudinal cline.

Not all adolescents are sleep deprived: a study on Brazilian rural populations

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Purpose: Several studies have shown that during weekdays adolescents generally sleep on average considerably less than the recommended 9h/day. This sleep deprivation has been attributed in great part to a sleep phase delay, which occurs for biological and socio-cultural reasons. The aim of this study was to depict some environmental factors related to adolescents sleep length. Methods: A total of 1140 students (569 males), aged 10-19 years, attending two schools of rural regions of Paraná State in southern Brazil, completed a questionnaire about their sleep habits. Demographic data were also obtained. Prevalence ratios (PR) were estimated for more than nine hours of sleep on weekdays. Sleep duration in adolescents with and without electric lighting at home was compared by means Kruskal-Wallis test. Results: Average sleep duration was 9.63(±1.64)h during weekdays and 10.14(±2,42)h during weekends. The prevalence of adolescents with more than nine hours of sleep during weekdays was 58.3%. On multivariate analysis, age, work and bedtime were factors associated with sleep duration. The prevalence of more than nine hours of sleep was lower in older students (16-17years) than in younger students (10-11years) (PR=0.93; CI95%:0.87-0.99) and lower in workers than in non-workers (PR=0.92; CI95%:0.89-0.96). A one-hour delay in bedtime was associated with significant differences on prevalence ratios. Adolescents with later bedtimes (10pm) showed shorter sleep duration when compared to those with earlier bedtimes (9pm) (PR=0.79; CI95%:0.76-0.84). Adolescents without electric lighting at home showed longer sleep duration on weekdays

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(p<0.001) and on weekends (p=0.01) when compared to those with electric lighting at home.

Conclusions: In contrast with data previously reported, a high prevalence of adolescents with more than nine hours of sleep during weekdays was found. Data on populations living in less industrialized regions reinforce the idea that technological advances are associated with the negative impact of the sleep phase delay.

Simulated night shift work under white, yellow and dim light

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Purpose: To investigate the effects of white, yellow and dim light on subjective ratings of sleepiness and activation, and melatonin suppression during simulated night shifts. Methods: 18 healthy subjects (22.7y ± 1.7) who did not work in night shifts during the 3 months preceding the study were selected and participated in a simulated night shift during three conditions: white, yellow and dim light. There was one week in between conditions to which subjects were assigned in random order. Baseline measurements were done during the first 2 hours of each night shift (21:00-23:00) during which subjects stayed in dim light. Subjective ratings of sleepiness (KSS) and activation (Thayer), and saliva samples for melatonin analysis were obtained each hour from 21:00 until 6:00 the next day. Results: Subjective ratings of sleepiness increased significantly over time ($F(7,10) = 21.38$, $p<0.01$), but did not differ between the 3 conditions ($F(2,15) = 1.73$, NS), nor was there an interaction effect over time ($F(14,3) = 0.82$, NS). Preliminary results indicate that there are no differences in subjective ratings of activation, and that melatonin production does not differ between the dim and yellow light condition while each of these two conditions do differ when compared to the white light condition. Conclusions: Subjective ratings of sleepiness increased significantly, and subjective ratings of activation decreased during the night, but did not differ between conditions. Melatonin on the other hand was suppressed in the white light condition compared to the dim and yellow light condition. These preliminary results suggest that working night shifts under yellow light does not affect sleepiness and activation differently than under white light, but that melatonin is less suppressed. This could benefit health.

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The effect of circadian phase on skin temperature and sleep inertia

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Purpose: To investigate the relationship between sleep inertia and skin temperature at different circadian phases. Methods: Five healthy males and four healthy females (age 20-25) were subjected to a forced desynchrony protocol, consisting of six 20h-days in which they stayed in dim light (< 10 lux) during the subjective day and in darkness during the subjective night. Sleep inertia severity was measured by subjective ratings of sleepiness (KSS) and activation (Thayer) at 1, 15, 30, 45, 60 and 90 minutes after waking up as well as every two hours during the subsequent day. Distal and proximal skin temperatures were measured at 2 min. intervals throughout the experiment. Results: Preliminary results indicate circadian rhythms in skin temperature and subjective ratings of sleepiness and activation. However, the subjective ratings during sleep inertia were little affected by circadian phase. Skin temperatures were masked due to sleep wake alternations. Conclusions: These preliminary results suggest that subjective ratings of sleepiness and activation during sleep inertia are at best marginally affected by circadian phase. This indicates that waking up from sleep increases sleepiness and decreases activation to such a degree that the circadian modulation of sleepiness and activation is of minor importance during this period. Further analyses will reveal whether skin temperature during sleep inertia is modulated by circadian phase.

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Cognitive performance in shift-workers and internal time

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Purpose: The circadian clock modulates human physiology, behaviour and performance. Yet, field research investigating the consequences of shift-work on human performance has so far rarely considered internal time. In this study, we investigate cognitive performance of shift-workers based on internal rather than on external time. Methods: We assessed cognitive performance, i.e. psychomotor vigilance and selective visual attention, in 21 young, rotating shift-workers in the field (every two hours for each shift over a course of four weeks). Daily sleep-logs and actimetry were also assessed across the four week study period as well the MCTQ (Munich ChronoType Questionnaire). The sleep-deficit corrected mid-sleep on free days after evening shifts (MSFEsc) was used as a marker for internal time (chronotype). This marker has been validated by sleep logs and actimetry. Results: A shift-specific modulation of psychomotor vigilance speed and attentional performance was observed as well as a strong chronotype-specific difference between the participants with regard to their performance within the distinct shifts. Conclusions: Cognitive performance is strongly influenced by individual phase of entrainment (chronotype). This is apparent both in the laboratory and in real-life settings. The effects of shift-work on cognitive performance can only be understood in the light of internal time.

Sleep and diurnal preference in type 2 diabetic patients

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Purpose: To evaluate diurnal preference and sleep pattern in type 2 diabetic patients. Methods: The Composite Scale of Morningness (CSM), the Pittsburgh Sleep Quality Index,

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the Pittsburgh Insomnia Rating Scale (PIRS), the Multifatigue Inventory, the Epworth Sleepiness Scale and the Beck Depression Inventory II (BDI) (all in Romanian translation) were given to diabetic patients suffering from a disturbed sleep pattern. Patients were recruited from outpatient and inpatient facilities at Baia Mare County Hospital, Romania. Volunteering control subjects were recruited from patients' families or acquaintances and students attending the Faculty of Psychology in Cluj-Napoca. Results: 77 diabetic patients (mean age ± S.D: 58.6±15.5; mean body mass index 30.2±6.0) and 174 controls (mean age ± S.D: 34.6±15.7; mean body mass index 23.6±4.6) were included in this study. Diabetic patients scored significantly higher in CSM (41.2±5.1) than controls (36.9±7.0). Diabetic patients also needed more time to fall asleep, had more disturbances during sleep, lower sleep efficiency and worse overall sleep quality (mean PSQI scores of 6.5). Sleep length however did not significantly differ between both groups (7 hours). Diabetic patients furthermore scored significantly higher in PIRS, and reported higher levels of fatigue, daytime sleepiness and depressed mood. Conclusions: These results indicate a higher prevalence of individuals with "morningness" among diabetic patients, who, at the same time, exhibit more sleep disturbances. Whether morningness is a premorbid trait or a characteristic of the diabetic state, needs to be further clarified.

Sleep and diurnal preference in depression

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Purpose: To evaluate diurnal preference and sleep pattern in depressed patients. Methods: A questionnaire consisting of the Romanian translations of the Composite Scale of Morningness (CSM), the Pittsburgh Sleep Quality Index, the Pittsburgh Insomnia Rating Scale (PIRS), the Multifatigue Inventory, the Epworth Sleepiness Scale and the Beck Depression Inventory II was distributed to patients suffering from major depression or dystimia, at Baia Mare County Hospital. Control subjects were recruited from patients' families or acquaintances and students attending the Faculty of Psychology in Cluj-Napoca. Results: 49 depressed patients (mean age ± S.D: 44.8±19.8) and 174 controls (mean age ± S.D: 34.6±15.7) completed the survey. Depressed patients scored lower in CSM (35.6±8.2) compared to controls (36.9±7.0). Depressed patients had clear difficulties in falling asleep (about 45 minutes, double of the controls' time), had shorter sleep length (under 7 hours), more disturbances during sleep, lower sleep efficiency and worse overall sleep quality (mean PSQI scores of 8.9). They scored significantly higher in PIRS (more than double compared to controls) and accused higher levels of fatigue and daytime sleepiness. Conclusions: These results confirm that depressed patients are more evening oriented and display more sleep disturbances and more severe sleep loss consequences than controls.

Spontaneous alterations in neuronal firing within the rat median raphe nucleus under urethane anaesthesia

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Purpose: Examine the hypothesis that individual serotonergic cells could spontaneously change their mode of firing and whether these alterations are related with different sleep-like brain state observed under urethane anaesthesia. Methods: In vivo extracellular single unit recordings were performed under 12:12 light/dark conditions within the serotonergic neurons of the median raphe nucleus (MRN) of urethane anesthetized rats. Putative serotonergic neurons were identified on the basis of their location and their electrophysiological characteristics of slow regular firing with broad bi- or triphasic action potential. During the experiment EEG signal were monitored to verify the physiological state of the animals. The changes in the activity types were computed off-line by analysis of the interspike interval histograms constructed for each 100 s of recording. Results: Individual presumed serotonergic cells of the rat MRN switch their firing pattern from a particular type of activity to another. In course of long lasting (up to 10 hours) recordings we have found that cells displaying classical clock-like pattern decreased their firing to become nearly quiescent, changed discharge pattern to less regular or started display action potential in bursting manner. Tendency to cease firing by regularly firing cells is supported by strong negative correlation between the regular and silent activity types. Furthermore, the different patterns of activity of these neurons are significantly distributed across the 24h light/dark cycle. Conclusions: The present data confirm formerly proposed supposition that individual serotonergic cells are able to switch between different activity patterns, earlier described as distinctive subpopulation of these cells.

Circadian modulation of vigilance state episodes under constant sleep pressure in the rat

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Purpose: Characterize the influence of the circadian clock on vigilance state episode frequency and duration. Methods: Rats (n=8) were implanted with EEG and EMG electrodes and subsequently adapted to constant dark conditions (DD) for at least 1 week. A baseline (BL) day was recorded followed by "short-day protocol" - 2h sleep deprivation followed by 2h rest for 2 days. Vigilance states were determined and EEG spectral analysis was performed. State episode frequency and duration was determined based on episode consolidation and interruptions. Data from the second day in the protocol is analyzed and compared with baseline. Results: During the protocol the animals slept 7.5% less over the 24-h circadian cycle. Vigilance states and slow-wave activity of the NREM sleep EEG (SWA 1-4 Hz) were evenly distributed across the day. REM sleep and SWA did not show a circadian modulation whereas the circadian modulation in NREM sleep and waking were markedly reduced. Waking and NREM sleep episode duration and episode frequency of all vigilance states showed a circadian modulation during the

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baseline. During the protocol episode frequencies of all vigilance states lost their circadian rhythm. NREM and REM sleep frequency increased to the highest values found in baseline, whereas waking frequency decreased to the lowest levels. REM sleep episode duration lost its circadian rhythm. The circadian modulation of waking episode duration remained intact except for CT20-24 where the duration was decreased. NREM sleep episode duration increased between CT16-18, reducing circadian amplitude. Conclusions: In the present protocol circadian modulation in sleep and waking is mainly caused by circadian modulation in vigilance state episode duration. Episode frequency was not under strong influence of the circadian clock. In contrast to humans, the influence of the circadian clock on REM sleep was weak compared to waking and NREM sleep.

Sleep in relation to age and chronotype in Polish adolescent and adults

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Purpose: To evaluate the distribution of chronotypes and sleep habits by age. Methods: Studies were carried out on subjects classified into three age groups: below 20 yr, 21-30 yr, and older than 31 yr. Data were collected on their sleep habits during workweek and free days, and on morningness-eveningness preference (Morningness-Eveningness Questionnaire; MEQ). Results: The distribution and mean scores on the MEQ advanced toward the Morning type from the young to the aged group. The self-estimated length of sleep was shorter for the Evening than the Morning types, especially in the adolescents. In the three age groups sleep duration differed markedly between workdays and free days. On average, subjects slept 2.5 h (adolescents and young adults) and 1 h (adults >30) longer on free days, with extreme individuals sleeping 4 h on workdays and 12 h on free days. Distribution of midsleep times (the midpoints between sleep onset and wake up) for different age groups fitted with a Gauss curve, both on workdays and free days. Midsleep times were significantly delayed on free days, in age- and chronotype-dependent manner. The biggest differences were found in the Evening adolescents. Conclusions: The phase of circadian rhythms had moved forward from the young to the aged group. Young subjects, especially the Evening types, may experience difficulty adjusting to early demands of the school/university schedule, and suffer from sleep debt that accumulates along the workweek.

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12. Circadian rhythm disorders; Diseases and circadian rhythm alterations

Preclinical model for the personalization of cancer chronotherapeutics

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Purpose: To identify and to characterize distinct classes of chronotoxicity of irinotecan (CPT11) according to gender and genotype in mice, as an experimental model for the personalization of cancer chronotherapeutics. CPT11, a Top1 inhibitor, is widely used against colorectal cancer, despite its severe toxicity which remains unpredictable through pharmacokinetics (PK) or pharmacogenomics assessments. Methods: Mice (270 ? and 270 ?) of 3 strains (C57BL/6, B6D2F1 and B6CBAF1) aged 6-8 weeks were synchronized with LD12: 12. They received a therapeutic dose of CPT11 (50 or 80 mg/kg/day x 4 days according to strain) at ZT 3, 7,11,15,19 or 23. Toxicity was evaluated with daily body weight loss. Additional studies in 132 ? and 132 ? B6D2F1 or B6CBAF1 investigated hematologic, intestinal toxicities , plasma PK of CPT11 and its active metabolite SN38, after CPT11 dosing at selected ZTs. Results: Maximum body weight loss (days 5-7) varied significantly according to ZT, strain, and gender, with significant interactions between these factors (3-way ANOVA). Circadian rhythms in CPT11 toxicity were validated separately in ? or ? mice of each strain, with different waveforms. Least toxicity, as assessed with body weight loss, leukopenia, bone marrow hypoplasia and intestinal lesions resulted from CPT11 dosing at ZT 11 for ? B6D2F1 and at ZT 15 for ? B6D2F1 and ? or ? B6CBAF1. The amplitude of the 24-h tolerability rhythm was least in ? B6D2F1. These differences were validated with Hoteling-test. Chrono PK of CPT11 and SN38 adequately explained chronotoxicity only in ? B6D2F1, but not in the other groups. Conclusions: We have identified 3 classes of CPT11 chronotoxicity in mice, which differ with regard to timing and magnitude of toxicity rhythm, main target organ of toxicity, circadian control of drug metabolism, and relevance of drug exposure for toxic outcome.

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Expression and functional analysis of circadian genes in mouse ovaries

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Purpose: To assess the presence of the circadian clock in ovaries and its involvement in ovulation. Methods: ICR wild-type and Clock mutant mice were individually housed under 14L/10D, and vaginal smears were checked to identify the estrous cycle. To study circadian ovarian oscillations, right ovaries of 3 wild-type mice were collected every 4 h for 4 days of one estrous cycle. Real-time RT-PCR of total RNAs extracted from ovaries was performed to examine expressions of circadian genes, Bmal1, Cry1, Cry2, Per1 and Per2. To determine the effect of the ovarian circadian clock on ovulation, oocytes in the oviducts of 3 wild-type mice and 3 Clock mutant mice were checked every 4 h from the pre-estrous to post-estrous stages including ovulation time. At the same time, right ovaries and kidneys and blood serum were collected for analyses of Per2 expression and LH concentration, respectively. For superovulation, mice that had been injected with 10 IU PMSG were injected with 10 IU hCG, equal to LH surge, 48 h after PMSG injection. Oocytes in the oviducts were checked at 15 and 19 h after hCG injection. Results: Circadian genes were periodically transcribed every 24 h during the whole estrous cycle in the ovaries. LH surge and ovulation occurred at 12 and 0 h and at 16 and 4 h after light onset in wild-type mice and Clock mutant mice, respectively. The expression of Per2 in the ovaries peaked at 16 h after light onset in wild-type and Clock mutant mice, although the amplitude in wild-type mice was 2-times higher than that in Clock mutant mice. To clarify the LH surge effect on ovulation delay in Clock mutant mice, hCG was injected into PMSG-treated Clock mutant mice at the time of LH surge in wild-type mice, but ovulation was still delayed. Conclusions: The results suggested the presence of an ovarian circadian clock and its importance for the mechanism of ovulation.

Circadian rhythms in adult attention-deficit/ hyperactivity disorder

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Purpose: To assess circadian rhythmicity in adult ADHD using actigraphy, gene expression and endocrine markers. Methods: Patients (n=12) attending an adult ADHD outpatient clinic were recruited, as were age-matched controls (n=24) who also underwent screening for ADHD. Informed consent was received from all participants. Subjects wore an ActiWatch (Cambridge Neurotechnology, UK) on the non-dominant wrist for a period of 7 to 14 days. Quantitative PCR was carried out to measure gene expression in buccal samples. Relative expression levels of the circadian clock genes hPer2 and hBmal1 were examined. Salivary levels of cortisol and melatonin over a 24 hour period were measured. Results: Preliminary results indicated significant differences in a number of circadian parameters in adult ADHD, in comparison to the healthy control group. The period length was significantly shorter in the patient group compared to the control group and the period deviation was significantly greater in the ADHD group. The relative amplitude of the rhythm, was also significantly weakened. There was no statistically significant change in other circadian parameters, nor in the average of maximum light exposure between ADHD and control groups. hPer2 and hBmal1 expression were found to cycle in a circadian fashion, however the rhythmic expression of hPer2 and hBmal1 were found to be significantly deregulated in the ADHD patient cohort. Cortisol secretion was shown to oscillate, peaking in the early morning in the majority of the control group, whereas the patient group exhibited varied profiles of cortisol secretion. Conclusions: These results provide novel, behavioural and molecular evidence that adult ADHD is associated with less-robust circadian rhythms, and that this circadian dysfunction

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might contribute to the poor sleep patterns that are associated with adult ADHD.

An implanted device for the adjustment of cancer chronotherapeutics to the patient'scircadian timing system

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Purpose: Chronotherapeutics improves tolerability and efficacy of cancer treatments through the adjustment of chronomodulated drug infusions to the Circadian Timing System (CTS). CTS function can vary according to gender, age, genotype, lifestyle, diseases and treatments. Yet a precise knowledge of the circadian phase is required for the personalization of cancer chronotherapeutics and can be provided by core body temperature rhythm monitoring. Methods: We have embedded a temperature sensor and an emitter into a commercially available implantable vascular access port, used worldwide to deliver chemotherapy (Celsite, B. Braun Medical). The device (Celsite® Rhythm?) is associated to an external receiver worn by the patient and a PC software for medical decisions. No specific surgery is required. Results For the industrialised system, the emitter uses advanced technologies to minimize volume (<1cm3) and to maximize lifespan (>2 y); resolution is 0.1°C within 35-42°C. Temperature is sampled every 10 min, for detection of possible observable periods of 20 min in circadian components. This device was validated in a pilot study in a rat with simultaneous recording of body temperature with Celsite® Rhythm? and a commercially implanted sensor (Data Sciences). Conclusions The device will help determine the internal circadian phase and CTS characteristics of individual cancer patients before, during and after the administration of cancer treatments. Celsite Rhythm® will be used for 1/ the rapid adjustment of chronotherapeutic drug delivery according to the CTS dynamics, 2/ the detection or monitoring of infectious processes, 3/ the development of real time circadian control of chronomodulated drug delivery, 4/ the development of a unique international prospective data base regarding the long term physiopathology of circadian function along cancer processes and their treatments.

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Correlation between wrist skin temperature circadian rhythm and ambulatory blood pressure monitoring (ABPM)

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Purpose: Wrist skin temperature (WT) has been proposed as an index for the circadian system status in normal-living subjects. WT increases in association with sleep and decreases during activity phase, following a close relationship with peripheral vasodilatation. Thus, the purpose of this work is to determine the relationship between WT and blood pressure (BP) circadian rhythms. Methods: A total of 11 voluntary subjects, aging from 21 to 60 years old participated in this study. A wireless data logger (ThermoChron®, IDC S.A., Spain) was placed on the non-dominand hand at wrist level to record skin temperature during five consecutive days. The ambulatory blood pressure monitoring (ABPM) was determined during 48 hours using a BP monitor (Spacelabs® Medical), with a sampling rate of one measure every 20 min during the day and every 40 min during the night. Cosinor and non-parametric analysis were used to characterize WT and BP rhythms. Results: Our results show for the very first time simultaneous recordings of WT and BP. These simultaneous measures have evidenced that both variables show a significant inverse relationship (systolic BP vs WT, r=-0.518, p<0.001, diastolic BP vs WT, r=-0.451, p<0.001). In addition, both variables daily patterns showed a good correlation in most subjects. Conclusion: The evident link between WT and BP rhythms turns WT into a new useful tool to study human pathologies related to autonomous deregulation such as hypertension. An adequate balance between sympathetic (vasoconstrictive) and parasympathetic (vasodilating) activation may be a prerequisite for a normal circadian pattern in BP. The ability to dissipate heat through vasodilating skin blood vessels would be the link between WT and BP circadian rhythms.

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Identification of an activator of Kv3.1 channel current from Androctonus australis hector scorpion venom required for circadian neural activity

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Purpose : Lack of Kv3.1 potassium channel current leads to sleep-loss with increase of cortical ?-oscillations besides a decrease of d-oscillations. Hence, the identification of a specific Kv3.1 activator seems to be important. Methods: Electrophysiological tests were performed on Kv3.1 injected oocytes using intracellular double microelectrodes technique. Scorpion Venom is known to contain toxins that are active on voltage-gated potassium channel. The crude toxic material AahG50 of Androctonus australis hector scorpion venom was tested on Kv3.1 current, and then fractionated by several chromatographies purification. Results: Aah G50 (10 µg/ml) inhibited the potassium current by 31%. After FPLC purification, we found that fraction 8 with low molecular weight (< 4 kDa) displays an activating activity, at the concentration of 1µg/ml, it increases K+ current amplitude by 18% (±3%). RP 18 HPLC of this fraction revealed the presence of four peaks that seem to have an effect on Kv3.1 current. Three of them (4 µg/ml) block Kv3.1 current recorded in Xenopus oocytes respectively by 17, 35, and 20% and the fourth peak (10 µg/ml) activates K+ current by 25% (n=3). Conclusions: The toxic fraction of Aah contains at least one peptide that could activate Kv3.1 channel expressed in brain regions involved in the modulation of the sleep–wake cycle, where they enable neurons to fire narrow action potentials at very high frequencies.

The utility of a midday salivary melatonin measurement as a diagnostic test for patients with the Smith-Magenis Syndrome

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Purpose: To determine the utility of daytime salivary melatonin as a diagnostic test in patients with the Smith-Magenis Syndrome (SMS). Methods: Thirty individuals with confirmed SMS [28 with del 17p11.2 and two with the retinoic acid induced 1 (RAI1) gene mutation] and five controls were studied. Single or serial daytime salivary melatonin levels were measured by radioimmunoassay. Results: Of the 30 patients participating in the study, there were 13 (43%) males and 17 (57%) females. Mean age at the time of their initial study was 9.8 y (4.5 m to 20 y). The mean midday salivary melatonin level was 79.0 pg/ml in SMS patients, compared with 16.3 pg/ml in controls, with 9 patients having values similar to controls. The median melatonin level in SMS patients was 49.0 pg/ml (first and third quartile values = 15.5 and 106.8 pg/ml). Twenty six (90%) of 29 patients had at least one value > 15.5 pg/ml, including 70 (78%) of 90 samples from patients with del 17p11.2 and 1 (20%) of 5 samples from the two patients with the RAI1 mutation. Neither the pattern of medication use, nor age had an effect on daytime salivary melatonin levels. Using a melatonin cut off of 25 pg/ml (the highest melatonin value in the control subjects was 24 pg/ml), elevated midday melatonin level was found in 20 (69%) of 29 SMS patients. With additional data from multiple time points sampling, two more patients (22 of 29, 76%) were found to have salivary melatonin levels above this cutoff. Conclusions: Although most SMS patients had elevated daytime salivary melatonin levels, the utility of a midday salivary melatonin level may be insufficient to distinguish patients with SMS from other conditions. Multiple sampling at additional time points may increase the sensitivity of a SMS salivary melatonin test.

Human in vitro model of molecular chronopharmacology of anticancer drug irinotecan

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Purpose: 1) To develop in vitro models for assessing the chronopharmacology of anticancer drugs in human cancer cells;2)to determine the critical parameter values of in vitro chronopharmaco-kinetics and -dynamics (chronoPK-PD); and 3 ultimately, to design modeled optimal drug delivery schedules in individual patients. We probe the relevance of this approach for irinotecan, a topoisomerase I (TOPO1) inhibitor, against colorectal cancer. Methods: We investigated whether colorectal adenocarcinoma cells (Caco-2) were amenable to circadian synchronization with 2 h-serum shock. We assessed the temporal relations between circadian clocks (Per2, Bmal1 and Rev-erb?) and irinotecan transport (ABCC1, ABCB1, ABCC2, ABCG2), bioactivation and detoxification (CES2, CYP3A4, UGT1A1) and molecular target (TOPO1). Gene expression at mRNA and protein levels was determined with qPCR and Western blots on cells sampled every 4h for 48h in 3 experiments. Serum shocked cells were exposed to irinotecan. Parent drug and bioactive SN38 were determined with HPLC. Results: Rhythmic mRNA expression of clock genes Rev-erb?, Bmal1 and Per2 was demonstrated after serum shock ($p<0.001$), with mean period length of 27.5 ? 0.3 h. The acrophase f of Rev-erb? occurred at 9.7 h and that of Bmal1 was located 7.7 h later, at 17.4 h, a finding which supports the known reciprocal regulation of both clock genes. Rhythmic mRNA expression was lacking for CES2 and ABCC1 but significantly validated for ABCB1 (f=15.0 h), ABCC2 (f=13.6 h), ABCG2 (f=11.9 h), UGT1A1 (f=12.9 h), and TOPO1 (f=13.3 h).The circadian transcriptional changes are being related to those in protein expression, enzymatic activities and in vitro pharmacokinetics. Conclusion: Synchronized human Caco-2 cells constitute an in vitro model for concurrent investigation of multiple cellular and molecular mechanisms of anticancer drug chronopharmacology.

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Identification of a quantitative trait gene underlying "behavioral despair" using CS mice with abnormal circadian rhythms

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The CS mice exhibit several distinct phenotypes of circadian behavioral rhythms, such as a long free-running period, spontaneous rhythm splitting, and entrainment of circadian rhythms, in response to a daily restricted feeding schedule under constant darkness. In addition, the sleep properties of CS mice are distinct from those of C57BL/6J and C3H/He mice, which have normal circadian rhythms. Because many mental illnesses are associated with abnormalities in the circadian system and sleep pattern, we characterized the behavioral phenotypes in CS mice with a battery of behavioral tests. Among these phenotypes, we found that CS mice exhibit an extremely low immobility time (almost no immobility) in both the tail suspension test (TST) and forced swimming test (FST), which are widely used for assessing antidepressant activity and depression -like behavior. Quantitative trait locus (QTL) mapping using CS and C57BL/6J mice revealed significant QTLs on chromosomes (Chrs) 4 (FST) and 5 (TST and FST). To identify the quantitative trait gene on Chr 5, we narrowed the QTL interval to 0.5 Mb using several congenic and subcongenic strains. Ubiquitin-specific peptidase 46 (Usp46) with a lysine codon deletion was located in this region. This deletion affected nest-building, alcohol preference, wheel-running rhythms under LD, muscimol-induced righting reflex, and anti-immobility effects of imipramine. The muscimol-induced current in the hippocampal CA1 pyramidal neurons and hippocampal expression of the 67-kDa isoform of glutamic acid decarboxylase significantly decreased in the Usp46 mutant mice. All these phenotypes were rescued in transgenic mice with bacterial artificial chromosomes containing wild-type Usp46. Thus,

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Usp46 affects “behavioral despair” and it is implicated in the regulation of GABA action.

Effect of a cell cycle inhibitor seliciclib on endogenous circadian timing system

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Purpose: To study the effects of seliciclib, a cyclin-dependent kinase inhibitor with anticancer properties, on two circadian biomarkers, rest-activity and temperature rhythms. To identify the respective roles of dosing time, mouse strain and gender, with a perspective of personalized cancer chronotherapeutics. Methods: Ninety six male and female B6D2F1 and B6CBAF1 mice had a temperature and activity sensor (Physio Tel, TA 10 TA-F20) implanted in their peritoneal cavity to monitor both variables every 10 min. After a week in constant darkness the mice received a single equitoxic dose of seliciclib: 600 mg/kg (B6D2F1) or 900 mg/kg (B6CBAF1) p.o. at one of 6 circadian times: CT3, 7, 11, 15, 19 or 23. Spectral analyses defined endogenous period t (h) and acrophase [radians (rd)] of thermic rhythm in each mouse during the week before seliciclib administration and during the week starting 2 days after treatment. Results: Before seliciclib, temperature t varied from 23.2 h in ?B6D2F1 to 23.5 h in ?B6CBAF1 (p= 0.016). Seliciclib lengthened t by 0.35 h in ?B6D2F1 and shortened it by 0.08 h in ?B6D2F1 (strain*gender, p=0.002), irrespective of the administration time (p= 0.076). Seliciclib caused a phase delay, which was less pronounced at CT3 (0.73 rd) than at CT7 (1.5 rd) or CT23 (1.68 rd) (p = 0.05). Mean phase delay was greater in ?B6D2F1 (1.2 ± 0.1 rd) than in ?B6CBAF1 (0.5 ± 0.07 rd) (strain*gender, p = 0.001). Conclusions: Seliciclib modifies the endogenous period and/or the phase of circadian coordination. This could result from its proven direct inhibition of casein kinase Id/e or interactions with other enzymatic targets under study. The circadian timing system constitutes a strain- and gender-dependent toxicity target for seliciclib.

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Melatonin and circadian rhythms in adolescent idiopathic scoliosis

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Adolescent idiopathic scoliosis (IS) represents the most frequently occurring form of scoliosis that occurs and progresses in puberty. Puberty is a developmental phase during which profound hormonal, physical and psychoneurological changes occur. The neuroendocrine hypothesis involving a melatonin deficiency as the source for IS has generated controversies. Purpose: To determine whether melatonin production related to circadian rhythms of hormones is altered in adolescent idiopathic scoliosis. Methods: Blood was collected every 4 h during day and 2 h during night of 24-h periods from fourteen patients (age 9-18y) with idiopathic scoliosis and an age-gender matched control groups. Serum melatonin, cortisol, LH, FSH, E2, T, DHEA, androstendione, SHBG, HGH, GHBP, IGF-1, IGFBP-3, PTH, osteocalcin, osteoprotegerin, sRANKL levels were measured and statistically analyzed. The individual profiles of circadian markers (melatonin and cortisol) and studied hormones were quantified by a fit cosine curve yielding mesor, amplitude and acrophase. Results: There were no statistically significant differences in the secretion of serum melatonin and hormones as the mean 24-h concentrations between the patients and the control group. The levels of hormones in blood samples collected at 8 a.m. showed statistically significant differences: higher levels of melatonin (24.56 vs 9.56 ng/ml), LH (7.26 vs 2.48 U/L), E2 (107.7 vs 27.6 pg/ml), IGF-1 (426 vs 245ng/ml), osteocalcin (174 vs 72 ng/ml) and sRANKL (3.7 vs 1.9 nM/L) in IS group compared to controls. Cosinor analysis of circadian profiles of hormones showed considerable changes in their rhythmic secretion; there was a considerable dispersion of circadian phase: the acrophases occurred across the day and nighttime. By affecting the synchrony of coherence of the circadian system components, the rhythmic secretion of hormones was significantly altered, probably contributing to the development of scoliosis. Conclusions: The results suggest that a defect in processing by the circadian system might affect the growing spine. More research is needed to determine the mechanism whereby neuroendocrine processes of circadian rhythms influence the development of scoliosis.

Tumor-associated cytokines and circadian rest/activity rhythm in colorectal cancer patients

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Purpose: Proinflammatory cytokines can induce circadian disruption in laboratory rodents, similar to that demonstrated in cancer pts, using rest-activity as a circadian biomarker. We searched for associations between disrupted rhythms and serum concentrations or tumor protein expression levels of cytokines in two separate studies (st) in pts with metastatic colorectal cancer. Methods: In both st, rest-activity was monitored with 3d wrist-actigraphy and its rhythm estimated with autocorrelation coefficientr24. Circadian disruption corresponded to r24<0.37 (lowest quartile in prior large st). In st1, morning serum concentrations of TGF?, TNF? and IL6 were determined with ELISA in 80 pts. In st2, the % labeled tumor cells for TGF?, TNF?, EGF, VEGF, IL6 and IL1B was scored on paraffin-embedded samples of primary tumors in 52 pts. Results: In both st, half of the pts had r24<0.37. In st1, circadian disruption was associated with higher median values of TGF? (x 4.6; p=0.002), TNF? (x1.7; p=0.03) and IL6 (x1.5; p=0.006). In st2, circadian disruption was associated with higher proportions of tumor cells expressing TNF? (x4.9;

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p=0.02). Conclusions: Tumor-associated cytokines, both circulating and in cancer cells, are associated with circadian disruption, as assessed with rest-activity rhythm monitoring in cancer patients.

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Valproic acid alters the rhythmic expression of PERIOD2::LUCIFERASE

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Purpose: To examine the effect of valproic acid on circadian expression of the clock gene protein PERIOD2, and to compare the impact of valproic acid with effects of lithium. Method: Fibroblasts and the SCN from transgenic PERIOD2::LUCIFERASE (PER2::LUC) mice were used to study PER2::LUC activity. Bioluminescence was recorded with photomultiplier assemblies in fibroblast cultures and organotypic SCN cultures before and after treatment with valproic acid or/and lithium chloride. The effect on phase, period and amplitude was calculated. Results: Bipolar disorder may involve alterations in circadian rhythmicity. Valproic acid (valproate) and lithium are widely used medications for treatments of bipolar disorder. Lithium has been shown to lengthen the period of circadian rhythms in several organisms. Valproic acid significantly phase shifted the PER2::LUC rhythm in fibroblast cultures at a time point corresponding to the lowest PER2 expression (trough), but had little or no effect when the drug was administered at the time of highest (peak) gene expression. Lithium chloride did not phase shift the PER2 rhythm but had a lengthening effect on the PER2 period that was dose dependent. Furthermore, the gene expression amplitude was increased by lithium. Conclusions: These results demonstrate that valproic acid, in contrast to lithium, can phase shift the clock gene rhythm at specific time points. This finding may in the future be important for the choice of drug when treating patients with bipolar disorder.

In humans, early morning blue light exposure leads to age-dependent alterations in PER2 levels

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Purpose: To study the effect of light on PER2 expression and whether this effect is wavelength- and/or age-dependent. Methods: Young and older participants were subjected to a 2-h intermittent either blue or green monochromatic light pulse in the morning 8.5 h after their individual dim light melatonin onset. Oral mucosa was sampled at clock times equivalent to 0.5, 5, and 10 hours after light exposure on a baseline day without light exposure and 24 hours later on the day of light exposure. The samples were then analyzed for PER2 expression using real-time PCR. Results: We found that PER2 was expressed significantly higher in young compared to older subjects 10 hours after a blue, but not green, light pulse. Conclusions: Our findings indicate that the involvement of the non-image-forming visual system in human circadian gene expression depends on age. Moreover, we demonstrate that human buccal samples are a valuable tool to study clock gene expression and the response of PER2 to light in humans.

Exfoliated epithelial cells, a source of information on clock genes expression by preterm infants to explore the onset of metabolic syndrome

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Purpose: In translational research, ethics as well as legal reasons are limiting the use of biopsies in favor of non-invasive technology like the recovery of exfoliated cells from digestive fluids. Our major goal is to study the link between perinatal denutrition and the regulation of clock genes. Methods: Gastric fluid aspirates were collected from preterm infants at day 1, 8, 15, 23 and 30 after birth and related to infants' growth and feeding. Exfoliated cells isolated according to Ped Res 2007 62: 564-569 in the frame of our Biocollection « Prématheque », were enumerated and characterized by immunofluorescence and confocal imaging. In parallel experiments, time series analysed by TSA Cosinor were obtained from exfoliated buccal cells of human adult volunteer and from gastric mucosa of rat pups submitted to restricted/refeeding cycle to induce exfoliation. Results: Gastric cells were quiescent epithelial cell phenotypes with rare figures of apoptosis. On typical samples of 50 cells, 30% were expressing H+/K+ ATPases, 7% Tryptophane Hydroxylases and 50% were positive for stem cell markers (Pouf5F1 (Oct4) and Survivin). CLOCK and NPAS2 were found both at the cytoplasmic and nucleus sites. Expression levels and colocalization with DNA-fluorochromes were quantified by image analyses relatively to H+/K+ ATPases and survivin levels. Conclusions: Our results are in favor of the expression of NPAS2 by gastric cells of preterm infants. As gastric fluids are collected every 3 hours in neonatal intensive care unit, the technique is relevant to explore the acquisition of circadian rhythmicity by the gastric epithelium of preterm infants.

Contribution of the circadian clock gene Bmal1 in the development of dilated cardiomyopathy

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Purpose: Disruption of circadian rhythms, such as occur with shift work, sleep deprivation or jet lag, has been associated with increased incidence of hypertension, coronary artery disease, dyslipidemia and other cardiovascular pathologies. The goal of this project is to determine whether loss of the circadian clock gene, Bmal1, leads to disruptions in the structural and functional integrity of cardiac

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muscle. Methods: Transthoracic echochardiograms were performed on 8 week old wildtype and Bmal1 knockout mice. Hearts were collected for electron microscopy and biochemical studies. Titin isoforms were assessed using a vertical agarose gel system. Results: The Bmal1 knockout mice develop signs of dilated cardiomyopathy. Echocardiogram data show increased left ventricular internal diameter, indicating enlargement of the left ventricle. Ejection fraction and fractional shortening are decreased, indicating systolic dysfunction. Furthermore, there is progressive thinning of the left ventricular posterior wall. Consistent with the functional data, electron microscopy shows sarcomere disorganization within the cells. This is characterized by diffuse M lines and A bands, wavy Z-lines and asymmetrically aligned thick filaments. At the protein level, there is down-regulation of the compliant N2BA and up-regulation of the stiff N2B titin isoforms in the left ventricle of Bmal1-/- mice compared to age matched controls, suggesting that titin-based stiffness is increased in the Bmal1-/- cardiomyocytes. Conclusions: Systemic loss of the circadian clock gene, Bmal1, is associated with the development of dilated cardiomyopathy. Ongoing studies are directed at determining if there are additional biochemical and functional indices of cardiomyopathy at the cellular level of the Bmal1-/- hearts. Future studies will determine if heart specific loss of Bmal1 leads to cardiomyopathy or whether this complex disease is due to multisystem dysfunction.

Control of cancer progression through rhythmic induction of tumor stress genes with circadian meal timing

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Purpose: To slow down cancer progression through the reinforcement of host and tumor circadian clocks or rhythmic signaling pathways with meal timing (MT). Methods: Pancreatic adenocarcinoma-bearing mice were synchronized with 12 hours of light/darkness. Mice were fed ad libitum (AL) or with MT from Zeitgeber Time (ZT) 2 to ZT6, with normal or fat diet. The circadian timing system was assessed through 1/ telemetered rest-activity and body temperature and 2/ liver and tumor mRNA expression, with qPCR for clock genes (Rev-erb?, Per2 and Bmal1) and clock-controlled genes (Hspa8 and Cirbp) at endogenous Circadian Time (CT) 0, 4, 9, 12 or 16. Tumor gene expression was determined with DNA microarrays (Affymetrix) at CT4 and at CT16. Results: Tumor growth was nearly halved in mice on MT as compared to AL (ANOVA p=0.01), without any influence of diet (p=0.5) or body weight. MT significantly modified the 24-h expression pattern of 423 genes in tumor transcriptome, mostly in stress, cell cycle and metabolism domains. MT advanced the phases of rest-activity, body temperature and liver molecular clock rhythms by 8 to 12 hours. MT nearly doubled the circadian amplitude of host body temperature ($p<0.001$) and induced strong rhythmic transcription of temperature-sensitive stress genes Hspa8 and Cirbp, two regulators of cell cycle and apoptosis, in tumor ($p<0.01$). Conclusions: MT inhibited tumor progression through enhanced host circadian coordination. MT bypassed tumor defective clocks and induced rhythmic stress and cell cycle genes transcription in tumor.

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Influence of light exposure on skin wrist temperature rhythm in humans under free-style living conditions

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Purpose: The increased number of pathologies associated to circadian disruption highlights the convenience to assess the circadian system status (CSS) in humans under normal living conditions. In addition, modern lifestyle promotes low contrast environmental conditions either in temperature or light. Skin temperature measured at wrist level (WT) has been proposed as a new index to evaluate human CSS. Since light-dark exposure is the main synchronizer of circadian rhythms to 24-h cycles, the aim of the present work is to determine the relationship between actual light exposure (time and intensity) and WT rhythm robustness. Methods: WT and light exposure of 12 healthy subjects were recorded during a week using two data loggers, Ibuttom (ThermoChron®, IDC S.A., Spain) and Hobo (HOBO® Pendant Temperature/Light), respectively. Results: Young adults receive only 153 ± 23 min of light exposure exceeding 500 lux per day, whereas they remained 11h and 51 ± 29 min under less than 10 lux, and 9 h and 36 ± 27 min between 10-500 lux. An inverse relationship between WT and light intensity was observed, both during day ($r= -0.769$, $p<0.001$) and night ($r= -0.974$, $p<0.001$). Skin WT response to acute light exposure occurs in less than 10 minutes and it depends on light intensity. Conclusion: The effect of circadian disruption due to improper timing, suboptimal spectrum or insufficient light intensity can be reliably evaluated when combining WT and light data in subjects under normal living conditions.

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Lithium impacts on the molecular circadian clockwork through induction of Per2

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Purpose: Lithium has been widely used in treatment of Bipolar disorder, which is associated with circadian disorders. Current study aims to investigate the impacts of Lithium on the central and peripheral molecular oscillators and the underlying mechanism. Methods: Behavioural rhythms of WT and Rev-erba knockout mice upon Lithium treatment were measured by wheel-running. Molecular rhythms in organotypic SCN, lung slices, primary lung fibroblasts (from PER2::LUC mice), and transcriptional rhythms for Bmal1::luc or Per2::luc (in Rat-1 cells) were recorded using PMT photon counting. Lithium action on the decay rate of PER2::LUC signal was measured following cycloheximide treatment. Expression of Per2 mRNA

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was measured using Q-PCR. Results: Lithium-induced period lengthening of wheel-running behaviour in WT animals, but not in Rev-erba knockout mice. In addition, we observed dose-dependent period lengthening of PER2::luciferase protein cycles induced by Lithium in the SCN, lung and fibroblasts. Lithium also caused significantly elevated PER2::LUC expression and increase of circadian amplitude (2.7±0.25 fold) in the peripheral clocks (lung and fibroblasts). Upon Lithium treatment or GSK3ß inhibition, there were no significant changes of PER2::LUC decay rate, but greatly enhanced Per2 mRNA level (~2 fold, P<0.01), indicating that augmented PER2 protein signal is most likely due to increased Per2 transcription. In contrast, although a selective GSK3ß inhibitor also enhanced the PER2::LUC signal, circadian period was shortened in a dose-dependent manner, suggesting important differences between Lithium action and GSK3ß inhibition on the circadian system. Conclusions: Collectively, these data demonstrate that lithium acts on the amplitude of the circadian PER2 signal, and we identify Per2 mRNA as an additional target for Lithium action, through a mechanism which is independent of GSK3ß action.

Activity/rest rhythm of depressed adolescents undergoing therapy: case studies

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Purpose: to evaluate whether there is a relationship between 24h spectral power of the activity/rest rhythm and the clinical course of depression in adolescents. Methods: Six adolescents with Major Depressive Disorder (DSM IV criteria) were selected using Schedule for Affective Disorders and Schizophrenia for School Aged Children: Present and Lifetime Version. Depressive symptoms were assessed using the Children's Depression Rating Scale–Revised and clinical evaluations. Locomotor activity was monitored over a period of thirteen consecutive weeks. Activity was measured during 10 minutes periods using wrist-worn activity monitors. Results: The measurements of the 24h spectral power of activity/rest data correlated significantly with scores of the CDRS-R (p<0.05) in five patients. Conclusions: The 24 h spectral power of the activity/rest rhythm correlated significantly (negatively) with the clinical ratings of depression. The importance of social cues to entrain circadian rhythmicity and improve depressive symptoms was also observed in one patient.

Circadian abnormalities in patients with cirrhosis: origins and consequences

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Purpose: Sleep disturbances are common in patients with cirrhosis. Abnormalities in the 24-hour melatonin profile have also been reported, but their pathophysiology and their relationship to sleep-wake behaviour remain unknown. The aim of this study was to evaluate central circadian clock function/hepatic melatonin metabolism and their relationship to sleep quality/timing in patients with cirrhosis. Methods: The study population comprised 20 patients with cirrhosis and nine matched healthy controls. Plasma melatonin/cortisol were measured hourly for 24 hours in light/posture-controlled conditions. Urinary 6-sulphatoxymelatonin, the main melatonin metabolite, was measured over the same period to determine melatonin clearance. The ability of light to suppress nocturnal melatonin synthesis was assessed to a standard protocol. Sleep quality/timing were evaluated using questionnaires and two-week monitoring with actigraphy/sleep diaries. Results: There was evidence of central circadian disruption in patients with cirrhosis: peak plasma melatonin/cortisol times were delayed compared to the healthy controls (04:48?02:36 vs. 02:48?00:54; p=0.01; 10:18?02:54 vs. 08:54?01:24; p=0.06) and the melatonin response to light was reduced (12?19 vs. 24?15% p=0.09). In contrast, there was little evidence of impaired hepatic melatonin metabolism, with comparable 24-hour plasma clearance in the patients and healthy controls. However, although the patients showed a degree of misalignment between sleep and circadian phase, there was no obvious association between circadian abnormalities and impaired sleep quality. Conclusions: Melatonin profile abnormalities of central origin are observed in patients with cirrhosis but appear unrelated to the sleep disturbances prevalent in this patient population

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Amphetamine-induced change in circadian rhythms of dopaminergic system in the rat striatum

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Purpose: To investigate the possible changes in daily rhythm of dopaminergic system in rat striatum, a well known part of brain reward region, following chronic d-amphetamine exposure. Methods: Adults male Wistar rats were housed under LD12:12, exposed to either saline or 5 mg/kg d-amphetamine subcutaneously at a fixed time daily for 6 days and sacrificed at ZT 3, 9, 15 and 21 on day 7. The striatum were dissected and prepared for western blot analysis. Antibodies raised against tyrosine hydroxylase, the rate-limiting enzyme in dopamine synthesis, dopamine D1 receptor and dopamine D2 receptor were used. Results: Western blot analyses revealed that the level of tyrosine hydroxylase, dopamine D1 receptor and dopamine D2 receptors in the rat striatum exhibits the circadian pattern. Moreover, the expressions of these dopaminergic contents were disturbed following chronic d-amphetamine administration. Conclusions: This findings demonstrate the involvement of circadian rhythm in associated with the d-amphetamine-induced changes in dopaminergic system in the rat striatum, which is possibly be under the control of circadian genes. However, the

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mechanisms for such interactions remain unknown.

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Impact of lead on the circadian rhythm of locomotor activity and prophylactic effect of melatonin and 5-metoxytryptophol

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Purpose: we attempted to demonstrate the impact of lead on circadian locomotor activity and the protective effect of melatonin and 5-methoxytryptophol against the toxic properties of lead. Methods: four groups of young's rats were used (control, lead acetate-treated [1 mg/animal], lead acetate plus melatonin [25 µg/kg], and lead acetate plus 5-metoxytryptophol [25 µg/kg]). we use the Actimetry system to record the locomotor activity Results: we observed a decreased in locomotor's activity and the synchronization of circadian rhythm of this activity by light is deeply disturbed by Lead, which makes it possible to suggest the deterioration of the transmission of photic information towards the suprachiasmatic nucleus. Moreover, Melatonin as well as 5-methoxytryptophol seems to be protective. Conclusions: Lead has a chronobiotic effect. The expression of locomotor activity rhythm and its synchronization by light is partially or totally restore by melatonin and 5-methoxytryptophol.

Involvement of the mPer2, but not the mPer1, gene in the circadian regulation of ethanol CNS sensitivity

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Purpose: To investigate, at first, the daily rhytmicity of the brain sensitivity towards alcohol, and, second, the implication of the clock genes mPer1 and mPer2 in this regard. Methods: We therefore first set out assessing the diurnal variations in ethanol sensitivity in mice analyzing loss of righting reflex (LORR) duration, and, concurrently, the ethanol elimination rates. Ethanol-induced (3.5 g/kg; i.p.) LORR duration was thus determined at several Zeitgeber time (ZT) points (ZT5, 11, 17, and 23) in C57BL/6N mice. In parallel, the corresponding ethanol elimination rates were also assessed. Successively, we checked the involvement of the clock genes mPer1 and mPer2 in conveying this rhythm in sensitivity, testing the LORR duration at ZT5 and ZT11 in the Per1Brdm1 and Per2Brdm1 mutant mice and in their respective wild-type littermates. Results: Our results display the existence of a distinct diurnal rhythm in LORR duration, peaking at ZT11, whereas no significant difference could be observed regarding the elimination rates of alcohol, revealing higher brain sensitivity for this time-point. Furthermore, the Per1Brdm1 mutant mice demonstrate a similar diurnal pattern as the control mice, with enhanced LORR durations at ZT11. In contrast, the Per2Brdm1 mice did not exhibit such a temporal variation to the depressant effects of ethanol, revealing a constant high sensitivity towards ethanol. Conclusion: The present study reveals a central role of the mPer2 gene in inhibiting the alcohol brain sensitivity at the beginning of the light phase.

Disturbed sleep/wake rhythms in the Park5 mutant gad mouse

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Purpose: Patients with neurodegenerative disorders like Parkinson´s disease (PD) or Alzheimer disease suffer from poor nocturnal sleep and excessive daytime sleepiness. These sleep disturbances are likely to be attributable to pathologies in the endogenous rhythm generator of the suprachiasmatic nucleus (SCN) or in sleep promoting and arousing brain regions. To investigate the cellular correlates for disturbed sleep/wake rhythms in neurodegenerative diseases, we used the gracile axonal dystrophy (gad) mouse with a mutation in the Park 5 gene. Methods: Locomotor activity of young (4-12 weeks) and old (14-20 weeks) gad mice and wildtype littermates (WT) kept in constant darkness (DD) or under 12 h light and 12 h darkness (LD) was analyzed using infrared detectors. In addition, the number of orexin-A positive neurons in the lateral hypothalamus (LH), a brain region controlling the switch between sleep and wake, was analyzed by immunohistochemistry. Results: Gad mice show a circadian rhythm of locomotor activity in DD similar to WT, demonstrating that the endogenous rhythm generator in the SCN is intact. However, old gad mice showed an increased (subjective) daytime-activity in DD and in LD, suggesting that the circadian control of the sleep/wake cycle distal from the SCN is impaired. In the LH of old gad mice, the number of orexin-A neurons was reduced as compared to young gad and old WT mice. Conclusions: (a) The gad mouse is an excellent model to study neurodegenerative disturbances in the sleep/wake cycle. (b) Neurodegeneration of orexin-A neurons in the LH might explain the instability of the circadian sleep/wake rhythm in gad mice.

Impact of calorie restriction or a mimetic (resveratrol) on daily rhythms of locomotor activity in a non-human primate

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Purpose Since calorie restriction (CR) extends lifespan and ameliorates many of the age-related diseases in short living mammals, we hypothesised that it could act on evolution of rhythms during aging and more particularly locomotor activity (LA). In the context of a long-term study of CR during aging, we aim to follow the effects of CR on LA during 2 years. We also investigate the effects of a mimetic of CR, resveratrol, a polyphenol found in grape. Methods Mouse lemurs are nocturnal primates with marked variations of daily rhythms. They are much appropriated to study rhythms variations during aging. Indeed, aging in the mouse lemur is accompanied with decreased amplitude in the LA daily rhythm. Animals are fed 3 different diets: control (CTL), calorie restricted (CR) and the CTL diet supplemented with resveratrol (RES). Animals were included in the study at the age of 3 years and have

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reached their half-life after 2 years of diet. LA of summer acclimated animals (light/dark 14/10) was monitored during 14 days after 1 and 2 years of treatment, using laboratory-made LA cages. LA results are correlated to basal metabolism data. Results: CR and RES diets significantly increase night LA, compared to animals fed the CTL diet. We observed an effect of age on the repartition of night LA: the 2nd y. CTL animals have a decreased LA at the end of the night phase compared to 1st y. This effect is not significant in RES animals, and is even inversed with the CR diet. Indeed, 2nd y. CR animals have an increased locomotor activity at the end of the night phase compared to 1st y. Basal metabolism of CR animal is significantly lower to CTL animal. Conclusions Our data demonstrate a stimulatory effect of CR and RES on LA with the first evidences of an amelioration of aging effects in our model. Decreased basal metabolism could be the direct consequence of an adaptive mechanism to CR.

Circadian time effects of general (propofol) anesthesia on hippocampus phosphorylation of Extracellular Regulated Kinase (ERK) in rats

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We previously demonstrated a reciprocal interaction between general (propofol) anesthesia and circadian rhythms in both animal model and patients. The circadian profile of phospho erk in hippocampus appeared to sustain some of the memory processes. Purpose: To characterize the effects of general (propofol) anesthesia on phosphorylation of ERK1/2 in hippocampus at two different circadian times. Methods: Male rats received either an intra peritoneal injection of 120 mg/kg of propofol to set a short-duration anesthesia state (20-30 minutes) or the equivalent amount of the lipidic solvent. For both groups of animals, anesthesia or control, the injections were performed either at CT0 or at CT12. One hour following the injection, the animals were euthanized, the brains removed, and immediately frozen. The amount of phospho erk was assessed using immunohistochemistry on brain slides. Results: We observed no modifications of phosphoERK immunostaining in prefrontal cortex, and striatum areas in anesthetized animals as compared to controls, whatever the time of injection CT0 or CT12. However the amount of phosphoERK density was significantly decreased (by about 40-60%) in CA1, CA2 and CA3 areas of the hippocampus, but only when anesthesia was performed at CT12. Conclusions: The persistent amnesic effect observed several hours following propofol anesthesia is considered now to rely on an hippocampus dysfunction that lasts. Our current results evidenced that the impact of propofol anesthesia on hippocampus may vary depending of the circadian time, suggesting a circadian effect not only for its anesthetic properties but also for its effects on memory processes.

Neuroprotective effects of pineal hormones in lead-induced neurotoxicity

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Purpose: To understand the lead-induced neurodegenerative processes in the rat circadian system. Methods: twelve Wistar male rats were reared at an age of 4 weeks, under photoperiodic conditions of 14:10 light–dark cycle and constant room temperature. The animals were divided into four groups (controls, lead acetate-treated [10mg/kg], lead acetate-treated + melatonin solution (Mel; 25µg/kg), lead acetate-treated + 5-Metoxytryptophol solution (5-ML; 25µg/kg). At the end of treatment (5 weeks) rats received a photic flash of 15 min, one hour later, animals were perfused and the brains removed for vasopressin and Fos immunochemistry. Results: Lead intoxication decreases the number of cells expressing c-fos induced in the SCN of treated rat when compared with the controls. In lead-treated animals we also obtained a reduction in the number of immunoreactive VP cells in the SCN. It clearly appeared that melatonin and 5-metoxytryptophol treatments restored the number of VP and fos immunoreactive cells in lead-treated groups, suggesting a neuroprotective effect of pineal hormones upon cell population of the SCN neurons. Conclusions: This finding demonstrats that the neurotoxic effects of lead involve the circadian system by reducing the activation of SCN as reflected in loss of c- fos and VP immunolabelling. This study suggests that melatonin and 5-metoxytryptophol have the potential to attenuate some aspects of lead neurotoxicity.

Effect of sunlight exposure in the morning on adolescent sleep-wake cycle and sleepiness

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Purpose: To evaluate the effect of sunlight exposure in the morning on the sleep-wake cycle (SWC) and sleepiness of adolescents in the return to the classes after mid-year vacation. Methods: 83 high school students volunteers aged 15 + 0.5 were enrolled (63% girls). Participants took part in 2 stages: 1a) Characterization about chronotype, sleep knowledge and habits; 2a) SWC and sleepiness evaluation for two consecutive weeks after mid-year vacation. The SWC was assessed by sleep log and the sleepiness levels were evaluated by the Karolinska Sleepiness Scale (KSS), on 5 times: wake-up time, 8h, 11h, 14h and bedtime. Besides, subgroups (n=14) underwent a psychomotor vigilance test on weekdays at 8h and 11h. At the second week after mid-year vacation the sunlight intervention group (n=45) attended the first class (7:15-8:00 h) at shadow in an outdoor environment exposed to sunlight (11.500 + 4.800 lux) while the control group (n=38) stayed in usual classroom (256 + 96 lux). Results: The intervention group anticipated the bedtime from 23h48min to 23h25min ($p<0.05$) and increased sleep duration in 16 minutes on weekdays ($p<0.05$). The sleepiness levels, measured by the KSS, showed no difference between the two weeks for both groups ($p>0.05$), but the PVT detected a decrease in reaction time at 11hs in the intervention group ($p<0.05$). Naps frequency diminished on weekdays from 46% to 31% on the intervention group ($p<0.05$). Irregularity remained for bedtime ($p>0.05$), but decreased to wake-up time for both groups due to the timing of classes' start in the morning ($p<0.05$). Conclusion: Sunlight exposure in the morning advanced the SWC and increased

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alertness at 11h. Thus, this intervention can be used as a school procedure to help students to adapt their sleep-wake cycle on the return to school after vacation.

Disruption of circadian rhythms: chronic constant light and a night-work model result in depressive like behaviors

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Purpose: To confirm that a disruption of circadian rhythmicity leads to depressive behavior. Methods: Three experimental groups of adult Wistar rats were kept in different lighting conditions for 6 weeks: constant light (LL), constant darkness (DD) to produce a condition of external desynchronization, and the last one in a regular 12:12 light dark cycle LD submitted daily to forced activity during the sleep phase to produce a condition of night-work and internal desynchrony (NW). A control group was kept in normal LD conditions. To evaluate depressive-like behaviors we assessed locomotor activity, body weight, food intake, anhedonia related with sucrose ingestion and open field activity. Animals were tested for a base line and in two moments during the experimental conditions (3 and 6 weeks). Blood samples were obtained for measuring plasma corticosterone and brains were removed for c-Fos immunohistochemistry. Results: Chronic LL and NW groups showed anhedonic behavior in the sucrose test, increase grooming and only NW exhibited less central stops as a stress response in the open field. In the LL group, high levels of corticosterone concentration were found; diminished food intake and a suppression of SCN activity. Experimental conditions indicate that chronic constant light and the night-work model produce disturbance of circadian rhythms and depressive-like behaviors, showing that this could be reliable rodent models to study depression and its relationship with circadian rhythms.

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Detrimental effects on adult lifespan and reproduction of the German cockroach by rapid phase-shifting

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Purpose: To investigate the effects of desynchronization between internal clocks and environment time signals on well-being of organisms. Longevity, reproduction and locomotor activity of the German cockroach were monitored as the indicators for long-term effect of circadian timing desynchronization by rapid phase-shifting schedules. Methods: With a 12 h light-dark reversal in every 3 or 7 days schedule was used to monitor the adult lifespan and female fecundity. Mating successful rate of males was tested by adults from a phase-shifting schedule paired with normal schedule ones. Locomotion of males was detected continuously under no phase-shifting condition thereafter switched into phase-shifting condition. Results: The longevity of male cockroaches was significantly reduced 6~26% by rapid phase-shifting. Furthermore, the shorter interval between phase-shifting caused the higher reduction on lifespan. The females did not show reduction on lifespan and offspring under the 7 days phase-shifting schedule. However, the 3 days phase-shifting schedule did cause lifespan and offspring reduction of females, even though the effect was significantly smaller than that of males. Mating successful rate of males was significantly affected by previously experienced phase-shifting. Rapid phase-shifting significantly increased daily activities, especially the activities during photophase. In addition, the elevation of daily locomotion coincided with the shorten lifespan. Conclusions: Rapid phase-shifting causes detrimental effects on longevity and reproduction of the German cockroach. But, it induced different levels of impact on males and females, suggesting the existence of sexual dimorphism in circadian regulation. The rapid phase-shifting promoted activity rate may act as a major factor to reduce lifespan by increasing metabolic rate.

Circadian rhythms of melatonin, blood pressure, heart rate and per2 expression in rats subjected to rotating shifts

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Purpose: Shift work may increase incidence of cardiovascular diseases but underlying mechanisms are not understood and animal studies are needed. Methods: Rats were housed at control 12L (light):12D (dark) regimen or placed on 12L:12D with the darktime prolonged by 8 h (phase delay) every second day. In the first trial rat males (36) were exposed to the shifts and the second half (36) was control. Blood pressure (BP) was measured non-invasively weekly and after 10 weeks rats were killed in regular 4h intervals over 24h cycle when the LD regimen was identical in control and experimental group. Melatonin and selected metabolites were measured in plasma and per2 expression in the heart. In the second trial older female rats (14 months) were kept on the shifted LD regimen. They were implanted with transmitters and BP, HR and locomotor activity (LA) was measured in freely moving rats for 7 weeks by telemetry (Data Sciences). Results: Melatonin rhythm was recorded in both control and shifted group indicating that the central circadian oscillator was able to entrain to the 8h phase delay occurring in short rotation shifts. Melatonin rhythm in the shifted group had a shorter period of increased nighttime concentrations. Per2 exerted rhythmic expression. Plasma creatinine was rhythmic in control but not in the shifted group. Shifts did not result in a BP increase. Continuous measurement of LA, BP and HR showed that all parameters were arhythmic during the first week after shift initiation and free-running afterwards with the period of 27h. Conclusions: Results suggest that in the environment without stressors the shifted LD did not increase BP. Data indicate that lack of prediction of stressful conditions related to shift work and unexpected stress can be responsible for cardiovascular diseases associated with rotating-shift work.

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Society for Research on Biological Rhythms

Meetings

SRBR 12th Biennial Meeting

May 22, 2010 - May 26, 2010

The Society for Research on Biological Rhythms was formed to promote the advancement of basic and applied research in all aspects of biological rhythms, to disseminate the important results of that research among scientists, to the agencies that fund research and to the general public, to enhance the education and training of students and researchers in the field and to foster interdisciplinary communication. Biennial meetings provide an environment for the exchange of ideas during scheduled scientific sessions, as well as during informal gatherings.

The SRBR meeting in 2010 will be held at the Sandestin Golf and Beach Resort. All conference activities will take place at the Baytowne Conference Center in the Sandestin Beach and Golf Resort.

http://www.srbr.org/Pages/SRBR_Meeting.aspx

Conference Contact Information Michelle Chappell Conferences & Institutes University of Illinois at Urbana-Champaign Phone: 217-333-2880Fax: 217-333-9561srbrconf@ad.uiuc.edu

26th Conference of the I.S.C.
Vigo, Spain; July 5-9 2010

Conference

- July 5, 2010: first day of conference
- July 9, 2010: last day of conference, gala diner

Abstract submission

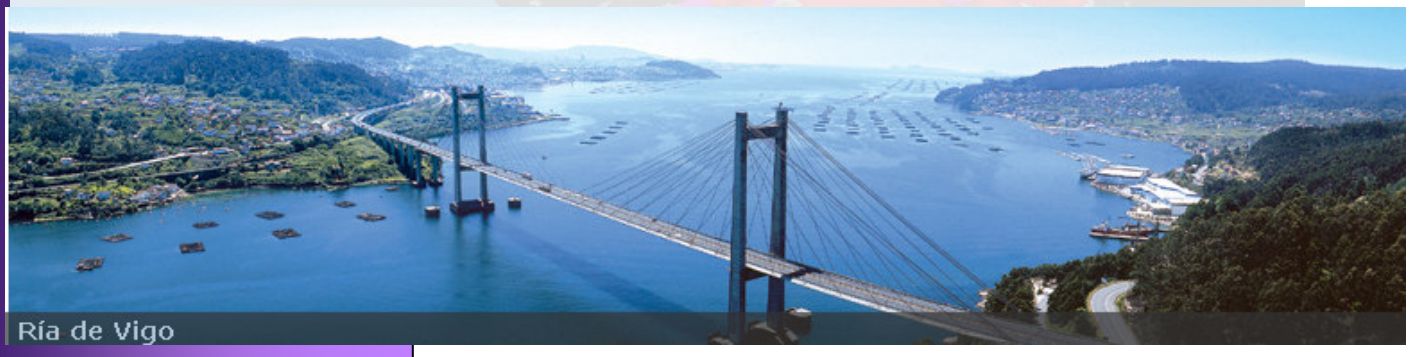
- December 1, 2009: on-line submission opens
- February 28, 2010: submission closes
- May 31, 2010: final decision on abstract acceptance

Registration information

- December 1, 2009: on-line registrations opens
- April 15, 2010: discount registration ends
- May 31, 2010: on-line registrations ends

The **26th Conference of the International Society for Chronobiology (ISC)** will be held at the E.T.S.I. Telecomunicación, Campus Universitario, University of Vigo, Vigo (Spain) on July 5-9, 2010.

http://webs.uvigo.es/isc2010/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Chronobiologistes…

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Vous devez participer à la vie de la Société Francophone de Chronobiologie en envoyant vos contributions à Fabienne Aujard, rédactrice en chef de Rythmes

Seules sont acceptées les contributions sous forme informatique, textes et figures, noir et blanc et couleurs. Cela assure la qualité de ce qui est produit, d'autant plus appréciable si vous optez pour la lecture électronique, qui, elle, est en couleurs !

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Enfin, vous enverrez vos contributions par courrier électronique à fabienne.aujard@wanadoo.fr avec copie à jean-francois.vibert@upmc.fr et jacques.beau@inserm.fr.

Fabienne Aujard
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Société Francophone de Chronobiologie

Ont contribué à ce numéro

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