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RYTHMES

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Éditorial

Le thème de la journée grand public de l'Institut National du Sommeil et de la Vigilance était cette année « sommeil et rythme de vie ». Par ailleurs, le congrès annuel Sommeil s'est tenu à Marseille du 19 au 21 novembre 2009. Cette manifestation qui a réuni plus de 2000 participants était co-organisée par la Société Française de Recherche et de Médecine du Sommeil (SFRMS) et la Société de Pneumologie de Langue Française. Pour la première fois, à cette occasion, la SFC a parrainé une session intitulée «Chronobiologie, performances et sommeil» qui a été suivie par une assistance importante, malgré la tenue simultanée d'une session sur l 'apnée du sommeil.

Le programme que nous avions construit en commun avec la SFRMS impliquait Kurt Krauchi, de l'équipe d'Anna Wirz-Justice (thermorégulation et rythme veille-sommeil), Antoine Viola, ancien lauréat de la SFC (polymorphisme de *per3* et performance cognitive), et Patricia Franco de Lyon (retard de phase ou retard d'endormissement chez l'enfant et l'adolescent?). Cette dernière conférence qui a abordé un problème majeur de société a fait la manchette du Quotidien du Médecin (voir plus loin le résumé dans Rythmes).

La chronobiologie était aussi présente dans 2 ateliers de formation des praticiens du sommeil consacrés à « médecine du travail » et « chronothérapie, photothérapie et mélatonine » animés par des membres de la SFC, et une session de communications orales.

L'une de ces communications, issue du groupe d'Isabelle Arnulf de Paris, était consacrée à l'influence des matines sur le sommeil et les rythmes chez les moines cloîtrés. Si les matines ne constituent pas un zeitgeber que nous prenons en compte dans nos protocoles traditionnels de chronobiologie, elles ont des conséquences comportementales importantes. Dans la pratique religieuse des moines cloîtrés, les matines ou messes du matin se situent fort tôt dans la nuit (0h15 à 2h15) et vont donc interrompre le sommeil. Les clercs séculiers doivent eux aussi réciter les matines, mais à l'heure qui leur convient, pour le bonheur des quelques fidèles courageux.

Cette communication nous a appris que les moines les plus adaptés à cette pratique ont un chronotype du matin. Néanmoins pour être présents à l'office, tous les moines ont besoin de plusieurs réveille-matin (e?), ce qui semble justifier le vieil adage populaire « être étourdi comme le premier coup de matines ».

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Les moines se couchent tôt (vers 20h), avec une difficulté d'endormissement et un temps de sommeil diminué (moins une heure), comparé aux sujets contrôles. Pendant les matines qui se déroulent dans une quasi obscurité (moins de 30 lux), les moines ne sont pas somnolents. Par contre, ils ont la sensation d'un sommeil moins rafraîchissant au matin et d'un moins bon fonctionnement intellectuel diurne. Leur courbe de température interne est biphasique, avec une interruption de la chute vers 23h qui anticipe le réveil des matines, puis présente une rechute avant la fin de la messe. Les moines cloîtrés constituent donc un modèle chronobiologique particulièrement intéressant.

De façon plus générale, au vu de l'intérêt montré au cours de différentes manifestations en 2009, la chronobiologie constitue désormais un point de rencontre incontournable de plusieurs disciplines, dont la santé publique. Notre Société se doit de renforcer ces interactions.

Bruno Claustrat
Président

In Memoriam Bernard Metz 1920 – 2009

Bernard Metz était un homme de grande stature, morale et scientifique.

Durant la guerre, il se consacra très activement, depuis 1943, à l'organisation clandestine du Groupement Mobile d'Alsace, dont les maquis constituèrent la Brigade Alsace-Lorraine ; c'est lui qui fit choisir André Malraux comme colonel de cette Brigade qui, intégrée à l'armée régulière, participa aux combats de la libération de l'Alsace et de la Lorraine. Il était sous-lieutenant.

Bernard Metz était professeur de physiologie appliquée à la Faculté de Médecine de Strasbourg. Il avait fondé et dirigé le Centre d'Études bioclimatiques, laboratoire propre de physiologie humaine du C.N.R.S. : intéressé de longue date par les rythmes biologiques, il faisait partie de la Society for Biological Rhythms. L'énumération serait longue des diverses responsabilités qu'il avait assumées, concernant la physiologie du travail, les maladies professionnelles, l'alcoolisme, la fondation et la présidence de l'International Ergonomics Association, etc.

En avril 1967, S. Jerebzoff, A. Reinberg, Th. Vanden Driessche et moi-même avions décidé de créer un groupement des rythmobiologistes de langue française, et m'en avaient chargé. B. Metz avait accepté de figurer dans le comité de patronage de notre association. Quelque 400 chercheurs se sont déclarés intéressés par notre initiative. Il était essentiel de constituer un Conseil solide. Qui choisir ? Bernard Metz connaissait beaucoup de chercheurs ; j'avais mes entrées auprès de lui et je savais qu'il méritait toute confiance. Je suis allé le consulter avec ma liste de quatre cents noms ; il m'a donné d'excellents avis, nous permettant d'établir un Conseil équilibré et de faire démarrer notre société, le 23 janvier 1969. Dans le groupe d'initiative il connaissait et il avait approuvé bien sûr le nom d'Alain Reinberg ; en outre il avait conseillé Maurice Fontaine, Ivan Assenmacher, André Hugelin (les quatre premiers présidents de la société !), ainsi que, pour la psychologie expérimentale, Mme Geneviève Oléron, aux avis toujours précieux. (Cf. Jean De Prins, dir., « Contribution à l'histoire de la Chronobiologie », *Bull. Gr. Ét. Rythmes Biol.*, 21 (1) : 1-70). En toute discrétion, il nous avait puissamment aidés.

C'est au plan de l'amitié que je veux exprimer la tristesse que me cause le décès de Bernard Metz.

Lucien Baillaud

Vos coordonnées accessibles sur le site de la SFC

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Utilisez ce formulaire pour une première inscription ;

Modifiez vos données en ligne si nécessaire (voir page 123).

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

Comment actualiser ses coordonnées sur le site.

Si vous connaissez votre identifiant et votre mot de passe, aller dans **Espace membres** et entrer l'identifiant et votre mot de passe, puis suivre les instructions.

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Aller dans **Espace membres** et cliquer sur **Login/Mot de passe oublié?** ; on vous demande alors le mail sous lequel vous êtes enregistré, et vous recevrez alors votre identifiant et votre mot de passe.

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 2010, 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 €.

Je vous remercie pour votre fidélité et profite de cette période de fin d'année pour vous présenter mes meilleurs vœux pour 2010.

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La physiologie saisonnière sous un nouveau jour

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Résumé

Chez les espèces saisonnières, si la fonction de reproduction est organisée de façon si précise par la photopériode, c'est pour que les « petits » puissent voir le jour au meilleur moment de l'année. Question de survie. Chez les mammifères, on sait depuis longtemps que cette tâche revient au circuit photoneuroendocrine qui, par une libération savamment dosée de mélatonine, coordonne le fonctionnement des différentes fonctions saisonnières - dont la reproduction. A l'inverse, les substrats anatomiques et les mécanismes cellulaires par lesquels cette hormone module la physiologie saisonnière restent encore obscurs. Récemment, plusieurs gènes ont été catalogués dans l'encéphale de mammifères saisonniers comme étant régulés par la photopériode, et sont désormais suspectés de jouer un rôle actif dans la régulation de la biologie saisonnière. Nous dressons ici un bref portrait de plusieurs d'entres eux, et examinerons comment, sous l'impulsion de la mélatonine, ils opèrent pour réguler la reproduction saisonnière. Enfin, nous verrons comment ces nouvelles données placent une structure hypophysaire, la pars tuberalis, à l'interface de la mélatonine et des fonctions saisonnières.

Abréviations :

AA-NAT: arylalkylamine N-acetyltransferase ; AVPV: Noyau periventriculaire antéroventral ; Dio2: Déiodinase de type 2 ; Dio3: Déiodinase de type 3 ; FSH : Follicle-stimulating hormone ; GnRH : Gonadotropin-releasing hormone ; GPR54: G protein-coupled receptor 54, ou KISS1R ; KISS1R: Kisspeptin receptor, ou GPR54 ; LH : Luteinizing hormone ; MT1: Récepteur à la mélatonine de type 1 ; MT2: Récepteur à la mélatonine de type 2 ; PC: Photopériode courte ; PL: Photopériode longue ; PT: Pars tuberalis ; RFRP: RFamide-related peptide ; SCN: Noyaux suprachiasmatiques de l'hypothalamus ; T3: Triiodothyronine ; T4 Thyroxine ; TSH: Thyroid stimulating hormone.

1. Contrôle saisonnier de la reproduction: des photons, de la mélatonine, et… ?

La reproduction est une fonction hautement complexe, orchestrée par l'axe hypothalamo-hypophysaire. Lorsqu'ils sont excités de façon synchrone, une poignée (moins de 2000) de neurones hypothalamiques secrète du GnRH (gonadotropin-releasing hormone) de façon pulsatile dans le système porte hypophysaire, afin de stimuler la libération des hormones gonadotropes par l'adéno-hypophyse (luteinizing hormone - LH, et follicle-stimulating hormone - FSH). A leur tour, ces hormones exercent une action biologique sur les gonades, où elles promeuvent la gamétogenèse et stimulent la production des hormones stéroïdes sexuelles (testostérone, oestrogène, progestérone). Ces dernières contrôlent les caractéristiques sexuelles secondaires, modulent le comportement sexuel, et contrôlent l'activité de l'axe hypothalamo-hypophysaire en retour. L'activité de cet axe biologique n'est pas fixe, mais varie à plusieurs occasions : chez l'homme par exemple, il est activé de manière transitoire lors de la période péri-natale, puis devient quiescent jusqu'à ce que la sécrétion de GnRH augmente de nouveau à la puberté, pour permettre la maturation finale des gonades (Sisk & Foster, 2004; Clarke & Pompolo, 2005). Pour beaucoup d'autres espèces, des régulations saisonnières s'opèrent par ailleurs afin de restreindre temporellement l'activité de reproduction à une période donnée de l'année.

Chez les espèces saisonnières, le contrôle annuel de la fertilité permet aux petits de naître à la période la plus favorable de l'année, généralement quand les ressources sont les plus abondantes et les conditions de vie clémentes. Le plus souvent, les organismes saisonniers utilisent les variations de la durée du jour, ou photopériode, pour discriminer et anticiper les saisons à venir (Malpaux *et al.*, 2001). Chez les mammifères, un circuit dît « circuit photoneuroendocrine » décode la photopériode ambiante et contrôle la production de l'hormone mélatonine (Figure 1), qui coordonne alors le fonctionnement des fonctions physiologiques saisonnières (telles que la reproduction et la prise de poids) avec la photopériode (Pevet, 1988; Goldman, 2001; Malpaux *et al.*, 2001; Schwartz *et al.*, 2001; Simonneaux & Ribelayga, 2003).

Le décodage de la photopériode par le système photo-neuroendocrine nécessite un pacemaker circadien fonctionnel dans les noyaux suprachiasmatiques hypothalamiques (SCN). Bien que son fonctionnement se calque sur des cycles de 24h, cette horloge est dotée d'une grande plasticité et s'adapte à un large spectre de photopériodes, entraînant par

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là même l'activité des fonctions physiologiques qu'elle contrôle. Des données récentes indiquent que les changements saisonniers de photopériode affectent le fonctionnement de l'horloge circadienne de façon durable (Johnston, 2005), tant aux niveaux moléculaires (Vuillez *et al.*, 1996; Pevet *et al.*, 1997; Pevet *et al.*, 1999; Schwartz *et al.*, 2001; Tournier *et al.*, 2003; Sumova *et al.*, 2004; Hazlerigg *et al.*, 2005) qu'au niveau de l'activité électrique neuronale (Jagota *et al.*, 2000; Mrugala *et al.*, 2000; Meijer *et al.*, 2007; VanderLeest *et al.*, 2007).

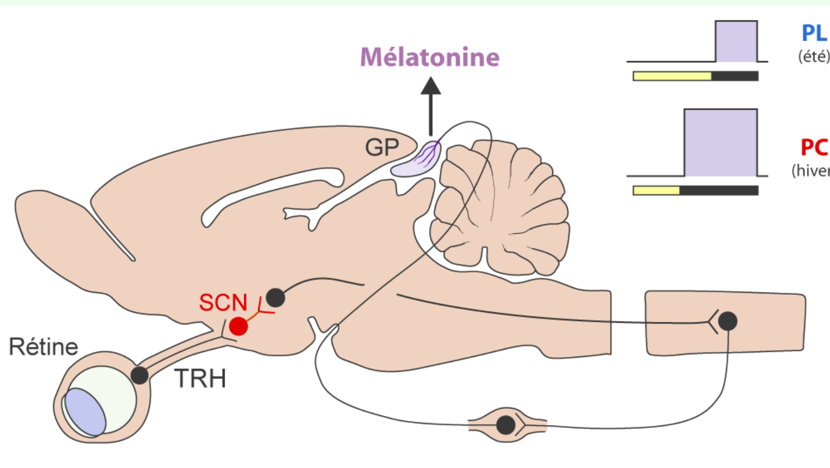

Figure 1. Le système photoneuroendocrine contrôle la libération rythmique de mélatonine. *Chez les mammifères, l'information lumineuse (alternance jour / nuit) est perçue par les photorécepteurs et les cellules ganglionnaires de la rétine. Leurs axones projettent, via le tractus retino-hypothalamique (TRH), vers les noyaux suprachiasmatiques de l'hypothalamus (SCN) où siège l'horloge circadienne principale. A leur tour, et grâce à un circuit nerveux complexe, les SCN contrôlent le rythme de synthèse de la mélatonine par la glande pinéale (GP). Les SCN restreignent la libération de cette hormone à la phase nocturne, de façon à ce que la durée de sa présence dans le sang et le cerveau varie avec la photopériode : en été, la photopériode longue (PL) est associée à une durée de libération courte, alors qu'en hiver, la photopériode courte (PC) est associée à un pic de mélatonine long (les barres jaunes et noires représentent le jour et la nuit).*

Les SCN sculptent le profil plasmatique circadien de diverses hormones (Kalsbeek *et al.*, 2006), en particulier celui de la mélatonine qui est l'hormone coordinatrice des fonctions saisonnières. Sous pilotage direct des SCN, la synthèse de mélatonine se limite aux périodes nocturnes, de telle manière que sa durée de libération varie directement avec la durée du jour (autrement dit, la durée de libération s'accroît avec les longues nuits de l'hiver…) (voir Figure 1) (Simonneaux *et al.*, 2002; Simonneaux & Ribelayga, 2003). Le profil de libération de la mélatonine fluctue donc annuellement, et c'est précisément cette information qui synchronise les fonctions physiologiques, telles que la reproduction, avec la photopériode ambiante. Alors que ce schéma est aujourd'hui bien établi, ce sont les cibles et mécanismes cellulaires décodant les signaux mélatoninergiques saisonniers qui restent flous (Malpaux *et al.*, 1999; Malpaux *et al.*, 2001).

La mélatonine est une petite molécule amphiphile produite par la glande pinéale, libérée dans la circulation générale et le liquide céphalo-rachidien dès qu'elle est synthétisée (Simonneaux & Ribelayga, 2003; Tricoire *et al.*, 2003). Des sites de liaison ont pu être identifiés par autoradiographie, avec l'utilisation de [^{125}I]-mélatonine comme radioligand. Alors qu'un grand nombre d'espèces a aujourd'hui été examiné, le nombre total des structures marquées se révèle particulièrement élevé : plus de 100 structures centrales et 30 structures périphériques semblent lier la mélatonine spécifiquement (Masson-Pevet & Gauer, 1994; Masson-Pevet *et al.*, 1994; Morgan *et al.*, 1994; Morgan & Mercer, 1994). Pourtant, de grandes différences existent entre les espèces, et seulement 2 structures sont invariablement identifiées: les SCN et la *pars tuberalis* de l'adénohypophyse (PT). Chez les mammifères, deux récepteurs à la mélatonine à haute affinité, de type récepteur couplé aux protéines G, ont pu être décrits : MT1 et MT2 (Masson-Pevet & Gauer, 1994; Morgan *et al.*, 1994; Morgan & Mercer, 1994; Reppert *et al.*, 1994; Reppert *et al.*, 1995; Barrett *et al.*, 2003; Witt-Enderby *et al.*, 2003). Une troisième classe de site de liaison de plus faible affinité, dite MT3, a aussi été purifiée et correspond à la quinone réductase 2 (Nosjean *et al.*, 2000; Nosjean *et al.*, 2001).

La manière dont les cellules cibles de la mélatonine interprètent les fluctuations photopériodiques de son signal, puis les utilisent pour induire des modifications physiologiques, reste encore mystérieuse. Jusqu'à présent, aucun locus cérébral entièrement dédié au contrôle des fonctions saisonnières n'a été caractérisé, à l'inverse de ce que représentent les SCN vis-à-vis des fonctions circadiennes. Des études de lésion indiquent que le temps saisonnier se mesure probablement dans différentes zones du cerveau, et l'on peut supposer qu'au moins certaines d'entres-elles disposent de sites de liaison à la mélatonine. Mais hormis les SCN et la PT, les sites de liaisons semblent différer de manière très importante entre les espèces (Masson-Pevet & Gauer, 1994; Masson-Pevet *et al.*, 1994; Morgan *et al.*, 1994; Morgan & Mercer, 1994). Deux handicaps majeurs font obstacles à la caractérisation des récepteurs à la mélatonine : 1) hormis pour la PT où les niveaux d'expression sont plus

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importants (Klosen *et al.*, 2002; Dardente *et al.*, 2003), les récepteurs à la mélatonine sont exprimés à de très faibles niveaux, et l'utilisation de techniques comme l'hybridation in-situ ne peut pas être envisagée ; 2) l'absence d'anticorps spécifiques dont le développement, techniquement difficile, n'a jamais été entièrement satisfaisant pour les modèles saisonniers tels que le hamster ou le mouton. Chez l'Homme, en revanche, un anticorps reconnaissant spécifiquement le récepteur MT1 a pu être décrit et utilisé pour des études d'immuno-localisation (Brydon *et al.*, 1999; Savaskan *et al.*, 2001; Savaskan *et al.*, 2002; Wu *et al.*, 2006).

Etablir où et comment la mélatonine contrôle les fonctions saisonnières n'est donc pas tâche facile. A l'approche « classique », consistant à localiser les

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Tableau 1. Principaux clusters photopériodiques.

Chaque cluster représente une zone anatomique où un ou plusieurs gènes sont exprimés différentiellement en fonction de la photopériode chez les principaux modèles saisonniers mammifères et aviaires : Caille japonaise (*CJ*), hamsters Syrien (*Syr*) et Sibérien (*Sib*), mouton (*M*), souris (S), rat (R). Pour chaque gène, des espèces chez lesquelles une régulation photopériodique a été démontrée sont indiquées. Le numéro dans la colonne référence renvoie aux références bibliographique correspondantes situées au bas du tableau.

Zone anatomique	Gènes photopériodiques	Espèce	Réf.
Pars tuberalis (PT)	- MT1 - TSHb	M, Syr, Sib M, S, CJ	1 2
Plancher du troisième ventricule / éminence médiane (tanycytes)	- TGFa - Dio2 - Dio3 - Crbp1 - Gpr50 - Nestin	CJ CJ, M, R, S, Sib, Syr CJ, S, Sib Sib, Syr Sib Sib	3 4 5 6 7 7
Région périventriculaire médiane aux noyaux hypothalamiques dorsomédians (DMH) – [Oiseaux : noyaux paraventriculaires (PVN)]	- Rfrp / GnIH	CJ, M, Sib, Syr	8
Noyau arqué (Arc)	- Kiss1 - PC2	M, Sib, Syr Sib	9 10
Aire périventriculaire antéroventrale (AVPV)	- Kiss1	Sib, Syr	11
Région postérieure dorso-médiane du noyau arqué (dmpArc)	- Crbp1 - Crabp2 - Rar(s) - RXRg - H3R - VGF - SOCS3	Sib, Syr Sib Sib Sib Sib, Syr Sib, Syr Sib	6 6 6 6 12 12 13
Plexus choroïde	- Transthyrétine	Sib	14

Abréviations : *Crabp2* : Cellular retinoic acid binding protein 2 ; *Crbp1* : Cellular retinol binding protein-1 ; *Dio2* : Déiodinase de type 2 ; *Dio3* : Déiodinase de type 3 ; GnIH : Gonadotropin-inhibiting hormone ; Gpr50 : G protein-coupled receptor 50 ; *H3R* ; Recepteur à l'histamine de type 3 ; *MT1* : Récepteur à la mélatonine de type 1 ; *PC2* ; Proprotein converting enzyme 2 ; *Rar* : Retinoic acid receptor ; *Rfrp* : RFamide-related peptide ; *RXR* : Retinoid X receptor ; *TGFa* : transforming growth factor alpha; *TSHb* : sous-unité β de la TSH ; *VGF* : Nerve growth factor inducible.

Références : (1) (Gauer *et al.*, 1994; Recio *et al.*, 1998a; Recio *et al.*, 1998b; Piketty, 2001; Schuster *et al.*, 2001) ; **(2)** (Hanon *et al.*, 2008; Nakao *et al.*, 2008); **(3)** (Takagi *et al.*, 2007) ; **(4)** (Yoshimura *et al.*, 2003; Watanabe *et al.*, 2004; Yasuo *et al.*, 2005; Revel *et al.*, 2006b; Barrett *et al.*, 2007; Watanabe *et al.*, 2007; Yasuo *et al.*, 2007a; Yasuo *et al.*, 2007b; Hanon *et al.*, 2008; Ono *et al.*, 2008; Yasuo *et al.*, 2009) ; **(5)** (Yasuo *et al.*, 2005; Barrett *et al.*, 2007; Watanabe *et al.*, 2007; Ono *et al.*, 2008; Yasuo *et al.*, 2009) ; **(6)** (Ross *et al.*, 2004) ; **(7)** (Barrett *et al.*, 2006) ; **(8)** (Ubuka *et al.*, 2005; Dardente *et al.*, 2008; Revel *et al.*, 2008; Smith *et al.*, 2008) ; **(9)** (Revel *et al.*, 2006a; Caraty *et al.*, 2007; Greives *et al.*, 2007; Mason *et al.*, 2007; Revel *et al.*, 2007; Smith *et al.*, 2007; Greives *et al.*, 2008; Wagner *et al.*, 2008) ; **(10)** (Helwig *et al.*, 2006) ; **(11)** (Revel *et al.*, 2006a; Caraty *et al.*, 2007; Greives *et al.*, 2007; Mason *et al.*, 2007; Revel *et al.*, 2007; Smith *et al.*, 2007; Greives *et al.*, 2008; Wagner *et al.*, 2008) ; **(12)** (Barrett *et al.*, 2005; Ross *et al.*, 2005) ; **(13)** (Tups *et al.*, 2004; Tups *et al.*, 2006) ; **(14)** (Prendergast *et al.*, 2002).

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cibles de la mélatonine pour ensuite en comprendre l'action, s'est récemment associée une autre stratégie, dite « inverse » (Figure 2). Celle-ci propose d'identifier les cellules dans lesquelles l'expression de certains gènes est influencée par la photopériode (via la mélatonine), afin d'appréhender les processus moléculaires gouvernant les fonctions saisonnières et, peut-être, de rendre possible la localisation et le phénotypage des cellules sensibles à la mélatonine (Figure 2).

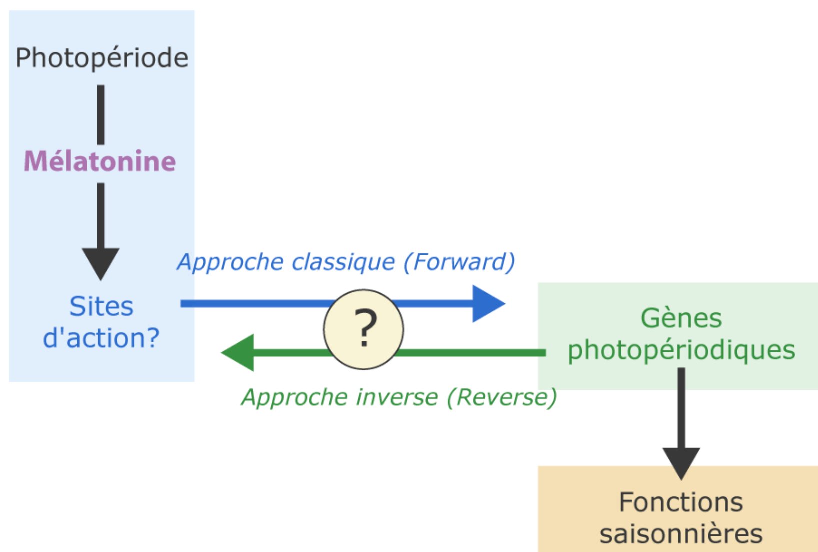

Figure 2. Des stratégies complémentaires pour comprendre comment la photopériode et la mélatonine contrôlent les fonctions saisonnières. *S'il est clair depuis plusieurs années que la mélatonine est indispensable pour le bon fonctionnement des fonctions photopériodiques, les cibles et mécanismes d'action de cette hormone sont restés obscurs. Pour résoudre cette énigme, deux types de stratégies existent. L'approche classique (ou Forward) a cherché à localiser les récepteurs de la mélatonine, afin de démasquer les processus moléculaires opérant plus en aval, et comprendre comment cette hormone contrôle les fonctions saisonnières. L'approche inverse (Reverse) se propose d'identifier des neurones dans lesquels l'expression de gènes est régulée par la photopériode (via la mélatonine), afin de comprendre les processus moléculaires opérant en amont (ou en aval), et de « remonter » jusqu'aux récepteurs de la mélatonine.*

Récemment, l'association de ces approches a permis de gros progrès dans le domaine de la physiologie saisonnière. Plusieurs gènes régulés par la photopériode et la mélatonine ont été identifiés dans l'hypothalamus de différents modèles saisonniers, mammifères et oiseaux, certains jouant des rôles importants dans le contrôle des fonctions métaboliques (Morgan *et al.*, 2006) ou de reproduction (Revel *et al.*, 2008; Revel *et al.*, 2009) (Tableau 1). En particulier, une évolution majeure a eu lieu dans le domaine de la biologie de la reproduction, avec la découverte et l'implication de la kisspeptine et du récepteur GPR54 (ou KISS1R) dans le contrôle de l'activité de l'axe hypothalamo-hypophysaire-gonadique. Il s'agit sans doute de l'avancée la plus importante dans ce domaine depuis la découverte du GnRH il y a 30 ans (pour revue, voir Gianetti & Seminara, 2008; Popa *et al.*, 2008; Roa *et al.*, 2009). Les kisspeptines, peptides issus du gène Kiss1, sont de puissants sécrétagogues du GnRH par l'action sur le récepteur GPR54. Des études récentes ont établi que ce système Kiss1/GPR54 joue un rôle clé dans le développement de la puberté (Popa *et al.*, 2008). Les neurones qui expriment Kiss1 dans le noyau arqué de l'hypothalamus sont aussi les principaux médiateurs du rétrocontrôle négatif des stéroïdes sexuels sur la sécrétion de GnRH, et ceux qui expriment Kiss1 dans le noyau périventriculaire antéroventral (AVPV) permettent la génération du pic pré-ovulatoire de GnRH / LH chez les rongeurs femelles. Les neurones à Kiss1 sont aujourd'hui établis comme les principaux intégrateurs des signaux internes et environnementaux régulant l'axe reproducteur (stéroïdes sexuels, facteurs métaboliques, etc…). Nous avons notamment démontré que le fonctionnement du système kisspeptinèrgique est aussi régulé par la photopériode, via la mélatonine (Revel *et al.*, 2006a), pour contrôler l'activité de l'axe sexuel sur un plan saisonnier (pour revue, Revel *et al.*, 2007; Simonneaux *et al.*, 2008; Mikkelsen & Simonneaux, 2009; Revel *et al.*, 2009).

Si toutes ces découvertes permettent aujourd'hui d'avancer dans la compréhension des mécanismes moléculaires de la biologie saisonnière, elles représentent des pistes sérieuses pour l'identification des structures nerveuses cibles (directes ou indirectes) de la mélatonine. Ces gènes sont exprimés dans différentes aires du cerveau, suggérant qu'un réseau de structures cérébrales (Tableau 1) plutôt qu'un seul centre nerveux assure la régulation de la reproduction saisonnière. La mélatonine agit-elle alors indépendamment sur chacune de ces aires cérébrales ? Un ensemble de résultats très récents vient contester cette idée et bouleverser certaines théories en places, suggérant que la mélatonine pourrait contrôler tous ces centres photopériodiques à distance. Voici donc quelques pièces du puzzle photopériodique qui se mettent place…

2. Une structure qui a plus d'un tour dans son sac : La pars tuberalis

Depuis longtemps, la PT est identifiée comme struc-

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ture clé pour les rythmes saisonniers (Morgan, 2000; Dardente, 2007; Duncan, 2007). Elle possède de nombreux atouts : c'est la structure qui présente la plus forte expression de récepteur MT1, elle exprime divers « gènes horloges », et module la sécrétion saisonnière de prolactine par l'adénohypophyse ; elle renferme même un oscillateur circannuel (Lincoln *et al.*, 2006). Mais de nouveaux résultats suggèrent qu'elle contrôlerait aussi les autres fonctions saisonnières, en relayant le message photopériodique véhiculé par la mélatonine à d'autres structures hypothalamiques. Cette section résume les différents travaux conduisant à cette nouvelle hypothèse.

2.1. Dio2 et Dio3 : Honneur aux cailles

Dans le cerveau, les déiodinases de type 2 et 3 (respectivement Dio2 et Dio3) sont les enzymes qui régulent les concentrations locales d'hormones thyroïdiennes T4 (thyroxine) et T3 (triiodothyronine). Ces hormones sont sécrétées par la glande thyroïde, majoritairement sous la forme T4, alors que T3 est la forme bioactive. T3 est alors produite localement par déiodination de l'anneau externe de la T4, réaction catalysée par Dio2 dans le cerveau. A l'inverse, la déiodination de l'anneau interne de T4 ou T3 par la Dio3 rendent ces molécules inactives. C'est ainsi qu'indépendamment des niveaux d'hormones thyroïdiennes circulants, l'action coordonnée de Dio2 et Dio3 permet un ajustement local des concentrations en T3 et T4 (Bianco *et al.*, 2002; Lechan & Fekete, 2005). Depuis longtemps déjà, les hormones thyroïdiennes sont suspectées de participer au contrôle des processus saisonniers (Karsch *et al.*, 1995; Dawson *et al.*, 2001). Elles permettent par exemple l'involution des gonades pendant la phase photoréfractaire chez les oiseaux et le mouton (Nicholls *et al.*, 1988; Moenter *et al.*, 1991; Webster *et al.*, 1991; Dahl *et al.*, 1995; Karsch *et al.*, 1995; Dawson *et al.*, 2001). Mais depuis peu, l'implication de Dio2 et Dio3 dans le contrôle de la reproduction saisonnière se précise encore.

Chez la caille japonaise, organisme modèle dans l'étude des rythmes annuels, le passage d'une photopériode courte (PC) à une photopériode longue (PL) stimule l'activité de l'axe hypothalamo-gonadotrope (Dawson *et al.*, 2001; Yoshimura, 2006). Des travaux récents montrent que cette modification physiologique s'accompagne d'une augmentation rapide de l'expression du gène de la Dio2, dans l'hypothalamus médiobasal (Yoshimura *et al.*, 2003; Yoshimura, 2006; Watanabe *et al.*, 2007), structure clé pour la mesure du temps photopériodique chez les oiseaux (Dawson *et al.*, 2001; Yoshimura, 2006). Peu de temps après, une diminution concomitante de l'expression du gène de la Dio3 a été mise en évidence dans la même structure (Yasuo *et al.*, 2005; Yoshimura, 2006; Watanabe *et al.*, 2007). Cette modulation coordonnée des expressions de Dio2 et Dio3 se traduit par des niveaux hypothalamiques de T3 augmentés en PL et diminués en PC, alors même que le taux plasmatique de T4 reste inchangé (Yoshimura *et al.*, 2003; Yoshimura, 2006). Logiquement, une infusion centrale de T3 chez des animaux maintenus en condition inhibitrice (PC) permis de mimer les effets de la PL sur la croissance testiculaire (Yoshimura *et al.*, 2003; Yoshimura, 2006). Cet ensemble de données suggère ainsi que chez les oiseaux, le contrôle de la conversion photopériodique de T4 en T3 par Dio2/3 dans l'hypothalamus médiobasal est une étape critique pour la modulation saisonnière de l'activité sexuelle.

2.2. Dio2 et Dio3 : Aussi chez les mammifères photopériodiques…

Chez les mammifères saisonniers, des variations photopériodiques d'expression de Dio2 et Dio3 ont aussi été observées. Chez les rongeurs, ces enzymes sont exprimées dans les tanycytes, cellules gliales spécialisées logés dans la couche de cellules épendymales du plancher et des murs infra-latéraux du troisième ventricule, ainsi que dans la zone acellulaire au dessus des sulci tubero-infundibulaires (Tu *et al.*, 1997; Tu *et al.*, 1999; Diano *et al.*, 2003; Lechan & Fekete, 2005). Plusieurs groupes de travail ont observé des variations photopériodiques d'expression des déiodinases chez le hamster Syrien (ou hamster doré) et le hamster Sibérien, deux modèles établis pour l'étude des phénomènes photopériodiques. Chez le hamster Syrien, l'expression de *Dio2* est maximale en PL et diminue fortement en PC, alors que l'ARNm de Dio3 n'est pas détecté chez cette espèce (tout du moins dans cette zone cérébrale), tant en PL qu'en PC (Revel *et al.*, 2006b; Barrett *et al.*, 2007). Chez le hamster Sibérien, en revanche, Dio3 est exprimé faiblement en PL et augmente significativement en PC. Pour Dio2, le niveau d'expression se maintiennent à des niveaux relativement constants en PL et en PC, (Watanabe *et al.*, 2004; Barrett *et al.*, 2007; Watanabe *et al.*, 2007). Chez les 2 espèces de hamster, les régulations différentielles de Dio2/3 dépendent directement de la mélatonine, qui peut modifier l'expression de ces gènes en très peu de temps : moins de 7 jours d'injections journalières de mélatonine inhibent l'expression de Dio2 chez le hamster Syrien

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(Revel *et al.*, 2006b). Enfin chez le mouton, modèle saisonnier dit de « jours courts » car sexuellement actifs en PC, l'expression de Dio2 est aussi nettement augmentée suite à un transfert de PC en PL (Hanon *et al.*, 2008). Chez les mammifères saisonniers comme chez la caille, la photopériode module donc aussi l'expression de Dio2 et/ou Dio3 de manière coordonnée, contrôlant ainsi la concentration de T3 dans l'hypothalamus médiobasal.

2.3. Et « non-photopériodiques »…

L'expression photopériodique de Dio2 et Dio3 à ensuite été examinée chez d'autres modèles. Chez le rat Wistar de laboratoire, la transcription de Dio2 apparaît insensible à la photopériode, tout comme peut l'être l'activité de reproduction (Revel *et al.*, 2006b; Yasuo *et al.*, 2007a). En revanche, des travaux effectués sur la souche de rat Fischer F344 indiquent que l'expression de Dio2 et l'activité testiculaire sont toutes deux inhibées par la PC (Yasuo *et al.*, 2007a). De façon surprenante, des injections journalières de mélatonine seraient suffisantes pour réduire les taux d'ARNm de Dio2, non seulement chez les rats Fischer F344, mais aussi chez les rats Wistar (Yasuo *et al.*, 2007a). Cela signifierait que chez les deux souches de rat, la concentration locale de T3 peut être modulée par la mélatonine via la modification de l'expression de Dio2, mais qu'en aval, les évènements contrôlés par T3 diffèrent. Si ces résultats sont confirmés, ils montreraient que le rat pourrait être utilisé plus largement pour l'investigation des mécanismes d'action de la mélatonine, ce qui présenterait certains avantages (génome entièrement séquencé, possibilités de manipulations génétiques, cerveau de taille supérieure à celui des hamsters).

Tout récemment, des résultats similaires ont été obtenus chez la souris de laboratoire (Ono *et al.*, 2008; Yasuo *et al.*, 2009). Ces rongeurs ne sont habituellement pas classés dans la liste des espèces saisonnières. Cela tient en partie au fait que la plupart des souches de laboratoire ne montrent pas de signes évidents de modifications saisonnières (reproduction, masse corporelle, etc) et ne produisent pas (ou peu) de mélatonine, à cause d'une mutation de l'enzyme arylalkylamine N-acetyltransferase (AA-NAT) (Roseboom *et al.*, 1998; Simonneaux & Ribelayga, 2003). Chez la souche CBA/N, qui produit de la mélatonine, il a toutefois été montré que l'expression de Dio2 et Dio3 change avec la photopériode (Ono *et al.*, 2008): en PL, les taux d'ARNm sont élevés pour Dio2 et réduits pour Dio3, cette situation s'inversant en PC. En toute logique, cette réponse à la photopériode n'est pas observée chez la souche C57Bl/6J, puisqu'elle ne produit pas de mélatonine. Malgré tout, et de façon inattendue, elle a pu être reproduite à l'aide d'un protocole d'injections journalières de mélatonine exogène, la mélatonine inhibant l'expression de Dio2 et augmentant celle de Dio3 (Ono *et al.*, 2008). Certaines souches de souris de laboratoire s'avèrent donc potentiellement capable d'encoder les informations photopériodiques en réponses neuroendocrines au niveau de l'hypothalamus médiobasal. Par ailleurs, et contrairement à ce que l'on avait pu croire jusqu'ici, ces données suggèrent que la souris représente un modèle tout à fait approprié pour l'étude des mécanismes moléculaires du photopériodisme chez les mammifères. Il s'agit d'un formidable progrès pour ce domaine, qui souffrait du manque de modèle biologique « classique », notamment pour les études génétiques. La possibilité d'utiliser la souris comme modèle photopériodique accélèrera encore la compréhension des régulations neuroendocrines saisonnières.

2.4. Dio2 et Dio3 contrôlent T3

Tout comme chez les oiseaux, les variations photopériodiques coordonnées d'expression de Dio2 et Dio3 chez les mammifères se traduisent par une augmentation des niveaux locaux de T3 en PL, et une diminution en PC. Or, l'implantation de capsules de T3 dans l'hypothalamus médiobasal de hamsters Sibériens placés en PC permet par ailleurs de renverser certaines (mais pas toutes) des réponses photopériodiques (Barrett *et al.*, 2007). Les animaux traités restent sexuellement actifs et ne perdent pas de poids, au contraire des animaux contrôles. La régulation photopériodique de la mue, dont on sait qu'elle est régulée au niveau de la PT, est en revanche inchangée, indiquant qu'elle est indépendante de T3. De quelle manière T3 affecte l'activité testiculaire et la régulation de la masse corporelle est désormais la question à éclaircir. On sait que les hormones thyroïdiennes jouent un rôle dans le développement et la plasticité du cerveau (Bernal, 2002; Bianco *et al.*, 2002; Lechan & Fekete, 2005), et il a été proposé que les variations locales en T3 pourraient induire des modifications morphologiques des pieds tanycytaires au niveau de l'éminence médiane, modifications qui permettraient de moduler la libération de GnRH (Lee *et al.*, 1995; Viguié *et al.*, 2001; Kameda *et al.*, 2003; Yamamura *et al.*, 2004; Yamamura *et al.*, 2006; Yoshimura, 2006).

2.5. La TSH entre dans la danse - Encore les cailles

Une question reste en suspend : celle de savoir comment la mélatonine pilote les variations d'expression de Dio2/3. Des données très récentes semblent déjà apporter des éléments de réponse, et encore une fois, c'est la caille japonaise qui permet de débloquer la situation. Chez ce modèle, le transfert de PC en PL s'accompagne d'une rapide augmentation de l'expression du gène codant la sous-unité β de la TSH (thyroid stimulating hormone) dans la PT (Nakao *et al.*, 2008). La sous-unité α étant également synthétisée dans la PT, il est probable que de

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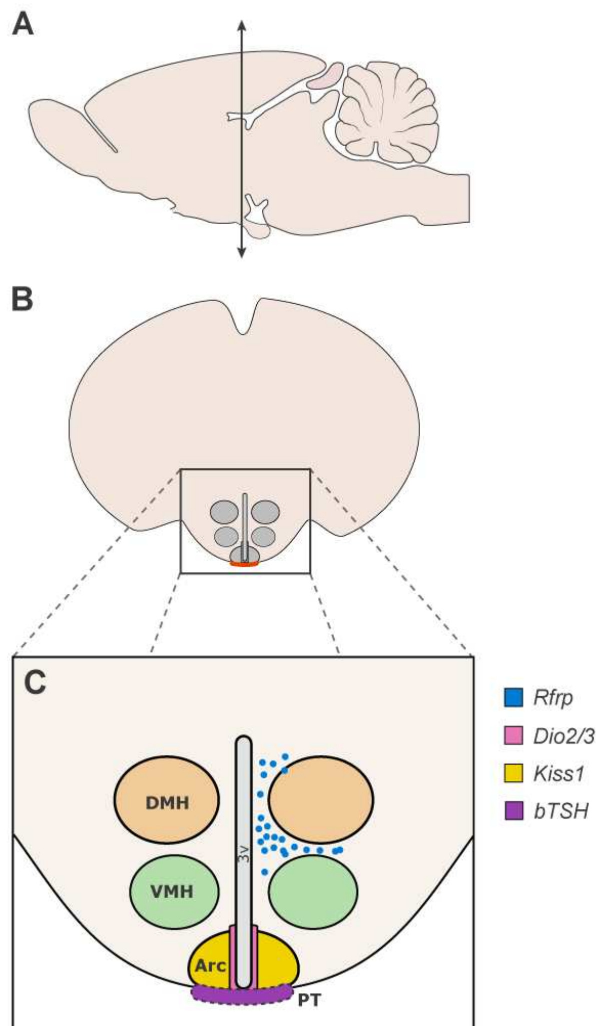

Figure 3. Localisation des neurones exprimant les principaux « gènes photopériodiques » associés à la reproduction saisonnière. A. *Schéma d'une coupe médio-sagittale d'un cerveau de rongeur. La double flèche identifie la position rostro-caudale de la coupe coronale présentée en B.* ***B.*** *Coupe coronale localisant l'hypothalamus (carré) et la pars tuberalis (PT ; trait rouge).* ***C.*** *Grossissement de la zone hypothalamique, où sont positionnés les noyaux ventromédians (VMH) et dorsomédians (DMH), le noyau arqué (Arc) et la PT. Des clusters photopériodiques sont définis par l'expression de « gènes sensibles à la photopériode », dont certains sont essentiels au fonctionnement de l'axe reproducteur : le gène codant les RFRPs (RFamide-related peptides), exprimé dans des petits groupes de neurones situés entre les DMH et les VMH (points bleus) ; les gènes codant les déiodinases de types 2 et 3 (Dio2/3), exprimés dans les tanycytes situés dans le plancher et les parois infra-latérales du troisième ventricule (3v) (zone en rose) ; le gène Kiss1 codant les kisspeptines, exprimé dans l'Arc (zone orangée) et le noyau périventriculaire antéroventral (AVPV, non localisé ici) ; le gène codant la sous-unité beta de la TSH (Thyroid-stimulating hormone ; b-TSH) est également exprimé de façon photopériodique dans la PT (violet), sous contrôle direct de la mélatonine.*

la TSH active soit alors formée localement. Nakao et al. ont alors montré que celle-ci interagit avec le récepteur à la TSH présent dans les cellules épendymaires à la base du 3ème ventricule, pour moduler l'expression de plusieurs gènes. Ainsi, une injection centrale de TSH à des animaux en PC permet, de façon dose-dépendante, de stimuler l'expression de Dio2 et de réprimer celle de Dio3, mimant l'effet d'un transfert en PL. A l'inverse, une infusion centrale d'anticorps anti-TSH à des cailles maintenues en PL inhibe l'expression de Dio2. Enfin, un traitement prolongé (2 semaines) à la TSH d'individus males en PC suffit pour réactiver l'activité testiculaire (Nakao *et al.*, 2008).

Peu de temps après la publication de ces résultats, des données similaires étaient rapportées chez le mouton (Hanon *et al.*, 2008): le transfert d'animaux de PC en PL augmente la synthèse de TSH dans les cellules de la PT sensibles à la mélatonine ; à son tour, la TSH stimulerait les cellules de l'hypothalamus mediobasal qui expriment de récepteur à la TSH pour induire l'expression de Dio2, et donc probablement élever les taux locaux de T3.

Ces résultats chez la caille et le mouton sont remarquables. Ils indiquent que la pars tuberalis est un locus bien plus important pour le contrôle de la saisonnalité que nous l'avions pensé. Cette structure contrôle non seulement la libération saisonnières de prolactine par l'hypophyse (Morgan, 2000; Dardente, 2007; Duncan, 2007), mais elle influence également l'expression de gènes hypothalamiques importants pour d'autres fonctions photopériodiques. Comme le soulignent Hanon et al., ce système s'oppose au modèle classique de Harris dans lequel l'hypothalamus contrôle l'activité de l'hypophyse antérieure par la libération de neuromédiateurs dans le système porte hypophysaire. Ici, un signal plasmatique (la mélatonine) est décodé par l'hypophyse (PT) pour contrôler la synthèse photopériodique d'une hormone hypophysaire (la TSH, et peut-être d'autres). A son tour, la TSH agit localement dans l'hypothalamus, où elle modifie la production de T3 dans les tanycytes pour moduler les fonctions physiologiques saisonnières. Au moins 2 questions restent à élucider : comment la mélatonine contrôle-t-elle la sécrétion de TSH par la PT, et par quels mécanismes la TSH transite-t-elle de l'hypophyse à l'hypothalamus ?

2.6. Le récepteur à la mélatonine MT1 : le retour

Dans un travail publié très récemment, Yasuo et al. ont examiné l'expression de Dio2 et Dio3 chez les souris C3H, qui synthétisent de la mélatonine (Yasuo *et al.*, 2009). Alors que l'expression de Dio2 dans les tanycytes y est constamment faible, celle de Dio3 est apparu augmenté en PC, rejoignant ainsi les résultats obtenus précédemment chez les sou-

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ris CBA/N (Ono *et al.*, 2008). Cependant, lorsque cette expérience est réalisée avec des souris C3H dont les récepteurs à la mélatonine MT1 sont invalidés (knock-out ; Liu *et al.*, 1997), la régulation photopériodique de Dio2/3 disparaît : les niveaux d'ARNm de Dio2 sont constitutivement élevés, ceux de Dio3 sont constitutivement réduits (Yasuo *et al.*, 2009). En revanche, ceci n'est pas le cas chez des souris dont le récepteur MT2 est invalidé (knock-out) (Jin *et al.*, 2003), où l'expression de Dio2 et Dio3 en PC est comparable à celle de souris sauvages dans les mêmes conditions. Enfin, chez des souris dont les récepteurs MT1 et MT2 sont tous deux invalidés (double knock-out; von Gall *et al.*, 2002; Jin *et al.*, 2003), le phénotypes est comparable à celui des souris mutantes pour MT1. Enfin, si des injections de mélatonine exogène à des souris C57Bl/6J (dépourvues de mélatonine endogène) en PL permettent de moduler l'expression de Dio2 et Dio3 (Ono *et al.*, 2008), ces effets sont abolis chez des ouris C57Bl/6J mutantes pour le récepteur MT1 (Yasuo *et al.*, 2009). Dans leur globalité, ces résultats indiquent donc que c'est par le récepteur MT1 que la mélatonine régule l'expression de Dio2 et Dio3 dans les tanycytes de l'hypothalamus médiobasal. Bien qu'une démonstration formelle soit encore requise, on peut légitimement supposer que les récepteurs concernés se situent dans la PT. Cette structure possèdent la plus grande concentration de récepteurs MT1 (voir chapitre 1), d'ailleurs co-localisés avec les cellules synthétisant la TSH (Klosen *et al.*, 2002). Par ailleurs, ces données confirment l'intérêt de l'utilisation de la souris comme modèle d'étude photopériodique, et notamment des souris génétiquement modifiées.

3. Régulation photopériodique de l'expression génique dans l'hypothalamus

En 10 ans, la liste des gènes « photopériodiques » dans le cerveau des modèles saisonniers s'est considérablement étoffée, essentiellement grâce aux progrès des techniques de criblages génétiques et moléculaires. Tous sont régulés par la mélatonine, directement ou indirectement. Leur expression tend à se concentrer dans un nombre restreint d'aires hypothalamiques, constituant ainsi des « clusters photopériodiques » (Figure 3). Le tableau 1 récapitule la répartition de ces clusters et leur contenu en gènes photo-

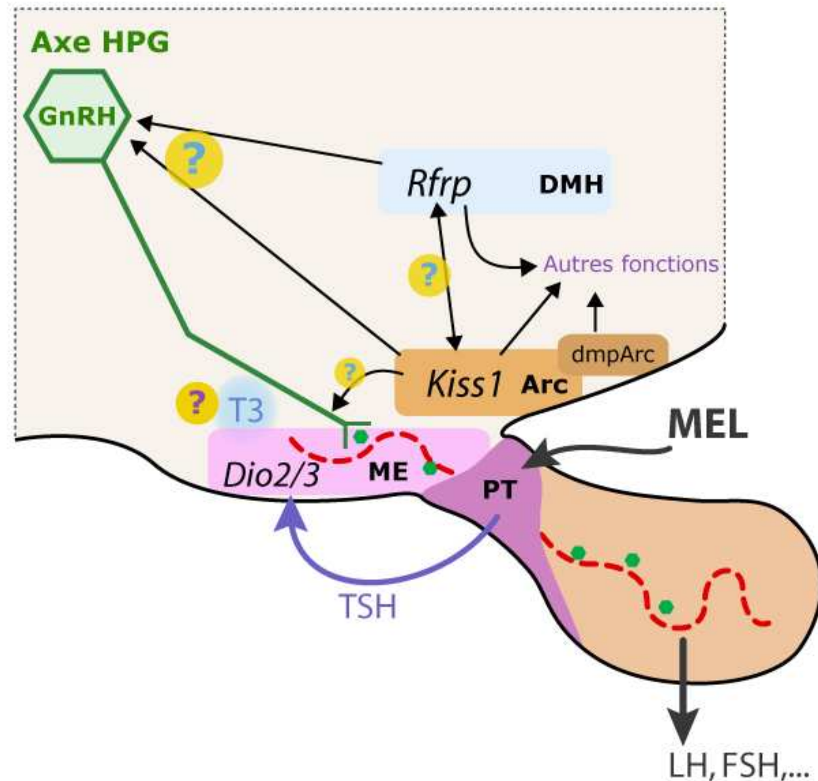

Figure 4. Un réseau photopériodique contrôlerait les fonctions saisonnières depuis l'hypothalamus mediobasal et l'hypophyse. *Sur ce schéma sont représentés l'hypothalamus et l'hypophyse en coupe sagittale. Les neurones à GnRH (gonadotropin-releasing hormone) sont les effecteurs centraux ultimes de l'axe reproducteur (axe HPG) : ils secrètent de façon pulsatile le GnRH (points verts) dans les capillaires de l'éminence médiane (ME) (système porte hypothalamo-hypophysaire), lequel GnRH active la libération des hormones gonadotropes par l'adéno-hypophyse (luteinizing hormone, LH ; follicle-stimulating hormone, FSH). Mais comment la sécrétion de GnRH est-elle modulée par les saisons ? Le profil de libération de la mélatonine (MEL), variable avec la photopériode (voir Fig. 1), est décodé par les cellules de la pars tuberalis (PT), grâce aux récepteurs à la mélatonine de type MT1. Ces cellules ajustent la synthèse de la TSH (thyroïd-stimulating hormone) en conséquence, laquelle agirait de façon « rétrograde » sur l'hypothalamus. Dans les tanycytes, la TSH modifie l'expression des déiodinases de type de 2 et 3 (Dio2/3), qui ajustent localement la concentration en hormone thyroïdienne T3. T3 permet l'ajustement de la fonction de reproduction, mais il reste à déterminer de quelle manière (?). T3 pourrait agir directement sur les terminaisons nerveuses des neurones à GnRH pour moduler la libération de cette hormone. T3 pourrait aussi agir sur l'expression de Kiss1 dans le noyau arqué (Arc) et Rfrp au niveau du noyau dorsomédian (DMH), dont les expressions dépendent aussi de la mélatonine. Ces deux gènes régulent l'activité des neurones à GnRH, mais les modalités de cette régulation et les intégrations possibles entre ces deux systèmes restent à préciser (?). Enfin, de manière tout à fait hypothétique, T3 pourrait également réguler l'expression photopériodique de gènes dans la région postérieure dorso-médiane du noyau arqué (dmpArc ; voir tableau 1), importante pour la régulation photopériodique du métabolisme énergétique.*

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périodiques identifiés à ce jour.

Ces diverses régions pourraient être interconnectées et définir un réseau nerveux complexe (Figure 3). Une question essentielle est alors de savoir si tous les gènes sont régulés directement par la mélatonine, ou bien si certains d'entres eux seulement sont capables de lire le message mélatoninergique, influençant alors secondairement les fonctions saisonnières d'autres clusters. Point important, il semble exister de claires différences inter-spécifiques quant à l'expression de certains gènes photopériodiques, non seulement entre « reproducteurs de jours longs » et « reproducteurs de jours courts » (par exemple), mais aussi entres des espèces proches telles que les hamsters Syriens et Sibériens (Ross *et al.*, 2004; Tups *et al.*, 2006; Barrett *et al.*, 2007). Ces variations pourraient (ou non) être corrélées avec des différences phénotypiques observées en réponse à la photopériode (par exemple, nette diminution de la masse corporelle en PC chez les hamsters Sibériens, mais pas chez les hamsters Syriens), ou avec des différences dans la répartition des récepteurs à la mélatonine. La physiologie comparative sera donc un aspect important des futures études.

Toutefois, les résultats récents impliquant la PT dans le contrôle de l'expression génique dans l'hypothalamus médiobasal, via la TSH, permettent d'échafauder un schéma attractif pour le contrôle des fonctions saisonnières par la mélatonine (Figure 4). Le message photopériodique véhiculé par la mélatonine serait perçu par les récepteurs MT1 de la PT, où il serait décodé pour moduler l'expression de la TSH. Ce messager transmettrait, de manière rétrograde par rapport aux schémas neuroendocrinologiques classiques, un message photopériodique à certaines cellules hypothalamiques, dont les tanycytes logés dans le plancher du troisième ventricule. Là, l'expression de Dio2 et Dio3 serait modifiée de façon coordonnée, afin de moduler la concentration locale de T3. A son tour, T3 contrôlerait les fonctions physiologiques saisonnières, comme la reproduction où le métabolisme énergétique. On peut alors spéculer que T3 agirait sur les autres clusters photopériodiques pour remplir cette fonction : effet dans le noyau arqué pour ajuster Kiss1, effet dans la région périventriculaire médiane aux noyaux hypothalamique dorsomedians (DMH) pour contrôler Rfrp, effet dans la région postérieure dorso-médiane du noyau arqué (dmpArc) pour commander l'expression des gènes métaboliques… Il est intéressant de noter que tous ces clusters se situent à proximité du 3ème ventricule, voie par laquelle T3 pourrait les atteindre facilement. Des expériences à venir montreront si T3 ou la TSH possèdent un quelconque effet modulateur sur l'ensemble de ces gènes photopériodiques (par exemple Kiss1).

Dans ce modèle hypothétique, la PT constituerait un point central d'intégration du message mélatoninergique, nécessitant des relais supplémentaires (TSH, T3…) avec les clusters photopériodiques contrôlant les diverses fonctions saisonnières, chacun des relais pouvant constituer un niveau d'intégration indépendant (observés par les effets photoréfractaires par exemple). Enfin, il a été montré que la PT renferme une horloge circannuelle (Lincoln *et al.*, 2006). Cette structure est donc idéalement située pour contrôler la physiologie des différentes fonctions saisonnières (via la TSH, T3, etc ?), en fonction d'un temps donné par une horloge annuelle endogène (pour certaines espèces), coordonnée aux saisons par la photopériode et la mélatonine.

Conclusion

La possibilité que la PT contrôle les autres fonctions saisonnières en relayant le message photopériodique véhiculé par la mélatonine aux clusters photopériodiques de l'hypothalamus est séduisante. Bien que de nombreuses expériences restent à mener pour consolider cette hypothèse, elle permettrait de combler le vide existant entre le circuit photo-neuroendocrine et la mélatonine d'un côté, et la régulation des systèmes physiologiques saisonniers de l'autre, tels que la reproduction ou le métabolisme énergétique.

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42ème Congrès de la Société Francophone de Chronobiologie

08-11 septembre 2010
La Colle-sur-Loup, France
Organisateurs : Franck Delaunay et Howard Cooper
Comité scientifique : X. Bonnefont, H. Cooper, O. Coste, F. Delaunay, E. Filipski, C. Gronfier, J.-C. Leloup, M. Teboul
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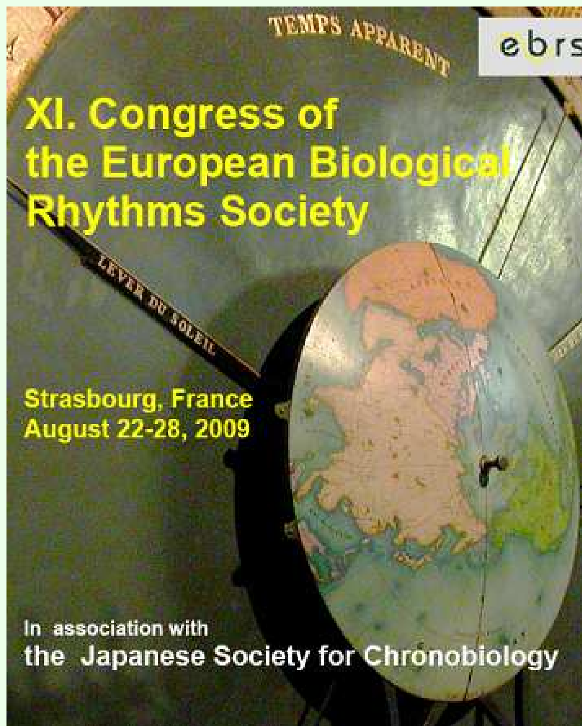
Postdoctoral position

A postdoctoral position in chronobiology and sleep research is available in the laboratory of Dr. Ralph Mistlberger at the Department of Psychology, Simon Fraser University (Canada). NSERC and CIHR-funded projects concern the neurobiology of circadian rhythms and sleep in rodents, with a special focus on 1. regulation of circadian rhythms by food and arousal state, and 2. interactions between circadian clocks and learning/memory systems. The successful candidate will have a doctorate in neuroscience or biological psychology and training in some or all of the following techniques/approaches: circadian and/or sleep science, small rodent handling and surgery, immunohistology and microscopy, bioluminescent imaging, EEG recording. Motivation, initiative and creativity are highly desirable. The initial appointment will be for 2 years, renewable for a third year. Simon Fraser University is located on top of Burnaby Mountain, overlooking Vancouver, BC on Canada's beautiful west coast. Application materials (research background, interests and a current CV with contact information of at least three references) should be directed to Dr. Ralph Mistlberger (*mistlber@sfu.ca*), Department of Psychology, Simon Fraser University, Burnaby BC V5A1S6.

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

Résumés des communications (suite)

Communications affichées : première partie



1. Clock genetics, molecular mechanisms, generation and synchronization of circadian rhythms

Genetic deletion of clock genes Per1 and Per2 does not lead to overt retinal degeneration

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Purpose: Many retinal activities are regulated by an endogenous circadian clock. We thus examined the retinal phenotype of clock gene knockout mice to search for possible modifications. Methods: In one series, eyes were obtained from adult wild-type (Bl/6), per1 -/-, per2 -/-, and per1-/-/per2-/- mice, and fixed for sectioning. Eyes were processed for conventional histology, or frozen sections for immunohistochemistry using a battery of cell markers. A second series of each mouse strain were adapted to a standard 12h light/12 h dark cycle, one group was exposed to intense white light, one group was killed 1h after lights on (zeitgeber time 1, ZT1). Some retinas were taken for western blotting. Results: Measures of cell density or layer thickness were similar between all strains. Qualitative images of rod (rhodopsin, transducin), cone (MW opsin, cone arrestin) and other retinal neuronal markers were also similar between all samples. Immunoblotting of rod and cone proteins showed no differences. Exposure to intense light did not induce photoreceptor damage in any strain. Finally, quantification of phagosomes showed equal numbers in all strains. Conclusions: Deletion of either per1 or per2 or both genes does not lead to any obvious loss in a given cell type, or affect retinal physiopathology.

Glucocorticoid receptors mediate the phase resetting effects of adrenal steroids on peripheral clocks, rather than mineralocorticoid receptors

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Purpose: To explore the action of glucocorticoids in the resetting of the circadian clock in mammals. Methods: Agarose perfused lung sections were taken from the PER2:luc mouse and treated with synthetic glucocorticoid dexamethasone (Dex), or corticosterone (Cort), to define a phase response curve (PRC). Further lung sections and Rat 1 cells were pre-treated with either glucocorticoid (GR) or mineralocorticoid (MR) receptor antagonists, followed by Cort. The actions of two novel GR specific agonists (GSK), on circadian clocks, were also investigated. Results: The pacemaker, in the mouse lung, shows a type 0 PRC with no definable "dead zone". The Gc induced phase changes, in the lung, can be partially blocked by a GR antagonist (30% reduction), but not by an MR antagonist. However, in the Rat 1 cells this phase change can be almost fully blocked (80% reduction) with the GR antagonist and partially blocked with the MR antagonist (50% reduction). The novel, highly selective, GR agonists are more potent, and have no MR activity. Our data show that both agonists can induce significant phase changes, which can be partially blocked by a GR antagonist (60% reduction). Conclusion: Gcs have the potential to induce significant phase changes on the peripheral circadian clock, in both ectopic lung slices and Rat 1 cell models, but the role of GR and MR remained unclear. Using pharmacological manipulation, our data suggests that in the lung Gcs mediate their effect through the GR

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rather than the MR. Here we have provided novel insights into the role of GR and MR in mediating Gc induced phase re-setting of the peripheral circadian clock.

Involvement of novel bZIP transcription factors in the zebrafish circadian clock

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Purpose: Characterization of the PAR and E4BP4 bZIP transcription factors in zebrafish, aiming to unravel their mode of action in core clock regulation and entrainment. Methods: Candidate members of the PAR subfamily and E4BP4 factors were searched for in the zebrafish genome based on their conserved domain sequences. 13 PAR and E4BP4 factors, including 10 novel ones, were identified and cloned, and their spatial and temporal expression pattern was described throughout development and the circadian cycle by whole mount in-situ hybridization. Light/ dark and constant dark regimes were applied in order to detect rhythmic expression patterns and to determine whether the expression is light-dependent or clock-controlled. Results: Remarkably, the majority of these factors displayed enhanced expression in the pineal gland along with additional tissues such as the retina and different cranial areas. Moreover, these factors exhibited various expression profiles, including clock-controlled and light-induced rhythmic patterns. For example, one novel E4BP4 demonstrated rhythmic expression in the pineal gland peaking at the end of the day (ZT10) in a light-dependent manner. Another novel E4BP4 displayed a light-induced rhythmic pattern in the pineal gland, with maximum levels at the begging of the day (ZT2), gradually decreasing to basal levels during the night. Two novel PAR factors exhibited clock-controlled expression in the pineal gland, interestingly, oscillating in almost opposite phases. Conclusions: PAR and E4BP4 bZIP factors were characterized in zebrafish in the context of the circadian clock. Loss-of-function and gain-of-function experiments are carried out to test whether these factors are involved in the circadian clock machinery, mainly regarding its entrainment and feedback to the core oscillator.

Molecular circadian clock in the Antarctic krill Euphausia superba

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Purpose: To perform the first characterization of the molecular circadian clock in the Antarctic krill Euphausia superba. Methods: Antarctic krill were collected in the Ross Sea during the XIX Italian Antarctic Expedition. Specimens were obtained at different times of the day (01:00, 06:00, 10:00, 15:00, 18:00, 23:00), over a complete 24-hour cycle. DNA, RNA and proteins extracted from different tissues (head, abdomen, thoracopods and photophores) were used to identify clock elements and to verify their temporal expression. Results: We identified homologues of Cryptochrome and Clock, two cardinal components of the clockwork machinery. The full-length EsCry cDNA-coding sequence consisted of a 1,638-bp ORF encoding a 545 aa sequence that contains pterin and FAD binding domains. EsCRY appeared to be an orthologue of mammalian-like CRY's and clusters phylogenetically with the insect CRY2 subfamily. EsCRY showed the canonical bipartite CRY structure, with a conserved N-terminal domain and a highly divergent C-terminus, that bears several binding motifs, some of them shared with insect CRY2 and others peculiar for EsCRY. Partial EsClk cDNA-coding sequence contained a 1,113-bp ORF encoding a 371-amino acid protein. Like all other orthologues, EsCLK had PAS and bHLH domains. Comparison of the predicted aa sequence of EsCLK with other arthropod orthologues indicated a high degree of sequence similarity (>75%). We also evaluated the temporal expression of EsCry and EsClk both at mRNA and protein levels over a complete 24 h cycle. Results showed a temporal expression of circadian elements in central and peripheral tissues. Conclusion: Our findings suggest the presence of an endogenous time-keeping mechanism that might allow this organism to synchronize its physiology and behaviour to the Antarctic environmental regimes.

New Drosophila clock genes identified by targeted expression of RNAi transgenes

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Purpose: Characterization of two new clock genes, identified by targeted expression of RNAi transgenes. Methods: A behavioral screen using RNAi expression targeted in the PDF-expressing clock neurons (Gal4-UAS system) was done in Drosophila. Locomotor activity rhythms were monitored in constant darkness. Candidate lines were behaviorally re-tested and positive were submitted to a molecular analysis of clock proteins cycling in brain clock neurons (immunocytofluorescence microscopy) or in head extracts with a relevant Gal4 driver (Western blots). Results: About 3500 genes have been tested so far and 16 RNAi lines were still positive after two behavioral tests. Results about two lines showing a strong behavioral phenotype are presented here. The first RNAi targets a gene encoding the USP5 ubiquitin protease and induces a strong lengthening of the behavioral period (> 30h). Molecular analysis reveals a degradation delay for CLOCK, TIM and most particulary for PER in head extracts, as well as a lengthening of the PER and TIM cycling period in PDF neurons. The second RNAi targets a gene encoding the Zelda transcription factor, and also induces a lengthening of the behavioral period (28h). zelda RNAi induces a decrease of CLOCK, PER and TIM protein levels in clock neurons. However, decreasing Zelda levels still affects PER oscillations in the absence of per transcriptional control, suggesting that Zelda does not act only as a per transcriptional activator. Conclusion: USP5 and Zelda are two new regulators of the Drosophila circadian oscillator.

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Characterisation of novel, candidate phase resetting genes

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Purpose: To identify novel candidate genes during phase resetting in the central circadian pace-maker by global transcription profiling of dorsal, ventral and central SCN sub-regions after a phase advance stimulus. Methods: C57BL/6J mice were kept in constant darkness for 7 days before receiving a light or sham treatment at CT22. Brains collected 3.5 hours after a light pulse were cryosectioned and SCN sub-regions isolated using laser microdissection. RNA was purified, amplified, labeled and hybridized to Affymetrix 430-2.0 arrays. Array data was interrogated for differential gene expression using a linear mixed effect model. Differentially expressed genes were assessed for significant over or under-representation of associated Gene Ontology terms. Results: We find light induced genes Per1, Per2 and fos in ventral SCN as well as GO terms 'circadian' and 'rhythmic process' in dorsal SCN indicating our technical approach is sound. The GO term 'memory' specifically associates with central SCN suggesting novel processes hitherto not associated with the SCN. Furthermore, we identified several genes upregulated upon light stimulus with known functions modulating synaptic efficacy. Neuronal pentraxin-1 (Nptx1), was selected for further characterization. Interestingly, Nptx-1 knockout mice do not phase advance after a light pulse at CT22. Conclusions: We present novel transcription profiles in SCN sub-regions after a phase advancing light stimulus, leading us to hypothesize that clock resetting after a phase advance light stimulus involves synaptic remodeling. Characterization of mice deficient in Nptx1 and other candidate genes support his hypothesis.

Ontogeny of Clock, Period 1 and aanat-2 expression in rainbow trout embryos reared under a natural photocycle or continuous illumination

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Purpose: To characterise the ontogeny of expression of genes central to clock cycling (Clock and Period 1) and melatonin synthesis (aanat-2) in the rainbow trout Oncorhynchus mykiss. Methods: Rainbow trout embryos were maintained at constant temperature, from fertilisation, under either a 12h:12h Light:Dark photocycle or continuous illumination (LL) until hatch. Clock, Period 1 and aanat-2 expression, measured every 4 hours over 24 hour periods, was measured by qRT-PCR at fertilisation, 60, 150, 300 and 420℃.days (post hatch). Results: Both the clock genes (Clock and Period 1) and aanat-2 were actively expressed in rainbow trout embryos. Period 1 mRNA expression cycled under both 12:12 and LL conditions with expression peaking at ZT 02:00 under the 12:12 photoperiod while the same cycling expression under LL appeared to be dampened and phase advanced by approximately 4 hours. Clock mRNA expression is arrhythmic under both photic conditions. Aanat-2 mRNA abundance showed a diel cycle elevating in the night under the 12:12 photoperiod while no such similar cycling was apparent under the LL treatment. Conclusions: This is the first functional demonstration of clock rhythms in rainbow trout embryos and as such it highlights a number of interesting possibilities. Period 1 cycling appears before the development of classical photoreceptors suggesting this cycling is initiated either by as yet unidentified photoreceptors or is passed to the oocyte via maternal mRNA. However the mismatch in period 1 expression between photic conditions adds further weight to the suggestion that unidentified photoreceptor mechanisms are in operation. There are striking similarities in the present results to those demonstrated in zebrafish suggesting conservation of the function of clock rhythms in teleost embryos.

Role of the Rev-erbα gene in the clockwork of food anticipation

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Introduction: When food is restricted to a temporal window during the day, rodents develop food-anticipatory activity (FAA) of different behavioural and physiological parameters. FAA has been proposed to be controlled by a circadian food-entrainable oscillator (FEO), whose anatomical localization and molecular clockwork are still unknown. Purpose: To evaluate the role of the orphan nuclear receptor Rev-erba gene in the expression of FAA. Methods: Rev-erba mutant mice (+/-: heterozygous, -/-: homozygous) and their control littermates (+/+: wild-type) were maintained on a 12-12 light-dark cycle, with food available ad libitum (AL), except during the period of restricted feeding (RF). During the RF condition, all mice had access to food during 6 h, from Zeitgeber Time 6 (ZT6, 6h after lights on) to ZT12 (lights off). To assess FAA, wheel-running behavior, general activity and body temperature were recorded. Results: In AL condition, no significant difference in the total amount of locomotor activity was found between the three genotypes. In RF condition, +/+ and +/- mice exhibited a strong FAA 1 h before mealtime (from ZT5 to ZT6),as shown by an increase of wheel-running activity as well as general activity. In contrast, -/- mice showed a significant reduction of both wheel-running and general activities. The rise of body temperature anticipating mealtime was also reduced in -/- mice. Conclusion: These results suggest that the Rev-erba gene may be a component of the clockwork of the FEO. The implication of Rev-erba not only in food anticipation but also in other feeding and metabolic processes should be considered.

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Entrainment elicits period after-effects in Neurospora crassa

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Purpose: Circadian clocks entrain to zeitgebers by synchronising their intrinsic period with the period of the entraining signal. In constant conditions, the circadian clock is capable of sustaining an intrinsic period (?) with remarkable stability. In addition to entraining the circadian clock, zeitgebers such as light cycles generate changes in ?, so-called after effects of entrainment. These effects have been previously reported for other model systems, e.g., for the mollusk Bulla gouldiana, rodents, and for human cell culture. However, after-effects in the fungus Neurospora crassa have not been reported so far. Methods: The Neurospora wildtype strain (bdA) was subjected to different T-cycles and photoperiods; the long period mutant frq7A was also subjected to different photoperiods. ? was assessed after release to constant darkness. Results: We found significant period changes as after-effects to prior entrainment compared to experiments without prior entrainment. For the wild type strain (bdA), ? was significantly lengthened, while it was slightly shortened for the long period mutant frq7A. Conclusion: Entrainment induces substantial period after-effects in Neurospora crassa, indicating that a multi-oscillatory network generates circadian oscillations at the molecular level.

Comparison of the locomotor activity rhythm of two sympatric Amphipod species from Bizerte lagoon (Northern of Tunisia)

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Purpose: The locomotor activity rhythm of two sympatric species of Amphipods living in the banks of Bizerte lagoon was studied under an aperiodic regimen: continuous light (LL). Materials and methods: Specimens belonging to the two species, Orchestia montagui (N = 14) and Orchestia gammarella (N=15), freshly collected during July, were put, at the laboratory, in actographs equipped with an infrared recording system and then they were placed in a climatic chamber. The experience was carried out on adult individuals, isolated during 13 days. Results: Periodogramm analysis showed that the circadian rhythmicity of Orchestia montagui and Orchestia gammarella individuals tested in summer was respectively equal to 100% and 76.9%. Whereas their circatidal rhythmicity was respectively equal to 46.6% and to 46.1%. In addition, actograms as well as the waveform indicated that Orchestia montagui activity was strictly unimodal and that of Orchestia gammarella was rather uni, bi and multimodal. The various circadian and circatidal period, determined, for these two species, by the two types of analyses, periodogramm and MESA, were appreciably equal. In addition, the study of the mean activity times calculated for the two species showed that the individuals of Orchestia montagui were statistically more active (aLL= 9h07 ± 1h30) than those of Orchestia gammarella (aLL = 5h53 ± 1h28). Conclusion: locomotor activity rhythm studied under continuous light was more stable for O. montagui (SNRLL = 0.837 ± 0.215) than for O. gammarella (SNRLL = 0.655 ± 0.266).

Does the master clock gene TOC1, function as a true response regulator in the picoeucaryote Ostreococcus tauri?

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Purpose: to study TOC1 phosphorylation, and its potential regulation by two component system in Ostreococcus tauri. Methods: The circadian clock has been extensively studied in the green lineage using Arabidopsis thaliana, leading to the identification of most of known plant clock genes. A 3-loops model accounts best for the central oscillator function. Green microalgae are alternative simple unicellular systems to study green clock architecture. We have developed the picoeukaryote Ostreococcus tauri, (Prasinophyceae) as a new model system by implementing molecular tools, including luciferase reporter strategy, overexpression (ox) and antisense (as) technologies to study gene function. Results: Orthologues of Arabidopsis master-clock genes TOC1 (timing of CAB expression 1) and CCA1 (circadian clock associated-1) were shown to be central clock components in Ostreococcus. Unlike higher plants TOC1 proteins, which belong to pseudo-response regulator family, Ostreococcus tauri TOC1 displays the conserved phosphate acceptor aspartyl residue found in receiver domain of true response regulators (RR). Overexpression of non-phosphorylatable TOC1 (D?N) abolished circadian rhythms of CCA1:Luc used as a reporter gene. RR phosphorylation is generally established by a phosphate transfer from histidine kinase to RR witch could involve a phosphorelay (HPt) in an intermediate step. Only two histidines kinases (HKs) HK1 and HK2 were identified in silico in O.tauri. HK2 has a (Light Oxygen Voltage) LOV domain, whereas HK1 exhibits a bacteriorhodopsin-like domain. HK1 and the only HPt displays a constant protein level under constant light, while HK2 protein peaks in the late afternoon and is under circadian control. Conclusion: Further environmental cues are tested to precise the function of TOC1 phosphorylation and a mass spectrometric approach will be used to analyse TOC1 phosphorylation status under circadian entrainment. The involvement of HKs in the regulation of TOC1 using -ox and -as strategy, as well as the characterisation of their chromophore binding domain has been undertaken.

Rat retina explants show rhythmic expression of clock genes and clock output genes in constant conditions

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Purpose: to demonstrate the presence of a circadian clock in the retina by evaluating rhythmicity in expression of clock genes and clock output genes in explants maintained for a few days in darkness. Methods: retinas were dissected from Wistar rats housed under a daily LD12/12 cycle and put in culture around ZT12 on semi-permeable membranes (photoreceptors down), with Neurobasal-A medium containing 2% B27 and 2mM L-Glutamine. Explants were collected every 4h (3 retinas per time point) over 3 days and frozen at -80℃. Total RNA was extracted, quantified and reverse transcribed. Gene expression was assessed by real time PCR using the TaqMan technology. PCR results were analysed with the qBase software by using beta-actin and pde6b as normalizing genes, followed by statistical analysis of relative mRNA amounts to evaluate the fit of sine-wave regressions. Results: Rhythmic expression was found for most of the clock genes and for downstream targets from the clock. Phases were calculated between the clock genes and showed small differences. Conclusion: Our work shows for the first time the free-running expression profile of clock genes and potential clock targets in mammalian retinal explants, in constant conditions. It further strengthens the notion that the retina contains a robust, self-sustained oscillator.

A Cullin-3-based E3 ubiquitin ligase is involved in the drosophila circadian clock

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Purpose: Identification and characterization of a new E3 ubiquitin ligase complex involved in the regulation of clock proteins. Methods: Locomotor activity was recorded in constant darkness after entrainment with light-dark cycles. Fly heads were collected every 3 hours in DD and protein extracts were analyzed by Western blots. Fly brains were dissected every 4 hours in DD and clock protein cycling in the clock neurons was analyzed by immunofluorescence. Results: In Drosophila, the control of PER and TIM cycling depends on their phosphorylation, which involves several kinases and phosphatases. Two F-box-containing ubiquitin ligases have been shown to target the proteasome-dependent degradation of PER and TIM in the absence of light (Slmb) and the light-induced degradation of TIM (Jet), by the proteasome. Here, we reveal a new pathway involved in the light-independent degradation of TIM, which relies on a Cullin-3-based E3 ubiquitin ligase complex. Deregulation of Cul3 in the clock neurons induces a loss of behavioral rhythmicity and of PER/TIM cycling in DD. Expression of a dominant-negative form of Cullin-3 leads to a persistence of phosphorylated TIM in head extracts. Conclusions: We identified a new E3 ubiquitin ligase complex involved in the control of Drosophila clock proteins cycling, which acts on the circadian control of TIM degradation.

Using lentivirus for restoration of function in the central nervous system of Per mutant mice

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Purpose: Develop a tool suitable for local, stable and long-term expression of a clock protein in the central nervous system of mice mutant for a designated clock gene. Methods: lentiviral vectors (derived from the HIV virus, devoid of pathogen elements) can infect non dividing cells and insert their genome in host's cells DNA. When bearing a gene of interest, those constructs can elicit a sustained, long term expression of the transgene in an area limited to the surroundings of the site of injection in the brain of an adult rat. Here we adapted this technique initially developed in rats to mice. To validate our tool, we aim at stably transfecting the SCN of Per1/Per2 double mutant mice with a lentivirus expressing the Per2 sequence. Considering that Per1/Per2 mutant mice are arrhythmic in constant darkness (DD), upon stable infection, a restoration of a rhythmic pattern of locomotor activity in DD should be observed, which will be assessed by wheel running activity. Results: The Per2 gene has been cloned into the transfer vector of the lentivirus construct (pLVPT-rt-TR-KRAB-2SM2). High titers of vector stocks have been produced as well as a control virus expressing GFP under the control of the same promoter. The obtained infecting solutions were injected stereotaxicaly into the SCN of Per1/Per2 double mutant mice. Lentiviruses expressing GFP stably transfects cells in the vicinity of the site of injection. Also, they do not alter behaviour in Per1/Per2 double mutant mice compared to sham operated animals. PER2-expressing viruses are currently being injected in those animals. Conclusions: Lentiviral constructs are new suitable tools for local expression of a protein in the central nervous system of mice. Using this tool in mutant mice will provide new insights in the importance of a gene when present only in one area of the brain.

Zeitlasser - a novel component of the Neurospora circadian clock

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Purpose: To characterise novel clock components in the fungus Neurospora crassa specifically those regarding temperature entrainment. Methods: The mutant zls (for German: zeitlasser) was generated in an insertion mutagenesis assay using a resistance gene (bar2) against a fungicide. We tested for a clock phenotype applying a frequency de-multiplication protocol of short temperature cycles (6h 20℃, 6h 28℃ in DD). Experiments determi n-ing the circadian behaviour on the physiological level where carried out in race tubes. The protein expression patterns in time series experiments where measured with SELDI-TOF. The gene responsible for the zls- phenotype is being identified by mapping with cleaved amplified polymorphic sequence (CAPS) markers and sequencing (Illumina GAII). Results: In comparison to the original zls+ strain, bdA, zls displays a delay of 15 h in the phase of entrainment in short temperature cycles. This entrainment behaviour depends systematically on the various structures of the applied symmetrical temperature cycles: the amplitude (0.5 to 4℃), the length,T and the order of temperatures (low/high or high/low). Protein expression levels reflect this mutated behaviour on the molecular level. The free running period of zls is not perfectly temperature

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compensated (Q10 [bdA] = 1,1; Q10 [zls] = 1,6). In contrast to its drastic phenotype in the temperature demultiplication protocol, the mutant shows only a slight delay (˜ 1h) in standard L:D conditions. It appears, however, more sensitive in skeleton photoperiod protocols (L:D 1:10:1:12 or L:D 1:12:1:10): While the mutant entrains with choosing the longer dark period as subjective night, bdA cannot be entrained by these conditions. Mapping identified a single mutation in linkage group I of the genome. Conclusions: The demonstrated circadian features prove zls as a completely novel component of the Neurospora clock system.

Contribution of ROS- and RASGEF-mediated signaling to the control of circadian rhythm in Neurospora crassa

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Purpose: In the transcriptional-translational negative feedback loop of the circadian clock of Neuropsora crassa the positive element White Collar Complex promotes expression of Frequency which in turn, inhibits the activity of the positive element. Reactive oxygen species (ROS) are known to contribute to signaling pathways and were shown to affect the circadian clock in different organisms. The generally used band strain of Neurospora displays robust conidiation rhythm on minimal medium whereas rhythmic conidia formation of wt requires addition of ROS generator to the medium. band was recently determined to be a point mutation in ras-1. Aim of our study was to analyze the interaction of the RAS- and ROS-responsive signaling pathways controlling the circadian rhythm. Methods: Conidiation rhythm was monitored by using race tube assay. Expression of clock and clock controlled genes was quantified by quantitative real time PCR. Immunoblot analysis was performed to follow protein expression and phosphorylation. RAS activity assay was applied to compare RASGEF activities of the strains. Results: ROS generation affects dose-dependently the phase of the conidiation under light-dark conditions and ROS-pulses induce transcription of clock genes. Effect of ROS on the circadian output is attenuated in a mutant strain deleted for a RASGEF homologue. Rasgef expression shows a low amplitude rhythm. Conclusions: ROS affect both input and output pathways of the circadian clock and under entrained conditions determine the phase of conidiation. RASGEF is a component of the circadian output and acts synergistically with ROS in the control of the conidiation rhythm.

Circadian clock proteins modulate GR transactivation properties at multiple control levels in murine fibroblasts

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Purpose: To investigate the effects of molecular clock proteins on the GR transactivation properties. Methods: NIH-3T3 cells, clock/clock mutant, pers KO, crys KO and bmal1 KO MEFs were transiently transfected with simple GRE reporter (GREtkLUC) and the effects of circadian clock proteins overexpression/deficiency on the maximal transactivation (Amax) and efficacy (EC50) of GRE-dependent transactivation by DEX were analyzed. Also, GR and various GR regulators (coactivators, corepressors and comodulators) expression levels were measured by quantitative real-time PCR in wild-type and clock genes-deficient MEFs. Results: We found that PER1, CRY1, and PER1/CRY1 combination reduces Amax, but not EC50, in a dose-dependent manner, while BMAL1 or CLOCK/BMAL1 combination significantly affects both parameters. CLOCK expression alone shows no effect. On the contrary, GR overexpression does not affect E-box-dependent transactivation by CLOCK/BMAL1, while strongly affecting Amax and EC50 of GRE-dependent transactivation. GR and various GR regulators expression levels are differentially affected in functional clock proteins-deficient MEFs. Conclusions: These results clearly indicate that circadian clock proteins modulate GR transactivation properties at multiple control levels in murine fibroblasts.

Heritable circadian period length in a wild bird population

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Purpose: The evolutionary ecology of circadian clocks is poorly understood. Experimental and association studies suggest that concordance of the free-running period (?) with the ambient light-dark cycle has major advantages for fitness and health. ? of wild-derived animals would therefore be expected to be close to 24 h, and heritable variation in ? should be counteracted by stabilizing selection. Methods: We addressed the evolutionary malleability of ? in a wild population of a widespread, diurnal songbird, the Great tit (Parus major). We examined 98 birds under constant dim light for variation in ? that originated from 20 broods and were raised in captivity. Family relations were known, and paternity was assessed by molecular methods. Results: Overall, ? was significantly shorter than 24 h. We found that there was ample variation and that ? was highly heritable. Four broods contained young that were fathered by the male that tended the nest as well as young that originated from extra-pair matings. Young from extra-pair matings had a much shorter ? than their half-siblings from within-pair matings. Conclusions: We thereby demonstrate that variation is not depleted by stabilizing selection, and that period length is potentially malleable by micro-evolution. The data from extra-pair matings suggest that sexual selection could contribute to maintenance of variation in circadian clocks and to selection for short period length.

Dexamethasone resets the circadian oscillation of Bmal1 via ROR-response elements

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Purpose: A single exposure of fibroblast cultures to a glucocorticoid agonist resets rhythmic cells, but not asynchronous cells, to a common circadian phase, and resets the circadian expression of clock genes. It has been suggested that the presence of glucocorticoid response elements (GREs) in the regulatory regions of core clock components is a likely mechanism for the resetting of the circadian expression of these components by glucocorticoids. However, the molecular mechanism that accounts for the resetting of the circadian clock remains to be elucidated. Methods: This study explores the underlying mechanism of the resetting of the circadian phase in cultured fibroblast cells by gulucocorticoid by means of a promoter analysis of clock genes. Results: The luciferase reporter assay showed that dexamethasone (DEX) enhanced Bmal1 promoter activity in a dose-dependent manner. In addition, the glucocorticoid antagonist mifepristone attenuated the enhancement caused by DEX. Real-time monitoring of the Bmal1 promoter activity in NIH3T3 cells revealed that application of DEX modulated the activity and circadian oscillation of the promoter. Mutation of a putative GRE in the mouse Bmal1 promoter minimized the effect of DEX stimulation on Bmal1 transcription. By contrast, mutation of two ROR alpha binding sites (ROR-RE) located upstream from the transcription start site of the Bmal1 gene essentially abolished the induction of the promoter activity. Deletion of one of the ROR-REs in the Bmal1 promoter attenuated the response to DEX treatment on the first day of the real-time analysis of promoter activity. Conclusions: These results indicate that DEX regulates the resetting of Bmal1 expression mainly via ROR-RE, and not via GRE.

Entrainment of KaiC phosphorylation rhythm by ratio fluctuation among Kai proteins

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Purpose: To elucidate the relationship between the ratios among Kai proteins and period of the KaiC phosphorylation rhythm. Methods: We employed in vitro KaiC phosphorylation system we previously reported (Nakajima et al. Science 2005). The concentration of recombinant Kai proteins was altered (Standard condition; 1.2 ?M KaiA, 3.5 ?M KaiB, 3.5 ?M KaiC) and we observed phosphorylation rhythm and its period. Result: We found that distinctive characteristics of KaiA and KaiB in determining period of KaiC phosphorylation rhythm. There was a definite concentration of KaiA and KaiB where the stable oscillation could be observed. The period was sensitive to the concentration of KaiA, but not to that of KaiB. Based on the experimental results, we examined the possibility of entrainment by periodic modulation of Kai proteins ratio. It was known that the ratio of Kai protein amounts circadianly oscillated under light conditions and the oscillation totally disappeared in dark conditions (Tomita et al. Science 2005). These facts means the period of KaiC phosphorylation rhythm under LL conditions could not be kept constant due to the oscillation of accumulation levels of KaiB and KaiC in a cell. Otherwise, under DD conditions the period of Kai oscillator is constant. These distinctive phase progression between light and dark conditions could make it possible to entrain the circadian clock to the external LD cycle. To validate whether this idea enables entrainment, we formulated a behavior of KaiC phosphorylation rhythm under light and dark conditions and checked the entrainability. Conclusions: Period of KaiC phosphorylation rhythm depends on the ratio among Kai proteins. It could contribute to entrainment of cyanobacterial clock to external LD cycles.

Circadian rhythm of the antioxidant components in the photosynthetic plant, Spinacia

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Purpose: To analyze, from the perspective of food products and nutritional science, circadian rhythm variation of the antioxidant components of spinach, Spinacia oleracea L. cultivated in two different environments-soil culture and hydroponics. Methods: The seeds of spinach, Spinacia oleracea L. were cultivated in a growth room at constant temperature, either 20℃ or 25℃, and 50% relative humidity. The spinach was germinated under LD 12:12 at a luminance of either 30 or 20 klux at the soil surface. Hydroponic spinach was also cultivated with LED light source having different light quality. Fresh leaves were extracted with 90% methanol for measurement of total phenol contents. An aqueous solution containing metaphosphoric acid (5%) was used for determination of vitamin C contents. The total phenol contents were measured by colorimetric methods. The vitamin C, oxalic acid and nitric acid contents were determined by HPLC. Results: There were considerable differences in the components contents depending on the position of leaves in the plant as well as the segment within a leaf. Therefore, the vitamin C contents changed markedly with growth stage as well as with aging within the same leaf. Moreover, a circadian rhythm of the vitamin C content was found in the cut into three segments of leaf blades, respective segments. The entrainment rhythm shows a maximum around subjective midday. The rhythm persisted with a period of 24 to 26 h under the free-running condition (LL) examined and was entrained to 24 h light-dark cycles (LD) [1]. Conclusions: As a result, various circadian rhythm variations were discovered in the components of spinach grown in different environments (soil culture and hydroponics), and thus a relationship was found between variation in vegetable components and time. By clarifying the mechanism of antioxidant components, as was done in this work, we can expect a further increase in the effectiveness of vegetables, and a more abundant dietary life.

[1] Kiyota M. et al. (2005) J. Photochem. Photobiol. B; Biol. 84:197-203

Neuroendocrine control of reproductive activity in a bivalve mollusc Perna perna: Impact of pollution

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Purpose: To evaluate the impact of pollution on the regulation of reproduction by studying the effect of cerebroid ganglia via neurosecretory cells on the sexual cycle, of P.perna, during an annual cycle. Methods: Studies performed in 3 populations of the Atlantic coast: the first taken as a control is developed in an unpolluted site. The two others are collected from areas contaminated with domestic and industrial wastewater. The histology of gonads identified the sexual cycle of P. p., and defines the periods of spawning release. The immunocytological approach against anti-LHRH a marked variation in the number of neurosecretory cells, depending on specific periods of gonadal activity. Biomarkers (AChE, CAT, GST, and MDA) were measured in P. p., to assess the degree of pollution in each station. Results: The latter occurs in 3 periods during the cycle in Reference Site. Where as among populations in the polluted sites, spawning takes place in 2 periods. Reproduction in polluted sites is disrupted, either through gametogenesis longer. The quantitative development of neurosecretory cells is correlated with the stages of sexual cycle. During the stages of maturation of gametes, it feels significantly, where as during the laying or sexual rest this number decreases. In area contaminated with domestic releases, this number is relatively less important compared to the other sites. In all sites, biomarkers show a seasonal cycle correlated to sexual cycle, and a significant site effect between the sites. The results show also that the reference site reveals a form of contamination. Conclusions: Pollution Disrupts the cycle of sexual P.p. by reducing the number of nesting and changes the length of the wave's gametogenetics. Pollution reduces the number of CNS containing the GnRH-like site in the very contaminated site.

Screening for polymorphisms in the Aanat gene and association with diurnal preference

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Purpose: To perform a screening of the Aanat gene to identify which polymorphisms are present in Brazilian sample and seek associations with diurnal preference. Methods: Individuals with extreme diurnal preference were selected according Horne-Ostberg questionnaire scores. DNAs are amplified by Polymerase Chain Reaction and analyzed in Denaturing High Performance Liquid Chromatography (DHPLC). Segregated association tests for each SNP or haplotypes blocks were performed. Results: A total of six polymorphisms were found. Two still not reported in data bank or in the literature. All polymorphisms in the Aanat gene found in this sample, except by one, presented very low frequencies, which maximum frequency 9%. The only one which is found more often, the rs4238989 is localized in the promoter region. This polymorphism is a Single Nucleotide Polymorphism (SNP), a C to G change in the position -263. A -263G allele is more present in evening preference volunteers. Conclusions: We observed that, except by one SNP in the promoter region, the frequency of variation of these SNPs in the AANAT gene are extremely low which indicate that it is very much conserved. The SNP C-263G in the promoter region is associated with extreme diurnal preference. The polymorphism in the promoter region may have an important role in the rate of transcription of Aanat.

Molecular characterization of phosphorylation mechanism of CRY2 at Ser557

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Purpose: To identify the protein kinase responsible for CRY2 Ser557-phosphorylation, which allows secondary phosphorylation at Ser553 by GSK-3? and leads to proteasomal degradation of CRY2. Methods: To investigate the phosphorylation level of CRY2 at Ser557, antibodies specific to the Ser557-phosphorylated form of CRY2 were generated. By using the antibody, we developed an assay detecting the Ser557-phosphorylating activity in combination with a recombinant substrate GST-CRY2. Results: We previously found that CRY2 is phosphorylated at Ser557 by ERK in vitro. However, neither activation nor inhibition of the ERK pathway altered the phosphorylation state of CRY2 Ser557 in cultured cells, suggesting that protein kinase(s) other than ERK contributes to Ser557-phosphorylation of CRY2 in vivo. To search for Ser557-kinase, we first examined subcellular localization of Ser557-phosphorylating activity in the mouse liver and found that the cytosolic fraction retained a larger proportion (81%) of the activities. DEAE column chromatography of the cytosolic fraction yielded three peak activities of Ser557-phosphorylation, among which we found one peak was devoid of active-ERK. The Ser557-kinase activity in the peak fraction was characterized by performing the in vitro kinase assay in the presence of various kinase inhibitors. Many drugs including the inhibitor of CK1, p38, PKA, PKC, PKG, and CaMKII had no significant inhibitory effect on the Ser557-kinase activity. On the other hand, a marked inhibition was observed with a drug that is known to inhibit CK2. However, recombinant CK2 hardly catalyzed Ser557-phosphorylation of GST-CRY2 in vitro.Conclusions: Our research corroborates the presence of Ser557-kinase activity other than ERK, and the activity was inhibited by a CK2 inhibitor. However, CK2 hardly phosphorylated GST-CRY2 at Ser557 in vitro, suggesting that other kinase sensitive to the CK2 inhibitor is responsible for the Ser557-phosphorylation.

A new mutation affecting rhythmicity in both FRQ-less and FRQ-sufficient Neurospora

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Purpose: To identify components of the oscillator that drives rhythmicity in FRQ-less strains of Neurospora crassa. Methods: Although a FRQ/WCC oscillator is said to be central to the circadian system in Neurospora, rhythms can be seen under some circumstances in FRQ-less (knockout) strains such as frq10. These rhythms are

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said to be driven by a FRQ-less oscillator separate from FRQ/WCC. In the chol-1 strain, the period of the conidiation rhythm lengthens on low choline, and the long-period rhythms continue in a chol-1 frq10 strain. We have previously reported that frq1 and wc-2 mutations significantly affect rhythmicity in chol-1, and prd-1 and prd-2 mutations significantly affect rhythmicity in both chol-1 frq10 and chol-1 frq+, demonstrating connections between rhythms in FRQ-less and FRQ-sufficient strains. To identify additional genes affecting the FRQ-less rhythm, we mutagenized chol-1 frq10 spores with UV light, selected colonies with altered rhythmicity, and identified a new arrhythmic mutation, named UV90. Results: Conidiation is arrhythmic or strongly damped in all UV90 strains, both chol-1 and chol+, and both frq10 and frq+. UV90 increases the phase-resetting response to both light and heat pulses in the damped chol+ frq+ strain, suggesting a decrease in the amplitude of the oscillator. Levels of FRQ protein are very low in UV90 frq+, consistent with a decrease in amplitude. UV90 therefore appears to be required for sustained, high-amplitude rhythms in both FRQ-less and FRQ-sufficient strains, demonstrating again that these rhythms are interdependent and may be driven by the same oscillator. The UV90 gene does not map near any known clock-affecting mutations. Conclusions: These results support a model of the Neurospora circadian system in which a single FRQ-less oscillator drives conidiation rhythms, and FRQ/WCC stabilizes amplitude and period and contributes to environmental input.

Identification of a HECT E3 ubiquitin ligase in the Drosophila circadian clock

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Purpose: Identification of a circadian role of TRIP12-like, a HECT E3 ubiquitin ligase. Methods: For all the experiments, flies were entrained in LD 12:12 cycles and analyzed in LD and DD conditions. Behavior was analyzed in activity monitors. Clock proteins cycling was tested in larval and adult brains and by Western blot analysis of head extracts. mRNA cycling was analyzed by quantitative RT-PCR on head extracts. Results: We identified two enhancer trap P-Gal4 insertions that are located in the same gene and showed strong expression in the PDF-expressing ventral lateral neurons (LNv). The target gene trip12-like is homologous to the human trip12 (Thyroid Interacting Receptor 12) gene, which encodes a putative HECT E3 ubiquitin ligase. Reducing trip12-like expression by targeted RNAi expression strongly lengthens the behavioral period as well as PER/TIM protein oscillations in the LNv. CLOCK protein levels are increased in the brain of RNAi-expressing flies and mutant larvae. In head extracts, CLOCK levels are increased in a per-independent manner and per/tim mRNA levels are accordingly higher. Surprisingly, TRIP12-like thus seems to increase CLK transcriptional activity and delay PER/TIM protein oscillations

Changes in circadian activity induced by the presence of sexual hormones in male crayfish: A mathematical model

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Purpose: To demonstrate that in the ERG circadian rhythm of crayfish Procambarus clarkii underlies ultradian activity which reappears as the consequence of some external disturbance. Methods: The ERG of an adult, male crayfish was recorded during 12 consecutive days. In the 5th day of recording, either an adult female crayfish or an ecdysterone (a sexual hormone) solution was introduced in the bath during 20 min. The experiment continued, at least, 5 days more. Results: After the presence of a female crayfish or an ecdysterone solution, the ERG circadian rhythm of the male crayfish shows an irreversible disturbance in amplitude, frequency and excitation level. The analysis of these results by time series shows a shortening in the length of the circadian period as well as the presence of ultradian periods of about 16, 12, 6 and 3 h superimposed on the circadian oscillation. These results let us to include a third oscillator in the mathematical model we have previously proposed to describe the generation of ERG circadian rhythm. This implies that the new model includes three main oscillators: two of them for describing the ERG rhythm and the third one to explain the presence (many times masked) of ultradian activity surged as a consequence of external disturbances. Conclusions: Our experimental work and its mathematical modeling let to propose that ultradian and circadian rhythms coexist and that together take part in the organization of the clock machinery in the crayfish.

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Circadian expression of permethrin-resistant genes in Aedes aegypti

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Purpose: To understand the relationships among circadian clock, detoxification enzyme and permethrin- resistant in mosquito Aedes aegypti. Methods: Use bioassay to measure the fluctuation of resistance against permethrin under LD condition. One of the resistant genes, cytochrome P450 (CYP9M9), was selected to monitor its detoxification effects during LD and DD conditions. To link the circadian clock with CYP9M9, RNAi technique against period gene was employed to investigate the diminishing circadian expression of CYP9M9. Results: the 50% knock down time (KT50) under lighting condition was significantly longer than that of dark condition in both susceptive and permethrin-resistant strains. This phenomenon implied the circadian control underlying the expression of the resistant gene. One of the detoxification genes (CYP9M9) expressed in fluctuation within one day which showed high level of expression during day time. RNA interference against period was employed to disrupt the circadian clock, and the expression of CYP9M9 lost fluctuation and kept at low level. Conclusions: The expression of permethrin-resistant gene (CYP9M9) is down regulated by clock genes.

The Neurospora circadian clock and functional genomics for discovery of new clock genes in both mating types

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Purpose: Many lines of evidence suggest that there are additional clock genes that remain to be discovered in Neurospora crassa. Methods: We have used a candidate gene approach: proteins coding for putative photoreceptors, some of them containing PAS domains, were identified by searching the Neurospora genome sequence. We obtained knockout mutants (some of these from the Neurospora Genome Project) and screened them in a variety of protocols for circadian rhythm and entrainment. Results: In addition to WC-1 and VVD, PHYI, PHYII and CRY all play a role in the Neurospora circadian clock. Surprisingly, we find differences in both entrained phase and free running period that map to near the mating type locus; the different mating types are essentially different chronotypes. The most extreme phenotype in our study is the cry phyII matA strain, which is completely arrhythmic under all conditions tested. This strain is not light blind but the kinetics of light-induced gene expression are abnormal. Conclusions: We show that chronotype in Neurospora is mating type dependent and -so far- all photoreceptors that we have assayed, have effects on chronotype.

Synchronise watches! Possible mechanism of communication between neurons and astrocytes in the suprachiasmatic nucleus of Phodopus sungorus

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Purpose: How do neurons and astrocytes in the SCN communicate and what is the role of astrocytes in the synchronisation of individual daily rhythms of single neurons? Methods: The SCN were removed from postnatal (P21-P28) Dsungarian hamsters (Phodopus sungorus), the tissue was prepared for cell-culturing and cells were cultivated on fibronectin-coated coverslips. Immuncytochemistry was performed for GFAP, NeuN, Per1, Connexin43, mGluR5 and IPR3. Single-labelling as well as double-labelling were carried out. Calcium-Imaging of the cell-cultures is still in progress. Results: The immuncytochemistry revealed a positive staining for Per1, NeuN, IPR3 and Connexin43 in Neurons. In astrocytes a positive staining for GFAP, Per1, IPR3 and Connexin43 was recorded. The results of the Anti-mGluR5-staining as well the results of the calcium-imaging have to be evaluated. Conclusions: With regard to previous studies and according to present results we favour the following mechanism of communication between neurons and astrocytes in the SCN: The release of Glutamat may increase the Ca2+ levels in neurons and astrocytes by activation of the metabotrophic glutamate receptor mGlu5. Glutamat stimulates the formation of inositoltrisphosphatate (IP3) which acts on the IP3 receptor (IPR3) to release Ca2+ from the endoplasmic reticulum. These elevated Ca2+ levels induce the gene transcription of clock genes in the nucleus via calmodulin kinase and phosphorilation of CREB and additionally the release of glutamate. Furthermore neurons and astrocytes are connected by gap junctions and thus they communicate via generated IP3 which passes the gap junctions and probably causes a Ca2+ elevation in the adjacent cell. In this way neurons and astrocytes could communicate and synchronise their transcription of clock genes by using Ca2+-waves. This hypothesis has to be confirmed by the still missing results from the calcium-imaging.

Aging and circadian system in zebrafish

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Purpose: To study age-related changes in brain melatonin content and expression of core circadian genes in a diurnal vertebrate, zebrafish (Danio rerio). Methods: Brain melatonin content was assessed using radio immunoassay in samples collected at daytime (ZT1 and 13) and night time (ZT16, 19, and 22) in young (1 year old) and aged (4 years old) zebrafish, maintained in 14L:10D photoperiod. The circadian patterns of mRNA abundance for core clock genes (bmal1, clock1, per1), and melatonin receptors (mel1a1 and mel1c) were measured in zebrafish eyes (and, in part, in brain tissue), using real-time quantitative RT-PCR. Samples were collected at 2-4 h intervals (ZT5, 7, 11, 15, 19, 23 and 3) in young and aged zebrafish. Results: The night time melatonin production showed age-dependent decline, leading to reduced daily amplitude of melatonin rhythm. The daily variation in mRNA levels for clock genes was present in both young and aged zebrafish. In young zebrafish, the peak time for gene expression was: ZT11 for bmal1, ZT15 for clock1, and ZT23 for per1. An age-dependent reduction in mRNA expression was found for bmal1 and per1, and this was associated with phase delay in bmal1 peak in aged animals. No significant age effects were found for either mel1a1 or mel1c expression. Conclusions: In zebrafish, melatonin production but not the expression of melatonin receptors declines with age. Aging also leads to reduced amplitude of mRNA expression for at least some core clock genes in zebrafish. Understanding the circadian aging in this genetically well characterized vertebrate, with diurnal lifestyle being similar to that in humans, provides new opportunities to address the role of circadian factors in aging. This might help in developing adequate prophylactic or treatment strategies to known age-dependent changes in the circadian system in humans.

Restricted feeding entrains rhythms of inflammation-related factors without promoting an acute phase response

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Purpose: To explore if an acute phase response (APR) or a pro-inflammatory state occurs during the FEO expression. First, levels of circulating cytokines were compared in rats under food restriction and rats treated with lipopolysacharide. Second, we characterized the influence of FEO expression on the diurnal variations of circulating cytokines and APR-proteins. Third, we tested if the feeding condition (22-h fasting and 2-h re-feeding) influences these parameters. Fourth, we explored measuring NF-?B if a cellular stress was established in the liver during with the FEO expression. Methods: Food access from 12:00 to 14:00 h for 3 weeks. Circulating levels of inflammation markers such as interleukin-1? (IL-1?), interleukin-6 (IL-6), tumor necrosis factor-? (TNF?) were determined by ELISA, and C-reactive protein and fibrinogen (APR-proteins) by agglutination assay and colorimetric method, respectively. Metabolic stress in the liver was established by immunohistochemical localization of NF-?B. Results: During FEO expression: 1) no APR was implemented; 2) food restriction modified the rhythmic 24-h fluctuations of IL-1?, IL-6, TNF-?, and fibrinogen; 3) 22-h fasting increased the level of most of the parameters tested, but this effect was not observed during FEO expression; 4) food restriction produced a significant peak in NF-?B signal in the liver, including its nuclear translocation. Conclusions: The stress condition associated with FEO expression is not sufficient to induce an APR, but it could be related to a local cellular stress response within the liver.

Circadian expression of the clock genes Per1, Per2, Per3 and Clock in sole Solea senegalensis

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Purpose: To clone and analyze the tissue and daily expression of the clock genes Per1, Per2, Per3 and Clock from Senegalese sole, a nocturnal flatfish species of interest in aquaculture. Methods: Adult sole specimens maintained at 19ºC under natural photoperiod were used. Total RNA from retina was extracted and reverse transcribed to cDNA. PCR amplifications were performed with degenerated primers for the first partial cloning of Per1, Per2, Per3 and Clock. Full length Per3 cDNA was obtained by RACE using specific primers. Tissue expression pattern was determined by RT-PCR in neural and peripheral tissues. Quantitative real time PCR analysis was carried out in optic tectum, diencephalon, retina, liver and gonads sampled every 4h using ß-actin and Rps4 as reference genes. Results: The full length Per3 cDNA contains a 3804bp ORF encoding 1268aa. Partial Per1, Per2 and Clock sequences obtained were 0.7, 2.1 and 0.6kb respectively. Predicted proteins analysis revealed the presence of a PAS domain in PER2, PER3 and CLOCK as well as a CLD domain in PER2 and PER3. Per1, Per3 and Clock expression was detected in all examinated tissues. qPCR expression analysis shows rhythmic daily profile of Per1, Per3 in retina, liver and optic tectum, Per2 in diencephalon and optic tectum, and Clock in retina and liver. The expression of the four genes in gonads did not show a rhythmic profile. Per1, Per3 and Clock display an inverted rhythm in retina and liver. Conclusions: We have isolated Per3 cDNA and partial Per1, Per2 and Clock cDNAs from Solea senegalensis. Daily expression studies of these genes indicate a clear circadian profile, showing an inverted rhythm in retina and liver. Our results reinforce the role of clock genes in the generation of rhythms in sole and suggest the existence of different synchronizers in this species.

The circadian variations in the anorexic effects of serotonin are related to the rhythmic expression of 5-HT1B receptors

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Purpose: Central serotonergic activity shows pronounced circadian rhythmicity with higher serotonin (5-HT) release during the nocturnal phase of the light/dark cycle. In relation to feeding behavior, the anorexic effects of 5-HT are stronger at the onset of the dark cycle which corresponds to the active period of the free feeding rat. Serotonin inhibits its food ingestion through 5-HT1B and 5-HT2C receptors located in different hypothalamic nuclei. We aimed to determine if the expression of these serotonin receptors in the hypothalamus is submitted to circadian control and if there is any link between diurnal receptor mRNA changes and diurnal rhythms in 5-HT1B and/or 5-HT2C receptor-mediated effects on food intake. Methods: The anorexigenic effects of, respectively, the selective 5-HT1B and 5-HT2C receptor agonists CP-94,253 (16mg/kg) and Ro 60-0176 (10mg/kg), were evaluated in male C57 mice at 4h intervals over a 24h period. The mRNA coding the 5-HT1B and 5-HT2C receptors in the hypothalamus was quantified in parallel by real time RT-PCR analysis. Results: As expected, C57 mice exhibited a circadian pattern of feeding characterized by the eating of 60-70% of their daily consumed food during the nocturnal phase of the light/dark cycle. The injection of CP-94,253 or Ro 60-0176 reduced food intake but the intensity of their anorexic effects was time dependent as indicated by one-way ANOVA analysis. Actually, the maximum inhibitory effect for both compounds was observed during the second 4h period following the extinction of the lights. The expression of 5-HT1B receptors in the hypothalamus exhibited also a circadian rhythm. Interestingly, the acrophase of 5-HT1B receptors levels coincided with the time point at which the injection of CP-94,253 induced the maximum inhibitory effects on food intake. In contrast, we observed no circadian variations in the expression levels of 5-HT2C receptors. Conclusions: These results suggest that the circadian variations in the anorexic effects of 5-HT are due, at least in part, to the rhythmic expression of 5-HT1B receptors.

Gene expression dissection of the circadian neuronal circuit of Drosophila

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Purpose: To profile selective gene expression within multiple elements of the neural circuit controlling circadian rhythms in Drosophila. Methods: We developed a novel method to manually purify specific sets of circadian neurons as well as mixed, control neurons with the Gal4-Gal80-UAS system. Genome-wide gene expression analysis was then used to identify three different sets of highly enriched mRNAs: one shared by all clock neurons, a second only enriched in PDF-positive clock neurons (ventral Lateral Neurons, LNvs) and a third only enriched in PDF-negative clock neurons. Results: From each of these three sets of mRNAs, we successfully characterized three novel circadian genes. d4E-BP (Drosophila 4E-binding protein) is enriched in all clock neurons, and loss-of-function and gain-of-function mutations alter behavioral rhythms. The uncharacterized transcription factor Fer2 is enriched in LNvs; it plays a pivotal role in the specification of a subset of clock neurons and therefore their ability to drive locomotor activity rhythms. The Drosophila homolog of the mammalian circadian gene nocturnin is expressed in a different subclass of the circadian circuit and has a role in the regulation of the circadian light response. Conclusions: The methodology is a powerful tool not only to dissect the cellular and molecular basis of circadian rhythms but also to molecularly characterize neuronal circuits that contribute to many different problems in Drosophila neurobiology.

The daily variations of tryptophan hydroxylase -2-mRNA in the Syrian hamster are glucocorticoid-dependent

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Purpose: We have assessed the role of glucocorticoids in the daily variations of tph2-mRNA (messenger of the rate-limiting enzyme of serotonin synthesis) within the dorsal (DR) and median raphé (MnR) of the Syrian hamster. Methods: Tph2-mRNA levels were measured by in situ hybridization in Syrian hamsters maintained in a 14 h-light 10 h-dark photoperiod. In a first group, hamsters were adrenalectomized and given intra-peritoneal cholesterol pellets containing 1% cortisol and 2% corticosterone, delivering constant diurnal levels of glucocorticoids. In a second group, adrenalectomized hamsters implanted with the same glucocorticoids-containing pellets were given glucocorticoids in their drinking water during the night. Sham hamsters implanted with 100% cholesterol pellets were used as controls. Results: Sham hamsters were characterized by daily variations of tph2-mRNA in the DR and MnR, which were completely abolished after removing the daily rhythmic pattern of glucocorticoids secretion in the first group of adrenalectomized hamsters. Furthermore, the re-induction of an artificial glucocorticoids day-night surge in the adrenalectomized hamsters of the second group was able to restore the tph2-mRNA daily variations. Conclusions: Through their action on the expression of the tph2 gene, glucocorticoids may constitute a synchronizing cue able to promote a rhythmic functioning of the Syrian hamster serotoninergic system.

Molecular mechanism of circadian oscillation reset by prolonged treatment of cycloheximide in mouse suprachiasmatic nucleus

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Purpose: The purpose of present study is to investigate molecular mechanism of circadian oscillation reset by prolonged treatment of protein synthesis inhibitor in mouse cultured suprachiasmatic nucleus (SCN). Methods: The brain slices including the SCN were obtained from Bmal1-luc mice which carried a bioluminescent reporter for Bmal1 gene expression. The circadian gene expression rhythms were monitored in a cultured slice by a photomultiplier tube detector. After confirming the robust circadian rhythms for 5-7 days, cycloheximide (CHX), a potent inhibitor of protein synthesis, was added into the culture medium. The inhibition was stopped by medium exchange after 24 or 48 h. The amount of Per1, Per2 and Bmal1 mRNA were measured before, during and after CHX application by quantitative RT-PCR. Results: The bioluminescence from the cultured SCN was decreased to the background level by CHX administration, and recovered after the washout of CHX. On the other hand, the Per1 and Per2 mRNA were increased during CHX treatment and reached the plateau level after 18 h. The mRNA levels were rapidly decreased on the withdrawal of CHX and remained low level for approximately 8 h. By contrast, the Bmal1 mRNA level was increased after CHX treatment and reached the plateau level after 6 h, but decreased spontaneously after 18 h. The mRNA was decreased to the basal level, but increased again 12 h after washout. Conclusions: These findings indicate that the increase of Per1 and Per2 transcription by CHX was probably due to the inhibition of respective inhibitory proteins (PER1, PER2, et cetera) by CHX. On the other hand, the Bmal1 transcription is also enhanced probably through the Rev-erba and RORa circuits, but the enhancement does not continue for a full circadian cycle. The balance between REV-ERBa and RORa seems to be involved in the time course of Bmal1 transcription.

The clock in the worm

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Purpose: Caenorhabditis elegans is an important model system for biological research in many fields including genomics, cell biology, neuroscience and aging. Several reports have shown evidence of a circadian clock, using either activity or resistance to osmotic shock. We find these nematode circadian phenotypes subtle so we are working to develop a more efficient system for circadian experimentation with worms. Methods: A fundamental property of circadian clocks is their pervasive regulation of physiology. We adapted a chemotaxis assay based on olfaction to monitor changes in olfactory sensitivity over the day. Age matched adult worms were raised in entraining temperature cycles. They were exposed to the repellant 1 octanol for 15-min, a sub-threshold condition. Re-

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sults: We find that 1 octanol is an effective repellant for C.elegans in presence of E. coli. Preliminary results show a bimodal response pattern to 1-octanol over the day. Conclusions: The response to a repellant olfactory cue changes through a mild, 24h temperature cycle in C. elegans.

Molecular analyses of Hiomt gene and its association with circadian phenotypes in humans

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Purpose: To search for polymorphisms in the Hiomt (hydroxyindole-O-methyltransferase) gene that encodes the last enzyme in the melatonin synthesis pathway and to verify possible associations between genetic variations in this gene and circadian phenotypes in a Brazilian population sample. Methods: Based on the questionnaire Horne & Östeberg (HO) score, we selected volunteers with extreme morning and evening preference. Volunteers that did not answer the HO questionnaire were selected as a sample of general population and seventeen DSPS (Delayed Sleep Phase Syndrome) patients participated in this study. The Hiomt gene was screened using DHPLC (Denaturing High Performance Liquid Chromatography) analysis system and direct sequencing. For six polymorphisms found with high frequency, we confirmed the genotype by TaqMan SNP Genotyping Assays. We used haploview software to performed haplotypic analysis. Results: We have found eight SNPs in our sample, being that one of them was not reported yet. We found also one VNTR (not reported in the literature) and one deletion. From the SNPs found in the screening, we observed an association of one them (rs28675287) with DSPS. Haploview analyzes shown that there is linkage disequilibrium between pairs of polymorphisms in the promoter region. The haplotype AG located in the first pair block 5` upstream is associated with evening preference. Conclusions: The analysis of our data indicates that polymorphisms in the Hiomt gene are associated with circadian phenotypes in humans. The likely mechanism is that these genetic variations affect the rate and/or level of melatonin synthesis. A next important step is look for the effects of these polymorphisms in the profile of melatonin synthesis and secretion in controlled conditions.

Per3 gene in Callithrix jacchus: full molecular analysis and VNTR comparison with other primates

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Purpose: To characterize Per3 gene in marmosets, to search for polymorphims in exonic regions, especially in one particular VNTR. To compare the VNTR length among new world primates species. Methods: Initially, to get the sequence of marmoset´s cDNA, the primers were designed based on human, chimpanzee and rat cDNAs. Total RNA was extracted from liver of a marmoset and the cDNA was made from this. When a marmoset Per3 draft contig became available in UCSC genome browser we started to use the draft sequence to help with the intronic regions. We use Phred/Phrap/Consed package to assembly all the gene sequence. To amplify intronic sequences the DNA was extracted from blood. And to search for length polymorphisms in VNTR region 70 marmosets, 33 capuchin monkeys and one Goeldi´s marmoset were analyzed. Now we started to look for SNP in 50 marmosets by DHPLC technique in exonic regions. Results: The entire cDNA sequence showed 4173 bp and the identity of marmoset cDNA with the human and marmoset of Per3 was 82% and 92%, respectively. More than 50% of intronic fragments were sequenced, this contig is incomplete and when compared with UCSC marmoset contig we can observe some relatively large differences from our sequences. These sequences showed up 81-89% of identity with the related sequence of the hPer3. In the VNTR region, only one marmoset presents a polymorphism (7 and 6 repeat), while the Capuchin monkeys carry two copies and Goeldi, six, but there were no polymorphism's variation in these two species. Conclusions: With the Per3 sequenced and characterized we will be able to study gene-environmental interactions in marmoset animal model of circadian rhythms.

Investigations on the circadian clock in Branchiostoma

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Purpose: To elucidate the evolutionary history of the clockwork on the behavioral, anatomical, and molecular level. Methods: Behavioral investigations, gene cloning, in situ hybridization, phylogenetic analyses, molecular modelling. Results: Branchiostoma (Amphioxus) is a close living evertebate relative of vertebrates. We found that Branchiostoma displays rhythmic locomotor activity persisting under constant darkness, suggesting the presence of a circadian oscillator. We cloned the amphioxus homologs of the clock genes Bmal (amphiBmal) and Per (amphiPer) and localized amphiPer and amphiBmal mRNAs in a restricted cell group in the anterior neural tube, in a region corresponding to the diencephalon of vertebrates. Both amphiPer and amphiBmal showed rhythmic and circadian fluctuations. In phylogenetic analyses we found that the overall amino acid sequences of AMPHIPER and AMPHIBMAL are more similar to the various vertebrate PER and BMAL proteins than to those of insects. However, computational modelling of the BMAL PAS domains showed that the AMPHIBMAL PAS domain retains a plesiomorphic trait in its molecular morphology that was not evident from the sequence analyses alone. This trait may affect the protein-protein interactions of the PAS domain and, thus, heterodimerization of BMAL with CLOCK. Among vertebrate BMAL proteins, the plesiomorphic trait is retained in the BMAL2 proteins, but is changed to a single new, apomorphic trait in the BMAL1s. Conclusion: The rhythmic behavior of Branchiostoma and the

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spatio-temporal pattern of clock gene expression suggest that Branchiostoma possesses a homolog of the SCN. The particular molecular morphology of the PAS domain of BMAL1 may be an evolutionary novelty that arose with vertebrates and may, among others, be responsible for the subtle differences in the interaction of the components of the clockwork that are observed between vertebrates on one hand and evertebrates on the other.

Circadian rhythm dissociation in a diurnal primate (Callithrix jacchus) under T cycles shorter than 24 hours

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Purpose: To evaluate circadian motor activity rhythm of the common marmoset under symmetric light-dark (LD) cycles shorter than 24 h. Methods: Motor activity of 6 adult females, in individual cages, was continuously registered. The animals were isolated in a room with controlled temperature and humidity, and sound attenuated. The light intensity during light phase was 146.5 ± 53.3 lx whereas dark phase consisted in total darkness. They were maintained under symmetric LD cycles with periods of 21 (T21), 21.5 (T21.5) and 22 h (T22) during 60, 35 and 48 days, respectively. Periodogram Sokolove-Bushell was used to measure the percentage of variance (VSP), an indicator of rhythm's significance. Results: Two components of circadian motor activity rhythm were seen for all animals on T21, one with the same period of external LD cycle, named light-entrained component (LEC), and the other in free-running, named non-light-entrained component (NLEC). In the other two conditions, only 83.3% (T21.5) and 33.3 % (T22) of the animals showed two significative components. The value of VSP of LEC was 8.2 ± 1.1, 14.2 ± 2.5, 24.8 ± 8.0, and of NLEC was 12.0 ± 5.3, 10.9 ± 3.9, 11.9 ± 1.5 to T21, T21.5 and T22, respectively. Conclusions: Motor activity rhythm of common marmoset can dissociate in two circadian components under T21, which may be driving by different groups of oscillators.

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The zebrafish pineal gland transcriptome: a focus on circadian genes

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Purpose: Towards understanding the regulation and function of clock controlled genes (CCGs) within a central clock organ, we analyzed the pineal gland transcriptome in zebrafish, an advantageous animal model for studying gene functions. Methods: Zebrafish pineal glands were collected every 4-hr throughout two daily cycles under constant dark and RNA was hybridized with affymetrix microarrays, providing a genome-wide examination of gene expression. The microarray datasets were normalized and searched for periodic time-dependence using Fast Fourier analysis (FFT). Results: The data analyzed by the microarray experiment yielded a subset of 82 transcripts which demonstrate circadian rhythmic profiles, including several known clock genes and CCGs such as bmal1, cry2a, cry3 and aanat2. Many transcripts in this list that are involved in different cellular and physiological pathways such as transcriptional regulation, phosphorylation, ubiquitination, and phototransduction were recognized as circadian for the first time. Some genes are clock-related family members which were only recently cloned in zebrafish and their possible role in the core clock mechanism and its output pathways is yet unknown. Few of the transcripts in the list were identified as novel genes, an intriguing result which warrants an in-depth study of their function. Conclusions: This genome-wide analysis emphasizes the ubiquitous role of circadian timing in pineal physiology and serves as a basis for further investigation of gene function and interactions within the clock system. In-vivo, in-vitro and bioinformatic approaches which are well established in the zebrafish model will be used to study the function of selected CCGs and promoter elements. Taken together, this research will increase our understanding of the intricate machineries that underlie circadian rhythmicity.

Expression and functional analyses of circadian genes in mouse oocytes and preimplantation embryos: cry1 is involved in the meiotic process independently of circadian clock regulation

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Purpose: In mammals, circadian genes, clock, arntl (also known as bmal1), cry1, cry2, per1, per2 and per3, are rhythmically transcribed every 24 h in almost all organs and tissues to tick the circadian clock. However, their expression and function in oocytes and preimplantation embryos have not been investigated. Methods: Presence of the transcripts of circadian genes in both oocytes and preimplantation embryos was analyzed by Real-time PCR. Immunofluorescence was used to analyze CLOCK, ARNTL, and CRY1 localizations in oocytes and preimplantation embryos. Knockdown of CRY1 was achieved by RNA interference. DAPI staining was used to identify the meiotic oocytes. Results: Amounts of the transcripts of circadian genes did not oscillate every 24 h in 1- to 4-cell and blastocyst-stage embryos. CLOCK, ARNTL, and CRY1 were localized similarly in the nuclei of GV oocytes and 1- to 4-cell stage embryos. Since CRY1 is known to interact with the CLOCK-ARNTL complex to suppress transcription-promoting activity of the complex for genes such as wee1, cry2, per1, per2, and per3 in cells having the ticking circadian clock, we hypothesized that if the circadian clock functions in GV oocytes and 1- to 4-cell stage embryos, CLOCK, ARNTL, and CRY1 might suppress the transcription of these genes in GV oocytes and 1 - to 4-cell stage embryos as well. However, knockdown of CRY1 in GV oocytes did not affect the transcription levels of wee1, cry2, per1, per2 and per3 but reduced maturation ability. Conclusions: Circadian genes are not involved in circadian clock regulation in mouse oocytes and preimplantation embryos but are involved in physiologies such as meiosis.

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Sequential and compartment specific phosphorylation controls the life-cycle of the circadian CLOCK protein

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The circadian clock facilitates a temporal coordination of most homeostatic activities and their synchronization with the environmental cycles of day and night. The core oscillating activity of the circadian clock in Drosophila and mammals is formed by a heterodimer of the transcription factors CLOCK (CLK) and CYCLE (CYC). Post-translational regulation of CLK/CYC has previously been shown to be crucial for clock function and accurate timing of circadian transcription. Here we report that a sequential and compartment specific phosphorylation of the Drosophila CLK protein, assigns specific localization and activity patterns. Total and nuclear amounts of CLK protein were found to oscillate over the course of a day in circadian neurons. Detailed analysis of sub-cellular localization and phosphorylation revealed a nucleo-cytoplasmic shuttling of CLK. Newly synthesized CLK protein is hypo-phosphorylated in the cytoplasm prior to nuclear import. In the nucleus, CLK is converted into an intermediate phosphorylated state that correlates with trans-activation of circadian transcription. Nuclear export promotes hyper-phosphorylation and degradation of the CLK protein. Surprisingly, CLK localized to discrete nuclear foci in cell culture as well as in circadian neurons of the larval brain. These sub-nuclear sites likely contain a storage form of the transcription factor, while homogeneously distributed nuclear CLK is transcriptionally active. These results show that sequential post-translational modifications and sub-cellular distribution regulate the activity of the CLK protein, uncovering a core post-translational timing mechanism of the circadian clock.

Resynchronization of circadian activity rhythms following various Zeitgeber shifts in phenotypically different Djungarian hamsters

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Purpose: In Djungarian hamsters bred at our institute two distinct phenotypes have been observed, the wild type (WT) and the DAO type. The latter is characterized by a delayed activity onset. As the activity offset remains synchronized with light-on, this delay leads to a compression of the activity time and finally to a free run or arhythmicity. One of the possible reasons may be a deficient mechanism of photic entrainment. Therefore, experiments with Zeitgeber shifts were performed. Methods: Investigations were carried out on WT and DAO hamsters being kept singly under standardized laboratory conditions (L:D = 14:10). Motor activity was monitored by means of passive infrared motion detectors. Advances and delays of the LD Zeitgeber were realized by single shortening and lengthening of the light (L) or the dark (D) period by 6 h. Results: Following a shortening of L or D, WT hamsters did re-entrain by advancing their activity rhythm, whereas a delay was observed following lengthening of L or D. DAO hamsters did re-entrain by a delay in both cases. Also, whereas all WT hamsters were able to resynchronize, a considerable part of DAO animals was not. They did instead start to free run, got arrhythmic or did switch to a diurnal pattern. Resynchronization was slower following an advancing LD shift compared to a delaying one and when changing L compared to changing D. Differences in the resynchronization time between phenotypes were found only when the Zeitgeber was advanced with DAO hamsters being much slower. Conclusions: The results support our hypothesis of a diminished ability of DAO hamsters to be entrained by the light-dark cycle. Also, they do fit with phase responses to light pulses obtained in hamsters of both phenotypes.

Synergic entrainment of Drosophila's circadian clock by light and temperature

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Purpose: Daily light and temperature cycles are the most important Zeitgebers for circadian clocks. The influence of each single Zeitgeber on the clock has been well studied; but little is known about the synergic effects of both Zeitgebers on the clock. In nature, light and temperature show characteristic daily oscillations with the temperature raising during the light phase and reaching its maximum in the late afternoon. In the present study, we studied behavioral and molecular rhythms in Drosophila melanogaster under simulated natural light-dark (LD) and temperature (T) cycles that typically occur during the equinox in October. Methods: Wild-type flies were either subjected to simulated LD or T cycles alone or to a combination of both (LD cycle: 12:12 with 1.5h dawn and dusk; T cycle: gradually changing between 16℃ and 26℃ with the maximum in the evening). Behavioral and molecular rhythms (TIM immunostaining of the clock neurons in wholemount brains) were assessed under the 3 different conditions. Results: Although behavior rhythms were entrained by all three conditions, the rhythms were most strongly set by the combination of LD and T cycles. The clock neurons responded differently to LD and T cycles. Some were not entrained by T cycles alone, others were barely entrained by LD cycles alone. The combination of the two Zeitgebers entrained all clock neurons, strongly enhanced the amplitude of TIM cycling in the majority of cells and set the oscillations in all clock neurons to a similar phase. Conclusions: Our results show that the two Zeitgebers synergistically entrain behavioral and molecular rhythms of Drosophila melanogaster.

Effects of DNA damage response on circadian rhythms

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Purpose: Cell division cycle seems to be gated by circadian clock, through the clock transcription factor (BMAL1/CLK) induced cell cycle kinase, WEE1. On the other hand, a clock component (i.e. FRQ in Neurospora crassa and mPer1 in mice) is phosphorylated by the cell cycle kinase, Chk2, upon DNA damage. Experiments re-

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veal that ionizing radiation treatments create predominantly phase advances in circadian rhythms. The molecular details of how DNA damage does not cause relevant delays, remains uncovered. Methods: In order to address the minimum criteria for DNA damage-induced phase advances in circadian rhythms, we employ our previous simple mammalian circadian clock model [1] and also a more detailed mathematical model [2] to study possible Chk2 dependent molecular mechanisms that produce the observed behavior. We hypothesize different Chk2 targets of PERs (monomer, dimer, and complex with BMAL1/CLK), and analyze the importance of regulatory feedback loops in the circadian system. Results: We show [3] that the unique phase advances of the circadian clock from DNA damage response are observed when Chk2 only affects PERs that are not bound to BMAL1/CLK. Furthermore, existence of an autocatalytic positive feedback mechanism in addition to the time-delayed negative feedback loop seems to be essential. Conclusions: The implications of the advanced phase responses of the circadian clock are to provide a mechanism to ensure more time for DNA repair. Our simulations suggest that Chk2 dependent phase advance is the best strategy to induce large induction of WEE1 that inhibits entry into mitosis. Cell cycle network is ingeniously wired with circadian rhythm for an optimal result upon DNA damage.

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The role of CREM/ICER in the circadian expression of Per1 and Per2 in the mouse liver

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Aim: To characterize the role of Crem gene isoforms (CREM activators and ICER repressors) on the circadian expresison of Per1 and Per2 in the mouse liver. Methods: Wild type and Crem knockout mice were sampled circadially every 4 h in DD, 6 animals per time point. Per1 and Per2 gene expression was evaluated by Q-PCR. Expression of ICER isoforms was monitored by PCR. CRE elements in mPer1 and mPer2 promoters were mutated and the role of individual elements studied by promoter-reporter analyses. Results: The expression of Per1 in w.t. mouse livers peaks at CT12. Per1 amplitude is lower in Crem -/- animals, suggesting the involvement of CREM activators in expression of Per1. The difference is most evident at CT12, which was confirmed in 2 experiments. Four potential CRE elements reside in the 1.8 kb mPer1 promoter. Mutation of mPer1 CRE2 lead to significant (p ? 0.05) decrease in CREM-mediated transactivation. The expression of Per2 in w.t. mouse livers peaks at CT12 – CT16. Per2 amplitude is higher in Crem -/- mice compared to controls, suggesting involvement of ICER repressors in circadian expression of Per2. The 1,6 kb mPer2 promoter contains four potential CRE sites, however, none of them is individually responsible for the CREM-mediated transactivation. Four ICER isoforms (I, I?, II, I?) reside in the mouse liver, the ratio between them is changing circadially. Conclusions: Different mechanisms are involved in the circadian regulation of Per1, Per2 by CREM/ICER. The circadial expression of Per1 is diminished and that of Per2 increased in livers of Crem-/- mice compared to wild types. Promoters of mPer1 and mPer1 each contain four potential CRE elements. To understand the CREM-dependent mechanism in circadian expression of Per1 and Per2, the importance of individual CRE elements has to be established and CREM/ICER isoforms that bind to CREs have to be identified.

2. Visual and circadian photoreception, non-photic cues

Evidence for a Zeitgeber-effect of passive heat loading in men

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Purpose: We aimed at testing the hypothesis that an external heat pulse in the evening, when core body temperature (CBT) decreases, induces a phase delay of the human circadian system, similar to the effect of light. Methods: Eight healthy male subjects (23-29y) were recruited for an intervention group (IG). Participants conducted a constant routine (CR) protocol including 40-h prolonged wakefulness under dim light conditions (<8lux). Passive body heating (PBH) took place at 14.83h after lights on (lon) for 30min. Subjects were immersed into warm water via a crane (39℃, only head out, minimal postural changes). A matched control group (CON) underwent the CR without PBH. CBT was registered via a rectal probe sampled at 30-s intervals. In order to determine melatonin concentration (MEL) saliva was collected every 30min throughout the protocol. Results: Time course analysis of CBT and MEL for the time segment before PBH (=baseline, BL) revealed no significant differences to CON (p>0.43). In contrast, CBT during the time segment after PBH (post-treatment day, PD) exhibited significant differences to BL (p<0.001). Both MEL and CBT time courses displayed significant interaction terms (BL vs. PD x IG vs. CON x TIME; ANOVA, p<0.01). Compared with CON, CBT and MEL exhibited an earlier decline and increase in the evening of PD, respectively. Different methods for analyses of circadian phase markers (mid range crossing time; CBT minimum; dim light melatonin onset, DLMO) revealed a phase shift of about 1.5h for CBT and 0.8h for DLMO compared to CON. Conclusions: We could demonstrate a circadian phase advance after a single heat pulse in CBT and to some extent in MEL in humans under CR conditions. Due to the fact, that there is no phase response curve to heat in diurnal mammals, this study marks the beginning of a systematic investigation.

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Motor learning during circadian wheel running: A fresh perspective using a new model rodent

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Purpose: To quantify and obtain a learning curve for motor

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learning during circadian locomotor activity in the Indian Pygmy Field Mouse, Mus terricolor. Methods: Adult mice were captured from crop fields and acclimated to laboratory conditions. Behavioral test-naïve animals were allowed continuous access to non-motorized running wheels for up to 30 days and locomotor activity was monitored using the Clocklab (Actimetrics) data recording and analysis system (Coulbourn, USA). A group of mice having 15 days prior wheel-running experience served as the control. The maximum wheel revolutions/min (counts/min) achieved for each day was noted using an inbuilt Clocklab function and a leaning curve was plotted. Results: M. terricolor exhibited a robust circadian locomotor activity rhythm with a ? of 23.80?0.26 h and acrophase at 00:26±1:42 h. Behavioral test-naïve animals showed a significant (P<0.001) increase in maximum counts/min increasing from 26.39?6.05 (counts/min) to 68.86?5.49 (counts/ min). The control group exhibited 63.8?7.1 (counts/min) throughout the duration of the experiment. Conclusions: M. terricolor, a known crop pest, is a good model organism. Motor learning is evident during circadian wheel running in M.terricolor, and was quantified. A plethora of research applications can be found for this phenomenon. The learning period in Clock mutant mice can be checked. Fast- and slow-learning lines can be created using this phenomenon as a screening parameter. The effect of therapeutics, aimed at treating motor-deficit disorders and other diseases, on the duration of motor improvement can also be studied.

Social context-dependent reorganization of the molecular clockwork in honey bees

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Purpose: The social environment interacts with the circadian clock of diverse animals, even in species with no elaborated social life style. However, little is known about the functional significance of this interaction, the specific social signals, or the dynamics of the ways in which the social environment modulates the circadian system. The aims of this study were to determine whether task-related plasticity in circadian rhythms in the honey bee is socially regulated, and to characterize the dynamics of context-dependent reorganization of behavioral and molecular circadian rhythms. Methods: We manipulated the social environment of young nurse-age bees and combined direct observations in the hive, analyses of locomotor activity in the laboratory, and measurements of brain clock gene expression with quantitative real-time PCR, to compare behavioral and molecular circadian rhythms. Results: Nurse-age bees that were restricted to a broodless honeycomb, but not nurses on an adjacent brood-containing honeycomb, showed robust circadian rhythms in activity and in clock gene expression. Nurses that were removed from the hive environment showed circadian rhythms in locomotor activity after a few hours outside the hive, and in clock gene expression after about 16 hrs. Conclusions: Plasticity in circadian rhythms in honey bees is regulated by specific social signals in the hive. These signals are non-volatile, and appear to be mediated by direct contact with the brood. Plasticity in behavioral rhythms is associated with relatively rapid reorganization of the molecular clockwork. The removal experiments emphasized the importance of an ecologically-relevant environment in studies on natural behavior because they show that the behavior and physiology change rapidly when an animal is removed from its natural environment.

Loss of photic entrainment at low illuminance in rats with acute photoreceptor degeneration

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Purpose: To investigate the effects of chemically-induced photoreceptor degeneration on the ability of rats to entrain to different light intensities. Methods: N-methyl-N-nitrosourea (MNU) (75 mg/kg) was injected into Long Evans rats, and then we measured the subsequent loss of rods and cones, quantified the number of melanopsin expressing cells along with the down-regulation of melanopsin and investigated the ability of these animals to photoentrain to different LD cycles (300, 15 and 1 lux). Results: Control animals entrained their locomotor activity rhythms to the three cycles. In contrast, MNU-treated animals could only entrain properly to the 300 lux cycle. For the 15 lux cycle, their phase angle was much altered compared to control animals. For the 1 lux cycle, MNU-injected animals showed no photoentrainment and an apparent free-run activity pattern with a period of 24.3 h. Subsequent to behavioural studies the animals were killed and rod, cone, melanopsin and melanopsin expressing cell loss were quantified. Rod and cone loss were almost complete, melanopsin protein was reduced by 83 % and melanopsin expressing cells were reduced by 37 %. Conclusions: Induced photoreceptor degeneration in adult rats dramatically affects their ability to entrain the circadian system at low light levels, presumably through rod-based mechanisms.

Mealtime entrains behavioral, hormonal, metabolic and neural parameters in the Rabbit pup

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Purpose: To study whether once a day nursing entrains hormonal, metabolic and neural parameters in newborn rabbits. Methods: Pregnant rabbit does were housed under a controlled light cycle (12/12 h LD; lights on at 07:00 = ZT0), stable temperature (23±2℃), provided with rabbit pellets and water ad libitum and were maintained in circadian recording cages equipped with two compartments one for the mother and one for the pups. Mother's compartment was in LD but pups were kept in constant darkness. Pups were scheduled to suck milk at either daytime (ZT03 group) or nighttime (ZT19 group). At PD7 pups were sampled every four hours to determine rhythms in stomach weight, corticosterone (CORT), ghrelin (GHRE), glucose, liver glycogen, free fatty acids (FFA) and PER1 protein in the suprachiasmatic nucleus (SCN) and dorsomedial hypothalamic nucleus (DMH). The same parameters were explored at PD9 in fasted subjects at PD8. Results: In both groups actograms showed a circadian component of 24 h with a maximum activity before nurs-

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ing. Stomach weight was highest 4 h after nursing; first 2/3 of their content was evacuated in the first 12 h after nursing. CORT was highest around time of nursing, then decreased and increased again before next nursing bout. GHRE was low around time of nursing and peaks 12 h after nursing (P<0.05 in ZT03 group; P<0.07 in ZT19 group) and then decreased. Glucose remained stable around 160-220 mg/dl in both groups. Glycogen had lower levels at nursing time, increasing significantly 8 h later and then decreasing. FFA had a peak 4-8 h after nursing. Rhythms of CORT and FFA, but not liver glycogen and stomach weight, persisted in fasting. Glucose remained stable with lower values but GHRE values increased. Timing of nursing induced a phase shift of 2.5 h in PER1 that affects mainly the amplitude in the SCN, but in the DMH induces a complete phase shift. Conclusion: Meatime is a potent non-photic zeitgeber for newborn rabbit pups.

Impact of the absence of melanopsin on the endogenous functioning of the mammalian retinal clock

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Purpose: The mammalian retina contains an endogenous pacemaker that regulates retinal physiology and adjusts daily the temporal phase of the central circadian timing system with environmental time. This entrainment process involves rods, cones and melanopsin-expressing retinal ganglion cells. In contrast with non mammalian retinas, in which the clock has been identified in photoreceptors, the location of the retinal circadian clock in mammals is still controversial. In addition, the impact of specific photoreceptor degeneration on the molecular machinery of the endogenous retinal clock is unknown. Methods: The experimental strategy is based on the isolation of the retina in two separate compartments: inner (inner nuclear and ganglion cell layers) and outer (cones and rods) using laser microdissection and real time RT-PCR. We investigate clock gene expression in these two retinal compartments during the 24hr cycle at six circadian times in the wild-type mouse.We next evaluated the impact of the absence of melanopsin on the endogenous functioning of the retinal clock by using Opn4-/- transgenic mouse model. Results: We find that clock genes and clock-controlled genes are expressed in a circadian manner not only in the inner nuclear and ganglion cell layers but also at the level of photoreceptors in the wild-type mouse. In addition, the absence of melanopsin results in dysfunction of the endogenous retinal clock, characterized by the alteration in amplitude and phase of the expression of certain clock genes. Conclusion: Our results suggest that the absence of a specific photoreceptor can contribute to a dysfunction of the retinal clock. Because circadian organization is widespread in the retina and controls fundamental pathways, disruption of circadian clock organization in the retina could potentially have a major impact on retinal functions such as gene cycling and photopigment regeneration.

Retinal melatonin synthesis impairment in streptozotocin-induced diabetic Wistar rats

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Purpose: Several studies have shown the physiological role of melatonin in the mammalian retina and its protective effect of diabetic STZ-induced rats. The aim of this work is to study the retinal melatonin content and AANAT activity in diabetic STZ-induced rats. Methods: Adult male Wistar rats were randomly allocated in two groups, control (citrate buffer) and diabetic (60 mg STZ /kg body weight). Tail blood was collected for glucose determination using a glucose meter, animals with glycemia > 200mg/dl were considered diabetic. Some of the diabetic animals were treated for 3 days with subcutaneous long-acting insulin (2U) in the beginning of the day and short-acting insulin (2U) plus long-acting insulin (2U) in the beginning of the night. All animals were sacrificed 3 days after streptozotocin and/or vehicle injection, every 3 hours throughout the 24h LD cycle. Retinal melatonin levels were measured by high performance liquid chromatography (HPLC) and AANAT activity was measured by a radiometric assay. Results: Our results show a circadian profile of retinal melatonin synthesis in the control group with a clear nocturnal peak. In the 3-days STZ-induced diabetic animals the retinal melatonin content was reduced on the dark period in comparison to the control group. In addition, insulin treatment restored the nocturnal melatonin levels to the same values of the control group. Regarding the retinal AANAT activity profile, diabetic STZ-induced rats showed the loss of AANAT rhythmicity which was restored on the insulin-treated animals. Conclusions: Our work is the earliest to show the diabetic reducing effects on retinal melatonin synthesis. Insulin treatment was able to recover both the levels and the rhythmicity pattern of retinal melatonin synthesis and AANAT activity.

Vesicular glutamate transporter 2 (VGLUT2) is co-stored with PACAP in central projections of the melanopsin containing retinal ganglion cells in the rat

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Purpose: Non-image-forming (NIF) functions including light entrainment of the biological clock, masking behaviour and the apillary light reflex are regulated via the retinohypothalamic tract (RHT) by a subset of retinal ganglion cells expressing melanopsin. Two neurotransmitters, glutamate and PACAP, have been identified in these intrinsically photosensitive retinal ganglion cells (ipRGCs), which upon activation releases the two neurotransmitters from nerve terminals in the brain. To further characterize the NIF system in rats, we examined the expression and co-localization of PACAP and VGLUT2, a marker for glutamate signalling, in ipRGCs and in nerve terminals in target areas of the NIF system including the suprachiasmatic nucleus (SCN), the intergeniculate nucleus (IGL) and the

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olivary pretectal nucleus (OPN). Methods: The anterograde tracer cholera toxin subunit B (CtB) was injected into both eyes of the rats to visualize RHT projections. By double and triple immunofluorescence against melanopsin, PACAP and VGLUT2 followed by confocal microscopy, we determined the degree of co-localization in the retina and in retinal target areas. Binocular enucleated animals were used as control. Results: PACAP and VGLUT2 were co-localized with melanopsin in ipRGCs in the retina. In the SCN, IGL and OPN, PACAP and VGLUT2 were found in retinal projections storing CtB. The majority of retinal projections contained both PACAP and VGLUT2. Conclusions: Our results demonstrated that PACAP and glutamate is co-stored in synapses in the SCN, IGL, and OPN. The study further indicated that VGLUT2 is involved in glutamate signalling in regulation of NIF functions.

The daily dynamics of melanopsin retinal ganglion cells

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Purpose: To analyze the daily variation of the mouse melanopsin-expressing cell types. Methods: Retinas of adults and during postnatal mice development were processed for immunochemistry. They were sampled every four hours under a 12-hour light-dark cycle and under constant darkness. Immunopositive neurons were classified and quantified attending to the localization of their soma and dendritic processes. A statistical analysis was carried out. Results: In the present study a daily oscillation in the number of melanopsin-expressing RGCs was observed in mice kept under a light / dark (LD) cycle. One hour before the lights were on (i.e., the end of the night period) the highest number of immunopositive cells was detected while the lowest was seen four hours later (i.e., within the first hours of the light period). We have also detected that these daily variations already occur in the early postnatal development, when the rod/cone photoreceptor system is not yet functional. The two main different melanopsin-expressing cell subpopulations (M1 and M2) also showed daily oscillations, although some differences between them were seen and their ratio changed along the 24-hour cycle. In order to find out whether or not the melanopsin rhythm was endogenous, other mice were maintained in constant darkness for six days. Under these conditions, no defined rhythm was detected. Conclusions: These findings suggest that some of the melanopsin-expressing RGCs “turn on” and “off” during the day/night cycle. The loss of daily variation in constant darkness suggests that either is light-dependent or is gradually lost under constant conditions.

Simulating aging of the eye in young subjects: experimental reduction of lens transmission and its effects on sleep and melatonin production in humans

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Purpose: To investigate how reduction in (blue) light intensity (as occurs during aging) affects sleep characteristics and melatonin rhythms in humans under real-life conditions. Methods: Via the use of soft orange contact lenses (OL) a decrease in light intensity, particularly in the blue range was obtained. 15 subjects participated in this study. In randomized order they started with the control (15 days of wearing their own contact lenses) or with the experimental condition (15 days of wearing OL). Actigraphy and sleep-diaries data were collected. For the last two nights of each 15-days session subjects came to our facility for collection of saliva samples to assess undisturbed melatonin profiles (<10 lux) and melatonin suppression in response to 2h of white light from 24h to 2h (600 lux, Osram tubes). On a separate night outside the 15-days sessions subjects came to our facility to assess the acute effects of wearing OL on melatonin suppression. Results: After wearing the OL for 15 days no shift in dim light melatonin onset was found in comparison to the control condition nor were there any changes in the amplitude of the melatonin rhythm. Furthermore, no significant differences were observed in the amount of melatonin suppression between conditions. However, the suppression of melatonin production immediately after starting to wear the OL was significantly reduced. Sleep parameters were changed by the use of OL; sleep was less efficient and more fragmented. Conclusions: The results show that after 15 days of wearing OL the system adapted to the change in light exposure and recovered its sensitivity to light. Although smaller in size the OL effects on sleep characteristics are reminiscent of the sleep changes that occur with ageing.

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A photoreceptor Clock shock: Differential roles of NPAS2 and Clock in the core circadian oscillator and regulation of a clock-controlled gene

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Purpose: Both NPAS2 and CLOCK are expressed in chicken retina. This study was conducted to explore the relative contributions of NPAS2 and CLOCK to the core circadian clock mechanism and to the regulation of clock-controlled genes (CCG) in chick photoreceptors. Methods: qRT-PCR was used to measure transcript levels. Western blot analysis was used to measure CLOCK and NPAS2 protein. The photoreceptor layer of 2 week old chick retinas was isolated by laser capture microdissection. Photoreceptor cell cultures were entrained to a light-dark cycle for 8 days before being transferred to constant darkness for measurements of mRNA of clock genes and the CCG Aanat. Artificial microRNAs (miRs) were transfected on day 4 in vitro to inhibit expression of CLOCK or NPAS2. Results: Clock and Npas2 were both expressed in photoreceptors in vivo and in vitro. In cultured photoreceptors, Npas2, Per2, and Bmal1 were all expressed in a circadian fashion, while Clock was constitutively expressed. NPAS2 miR significantly damped the rhythm of Npas2 mRNA and markedly reduced NPAS2 protein expression. However, NPAS2 miR had no effect on Clock mRNA levels or the rhythms of Per2 or Bmal1 transcripts. Never-the-less,

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NPAS2 miR significantly reduced but did not abolish the rhythm of Aanat mRNA. In contrast, CLOCK miR not only significantly reduced the expression of CLOCK protein, but also decreased the level of NPAS2 protein and nearly abolished the rhythm of Npas2 mRNA. In addition, CLOCK miR damped the rhythm of Per2 and Aanat expression, but not that of Bmal1. ChIP analysis indicated that both CLOCK and NPAS2 bound to the Aanat promoter in situ. Conclusions: CLOCK plays a dominant role in the core circadian oscillator of chick photoreceptor cells, while CLOCK or NPAS2 are capable of driving the rhythms of the CCG Aanat. Npas2 rhythms appear to be CLOCK-dependent. Bmal1 rhythms do not require normal expression of either CLOCK or NPAS2.

Intrinsically photosensitive ganglion cells in the retina of the diurnal rodent, Arvicanthis ansorgei

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Purpose: To characterize intrinsically photosensitive retinal ganglion cells (ipRGCs) in the cone-rich retina of the diurnal rodent Arvicanthis ansorgei. Methods: Retinae of A. ansorgei at different ages (from P1 up to few months old) were studied by electrophysiology ex vivo using multielectrode array (MEA) recording techniques. Multisite MEA recordings allowed us to study possible physiological heterogeneity of ipRGCs by monitoring large numbers of photosensitive ganglion cells simultaneously, and also to characterize these responses during development. Results: Recordings from ipRGCs show considerable differences in their response behaviour in comparison to rod- and cone-driven ganglion cell responses. Response onset of spike discharges is delayed and response duration elongated in comparison to normal RGC. Light responses persisted after blocking transmission of signals from rods and cones to the inner retina by applying APB (100 µM), NBQX (40 µM) and D-AP5 (30 µM). Furthermore, recordings from early postnatal retinae (P1-P8), when synaptic connections between the photoreceptor layer and inner retina are not yet established, confirm the intrinsic nature of the photoresponse of ipRGCs. In newborn A. ansorgei where photosensitivity of ipRGCs is not influenced by conventional photoreceptors, several types of ipRGCs could be observed, as distinguished by their response kinetics. Up to 40 light-sensitive units could be recorded from a single retina at age P1 and the responses were conspicuous even for light pulses as short as 100 ms. Conclusions: Retinae of newborn A. ansorgei show prominent light responses of ipRGCs that possibly consists of several subtypes.

Daily expression patterns of rhodopsin and its transcriptional regulators in Wistar rat retinal photoreceptors

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Purpose: to characterize daily expression patterns of RevErb-alpha, Nrl, Crx and Nr2e3 transcription factors that together constitute a major regulatory complex for the expression of rhodopsin gene in retinal photoreceptors. Methods: whole retinas or isolated photoreceptor layers from adult Wistar rats were collected over a 24h cycle in LD or DD and expression analysed by Taqman real time PCR and western blotting techniques. Results: transcription of rhodopsin gene shows a 24h rhythm, together with the amounts of three of its regulators: Nrl, Crx and Rev-Erb-alpha. In addition Nrl transcription factor displays specific circadian regulation likely involving post-translational regulation. It is known, indeed, that Nrl activity is strongly regulated by phosphorylation processes, dephosphorylation correlating with maximal transcriptional activity. We show here that the Nrl isoform showing the lowest apparent molecular weight in western blot and corresponding to the non-phosphorylated form of the protein, displays sustained rhythmicity, the maximum being attained by the end of the night, in synchrony with the amount of AA-NAT protein. We also show that nrl and crx display rhythmic transcription in photoreceptors, indicating they are themselves regulated by the clock. Conclusions: our results show that most factors in the Nrl/Crx/Rev-Erb-alpha/Nr2e3 complex display rhythmic expression and indicate this complex might be responsible for the cyclic transcription of rhodopsin gene in the retina. They further indicate that the rhodopsin gene, together with its regulators, constitutes a valuable model to investigate signalling pathways originating in the clock and affecting the expression of secondary order clock target genes.

Cone phagocytosis in neural retina leucine zipper (Nrl) knockout mice

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Purpose: Evaluation of circadian rhythm of cone phagocytosis in a S-cone rich retina model, Nrl-/- mice, during a 24 -h light/dark cycle. Methods: In these phagocytosis studies, eyes from young adult (2 months) Nrl-/- mice were taken, fixed every 3 hours under a 12-h light/dark cycle and stained by immunohistochemistry using anti-mouse S cone opsine antibody. The number of phagosomes in the retinal pigmented epithelium (RPE) was quantified using a morphometric system. Results: The number of S-cones (S -opsin) phagosomes present in the RPE showed a small peak 1 or 2 h after light offset and then a large peak 1 or 2 h after light onset, thereafter decreasing to low baseline levels by mid-day. S-cones (S-opsin) phagosomes in Nrl-/- mice were less numerous than rods in wild type mice where a broad peak of rod phagosomes has been demonstrated 1 to 2 h after light onset in previous studies. Conclusion: Cone outer segment phagocytosis and shedding can be observed in Nrl-/- mice and do not show similar profiles and amplitudes compared to rods in wild type mice. This strain may constitute a valuable novel animal model for investigating cone photoreceptor turnover.

Circadian photoreception in mitochondrial optic

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neuropathies: relative sparing of melanopsin-containing retinal ganglion cells

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Purpose: To test the integrity of the retino-hypothalamic tract (RHT), originating from the intrinsically photosensitive melanopsin-containing retinal ganglion cells (m-RGCs) in mitochondrial optic neuropathies (ON), which selectively affect retinal ganglion cells (RGCs). Methods: Clinical ON severity was determined by Optical Coherence Tomography. Melatonin suppression test was performed in 5 Leber hereditary optic neuropathy (LHON) and 4 dominant optic atrophy (DOA) patients, and in 9 gender and age-matched controls. We performed baseline and suppression night tests, the latter using monochromatic (470 nm) blue light between 1:30 and 3:30 AM. Melatonin was assayed by radioimmunoassay on plasma samples collected hourly from 12:30 PM to 3:30 AM. Immunohistochemistry was carried out on serial retinal sections from post-mortem eyes of two LHON and two control subjects. Human melanopsin antibodies were used to identify and count m-RGCs. Results: All patients had severe optic atrophy and normal pupillary light reflex (PLR). A significant suppression of melatonin plasma levels by light was observed both in control subjects (67± 17%) and patients (LHON 65±25%; DOA 53±33%). The suppression score was not statistically different among groups. m-RGCs in LHON were relatively preserved compared to controls despite the severe loss of total RGCs (98 and 75%, respectively). Conclusions: We demonstrate in both hereditary ONs, a substantial preservation of circadian photoreception and PLR, which in LHON relates to sparing of m-RGCs. It remains to be elucidated as to how m-RGCs are selectively spared by mitochondrial neurodegeneration in these disorders. Supported by Telethon grant GGP06233 to VC

Effects of feeding (time-scheduled vs random) on daily rhythms of locomotor activity and plasma cortisol and glucose in a teleost, the gilthead seabream

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Purpose: Food is hardly available continuously in the wild and so most animals show a wide variety of circadian rhythms which can be entrained to feeding time. The aim of this research was to study the influence of meal timing on gilthead sea bream behavioral and plasma metabolic and hormonal rhythms. Methods: Fish were subjected to different feeding regimes: scheduled at ML or MD, random, and restricted to a period of the day. Self-feeding and locomotor activity, as well as plasma cortisol and glucose were evaluated. Results: Scheduled-fed fish showed an increase in locomotor activity, known as food anticipatory activity (FAA), a few hours before mealtime. In addition, sea bream displayed dual behavior, with the animals fed during the light phase being diurnal and the animals fed during the dark phase nocturnal. The analyses of plasma cortisol and glucose showed marked differences between the groups, with the random feeding fish showing much higher concentrations. Cortisol showed daily rhythms which peaked at different times, depending on the light and feeding schedule in animals under a restricted phase of feeding. In animals fed daily at ML or at MD, the acrophase of cortisol was shifted several hours, synchronizing to the feeding cycle despite the lighting conditions. Plasma glucose rose much higher after meals in the random-fed group. Conclusions: Meal cycles entrained activity rhythms, with fish showing FAA, higher growth performance and maintaining low levels of plasma cortisol and glucose. The rhythm of cortisol, whose acrophase depends on the daily habits of the species studied (diurnal or nocturnal), was shifted in the gilthead sea bream by the mealtime. These results indicate cyclic feeding may be beneficial for fish welfare, while chaotic feeding regimes may lead to the loss of temporal integration and stress.

Disrupted phagocytosis of retinal rod and cone photoreceptors under constant light

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Purpose: To examine the effects of constant light upon the amplitude and profile of rod and cone photoreceptor phagocytosis. Methods: Two groups of adult A. ansorgei were maintained under two light conditions, standard 12/12 light/dark (LD) cycle and constant light for 7 days. After 7 days, retinas of animals were sampled every 3 hours (at zeitgeber time (ZT) 22, 1 and 4 for LD groups; and corresponding circadian time, CT, 1, 4, 7, 10, 13, 16, 19, 22 for LL groups. Eyes were enucleated and fixed and then were processed for double-label immunohistochemistry using rod- and mouse cone-specific antibodies (directed against rhodopsin and cone MW opsin respectively). The numbers of phagosomes in the retinal pigment epithelium were quantified using a morphometric system. Results: LD groups showed the expected large peak of rod and cone phagocytosis at ZT1, but with a previously unobserved non-uniform distribution: rod phagosome numbers in peripheral retina were higher than central retina. Rod phagosome numbers exceeded those of cones by about tenfold. LL groups showed scattered patterns of low rod and cone phagosome numbers throughout the 24 hr period, with no evidence of a major peak in the subjective early morning. There was no obvious degeneration of rods or cones (either cell loss or outer segment shortening) after extended continuous light exposure. Conclusions: Prolonged (7 day) exposure to constant lighting abolishes cyclic phagocytic activity in rods and cones, but does not induce detectable damage to either photorecep-

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tor type. Thus constant light leads to retinal clock malfunction.

Comparative study on free-running locomotor activity circadian rhythms in Brazilian subterranean fishes with different degrees of specialization to the hypogean life (Teleostei: Siluriformes; Characiformes)

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Purpose: Verify the presence of rhythmicity in the locomotor activity in six species of cave-dwelling fishes presenting different degrees of specialization to subterranean life. Methods: Locomotor activity was continuosly and automatically recorded with infra-red light beams for ten consecutive days of individuals kept in constant darkness. Results: Species tested show varied degrees of specialization to subterranean life and we found varying degrees of the circadian components of locomotor activity as measured by the periodogram algorhythm of Lomb-Scargle. Conclusions: Both the presence and robustness of the circadian components seem to vary according to the degree of specialization to subterranean life, the more specialized, the less circadian rhythmicity was detected.

Anticipation of two daily meals: circadian properties and neural correlates

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Purpose: The role of food as a 'zeitgeber' has been explored primarily by restricting food to a single daily meal. This paradigm has revealed circadian properties of food anticipatory rhythms, and the capacity for circadian function in the absence of the suprachiasmatic nucleus. However, natural environments presumably provide multiple daily feeding opportunities. Rats can anticipate two daily scheduled meals, which could involve multiple oscillators, clock consultation, interval timing or some combination. Experiments were conducted to assess the role of interval timing in anticipation of a second daily meal, and neural correlates of 2-meal anticipation at the level of cFos and Per1 expression. Methods: Male rats (n=16) were housed in running wheel cages under LD 14:10. The rats were food deprived for 34 h, then provided food (7 g) for 1 h twice/day, beginning 3 h and 13 h after lights-on. The first daily meal was omitted on day 13 of restricted feeding, both meals were omitted on day 16 (with no light that day), and the first meal was advanced by 3h on day 20. After day 22, the rats were sacrificed and perfused in groups of four at 4 time points. Results: All rats exhibited robust food anticipatory wheel running prior to both meals, of similar duration and magnitude. The timing of anticipatory activity to the second meal was not affected by omitting or advancing the first meal, and neither bout of anticipation was affected by omitting both meals for one day in constant dark. Conclusions: These results indicate that rats do not anticipate either of two daily meals by interval timers ('stopwatches') reset each day by feeding or LD cues. Whether anticipation of 2 meals is based on entrainment of 2 oscillators, or a combination of entrainment and clock consultation (discrimination of the phase of one entrained oscillator) remains to be clarified. Neuronal mapping of cFos and Per1 expression will reveal whether 2-meal anticipation is associated with unimodal or bimodal rhythms at the cellular level.

Effects of evening exposure to polychromatic white and blue-enriched light on the sleep and wake EEG in healthy older adults

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Purpose: Evening bright light exposure is reported to ameliorate age-related sleep complaints and daytime sleepiness. We aimed to examine the impact of evening light exposure and to compare two different light sources on the sleep- and wake EEG. Methods: Ten healthy older subjects (mean age=63.3 yrs; 6F) participated in a 13-day study. Three baseline days were followed by a constant posture (CP) in dim light to assess circadian phase. In the evening of days 5 to 8, the subjects were exposed for 2h to either polychromatic blue-enriched or white (4100k) fluorescent light of the same photon densities (mean 1.048E+15photons/cm2/s). A second CP on day 9 was followed by 3 more study days. Subjects were free to leave the hospital during the daytime except on CP days. EEG sleep recordings were visually scored according to standard criteria. Artifact-free wake EEG recordings were subjected to spectral analysis. Results: Wake EEG activity in the alpha range between 9.75 and 11.25 Hz was significantly higher during light exposures when compared to dim light condition ($p<0.05$) without differences between the light sources. Sleep latency was slightly longer in the night after light exposures for both light groups ($p<0.1$) and latency to REM sleep was significantly prolonged ($p<0.05$). Slow wave sleep tended to be longer after the white light exposures than to baseline. As reported elsewhere, light exposure produced phase delays of similar magnitude in both light groups. Conclusion: The evening light exposure had small effects on both the wake EEG and on sleep. We were unable to detect differences between the light sources, may be due to the small group size.

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A fly's eye technology in the human retina

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Purpose: To explore the hypothesis that melanopsin's bistable properties observed both in vitro in human and mouse melanopsin and in vivo in mouse are also ex-

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pressed in vivo in humans. Methods: We assayed the pupillary light reflex (PLR) in humans as a tool and applied a light stimulation strategy and mathematical models originally designed to demonstrate bistability and define photopigment states in invertebrates. Results: We show that the pupil only attains a fully stabilized state of constriction after several minutes of light exposure, a feature that is consistent with typical IRP photoequilibrium spectra. We further demonstrate that previous exposure to long wavelength light increases, while short wavelength light decreases, the amplitude of pupil constriction, a fundamental property of IRP difference spectra. Modelling these responses to invertebrate photopigment templates yields two putative spectra for the underlying R and M photopigment states with peaks at 481 nm and 587 nm respectively. Conclusions: These results suggest that the human retina exploits fly-like photoreceptive mechanisms that are potentially important for the modulation of non-visual responses to light and highlights the ubiquitous nature of photoswitchable photosensors across living organisms. Furthermore, this bistable mechanism may confer a novel form of “photic memory” since information of prior light conditions is retained and shapes subsequent responses to light.

Diminished circadian sensitivity to light in the elderly: aging of the ocular lens?

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Purpose: Aging is often associated with sleep and circadian rhythm disturbances that may result from inappropriate circadian entrainment related to impaired photic input. Although decreased sensitivity of the circadian system to short wavelength light (456 nm) has previously been reported in the elderly1, the origin of this diminished circadian response remains unidentified. The aim of this study is to investigate whether age-related changes in circadian sensitivity to light are the consequence of an increase in ocular lens density. Methods: Eight participants (55 to 63 years old) were exposed to 60-min of monochromatic light at nine different wavelengths (420–620 nm) of equal photon density (3.16 x 1013 photons/cm2/sec). Plasma melatonin suppression, as an index of circadian sensitivity to light, was compared across light treatments. Photic sensitivity spectra were then established for each subject. Lens density measurements were assessed using a validated psychophysical technique that eliminates the effects of inaccurate refractive correction and misalignment, and avoids effects of pupil size on measurements. Results: Compared to young subjects2,3, our preliminary results in the elderly show a specific decrease in circadian sensitivity to short wavelength light (< 500 nm), but no change of sensitivity for long wavelengths. Lens density measurements show a relative decrease in transmittance of the aged ocular crystalline lens for the same range of short wavelengths. Conclusions: Taken together, our results suggest that a decreased ocular lens transmittance selectively reduces circadian sensitivity to short-wavelength light in the elderly, and may contribute to impaired sleep and circadian rhythms.

This work was supported by grants from FP6-EUCLOCK

References: 1Herljevic M et al. 2005, Exp. Gerontology, 40 : 237-242 ; 2Brainard GC et al. 2001, J. Neurosci., 21(16) : 6405-6412 ; 3Thapan K et al. 2001, J. Physiol., 535 : 261- 267

Non-visual responses to simultaneous short and long wavelength monochromatic light in humans

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Purpose: Pre-exposure to long wavelength red light has been demonstrated to enhance non-visual responses to subsequent short wavelength blue light exposure in mice and this effect is proposed to arise from a long wavelength sensitive photoreversal function of melanopsin. The aim of the current study was to assess whether simultaneously presenting long wavelength red light with blue light will enhance non-visual responses to blue light in humans. Methods: Young, healthy males (18 – 35 yrs) were studied in a strictly controlled laboratory setting. Each study night a 30 min light pulse was administered on the rising phase of each individual’s melatonin profile. Subjective mood and alertness, heart rate and plasma melatonin levels were assessed at regular intervals before, during and after the light stimulus. Two different groups (Group A, n = 10; Group B, n = 6) were exposed to two different sets of light conditions consisting of single or simultaneous presentations of blue (?max 479 nm) and red (?max 627 nm) monochromatic lights at varying intensities. Results: Group A received photon matched light pulses (2.5 x 1013 photons/cm2/sec) of 479 and 627 nm light administered singly and in combination (479 + 479 nm and 479 + 627 nm; 5 x 1013 photons/cm2/sec). Significantly lower melatonin suppression was observed with 627 nm red light compared to all other conditions ($p < 0.0001$); no other significant differences were observed. Conclusion: Simultaneous presentation of equal photon density red and blue monochromatic light failed to significantly enhance non-visual responses to blue light at night in humans.

The compound eyes mediate appropriate entrainment of Drosophila’s circadian clock to twilight and moonlit nights

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Purpose: Fruit flies like to be active at dim light. If the nights are enlightened by artificial moonlight, wild-type flies become almost nocturnal (Bachleitner et al 2007, PNAS 9, 3538-3543), and if they are offered 1,5h of dawn and dusk they spend most activity around 5-10 lux during the twilight phase (Rieger et al 2007, JBR 22, 387-399). Eyeless flies do neither shift their activity into the night nor into twilight, indicating that the compound eyes are important to see dim light as well as the changes in light-intensity during twilight. We wanted to reveal the photoreceptor cells that responsible for this sensitivity. Methods: Wild-type flies and different photoreceptor mutants (clieya, ninaE117e, sev311, rh5, rh6 and rh5;rh6 double mutants and 3 different cryptochrome mutants) were recorded un-

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der rectangular 12:12 LD-cycles (at 10 to 10000 lux daylight intensity) with or without moonlight during the night, and under the same LD-cycles with simulated dawn and dusk with or without moonlight. Peaks of morning (M) and evening (E) activity were determined as well as mean activity levels for the day, the night and the twilight phases. Results: Except for the eyeless mutants, all mutants responded to twilight with a shift of M and E activity peaks into twilight; but the phases of M and E peaks occurred at higher twilight-intensities than in wild-type flies, indicating that all photoreceptor cells interact in perceiving twilight. rh5 and rh6 mutants as well as rh5;rh6 double mutants did not respond at all to moonlight, suggesting that photoreceptor cell 8 plays an important role in the perception of constant dim light.

Effect of lighting conditions on daily expression of clock genes and clock controlled genes in rat photoreceptors

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Purpose: Identification of the clock genes expressed specifically in rat photoreceptors and investigation of their daily expression patterns together with those from photoreceptor-specific clock controlled genes under different lighting conditions, in order to evaluate the existence of a circadian clock in these specialized cells. Methods: To investigate the expression of clock genes and clock controlled genes, two groups of 6 week old Wistar rats were housed under light/dark (LD) 12:12 and constant dark (DD) conditions. Animals were sacrificed every 4 hours during a 24h cycle and photoreceptor layers (97% rods) were isolated from the retinas using a tangential vibratome -based sectioning procedure. Circadian patterns in transcript levels of clock genes Clock, Bmal1, Per1, Per2, Cry1, Cry2, Rev-Erbalpha and Ror-beta and clock controlled genes NAT, AC1, c-Fos, rhodopsin, Nrl, Crx, Crem and recoverin were determined by quantitative real-time PCR. Beta-actin and PDE-6beta were used as reference genes for data normalization. Results: All tested clock genes are expressed in the photoreceptor layer of rat retina and show statistically significant daily changes in their transcript levels in LD conditions. In DD conditions, Cry2 and Ror-beta continue to show rhythmic expression. Clock controlled genes also show rhythmic expression in LD and similar patterns are retained in DD for NAT, c-Fos and Crem. Conclusions: rat photoreceptors contain the core clock genes of a circadian clock. Clock and clock controlled genes display rhythmic expression in LD and only a few of them show rhythmic patterns in constant dark, suggesting that, at the global level of the photoreceptor layer, lighting conditions critically affect the molecular clockwork and its outputs.

Modelling the effects of prior light exposure on melanopsin responses to light

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Purpose: In bistable photopigment systems, light elicits photosensory responses and drives photoregeneration of the chromophore to restore photic responsiveness. Melanopsin in the human retina has been shown to express bistable properties both in vitro and in vivo (Melyan et al 2005; Mure et al, submitted). These studies have shown that prior light exposure can modulate the amplitude of subsequent photic responses of melanopsin. In the present study, we attempt to model the capacity of the melanopsin photopigment system to respond to light following adapting pre-exposures to different light sources. Methods: We modelled the responses of the melanopsin photopigment system based on data for the equilibrium and difference spectra of melanopsin obtained by Mure et al in our laboratory, from studies of the human pupillary light reflex. Light spectra of broadband natural and artificial light sources were used to generate prior light stimulations to drive the melanopsin system to a defined state of equilibrium. Theoretically, this corresponds to the proportions of melanopsin isoforms in the 11-cis and all-trans retinal bound states. Mono- or polychromatic spectral templates were subsequently applied to examine the modulation of photic responsiveness. Results: The results suggest that prior exposure to light sources dominated by long wavelength light increase the ability of the melanopsin system to respond to subsequent light exposures, while light sources dominated by shorter wavelength light decrease the response. Exploiting the bistable properties of melanopsin could allow for optimization of spectral light distribution in industrial, domestic and clinical phototherapy applications by appropriate use of the potentiating effects of long wavelength light.

Development of daily body temperature and locomotor activity pattern's in newborn rabbits

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Purpose: To characterize the development of the diurnal pattern of the core body temperature and the locomotor activity in European newborn rabbits Oryctolagus cuniculus. Methods: The core body temperature and the locomotor activity rhythms were simultaneously measured by means of a telemetry system (VitalView, Respironics Minimitter, USA). The study was performed in 10 newborn rabbits from the postnatal day 2 (P2) to P15. The subjects were kept under constant light conditions and were fed by lactating does every 24hrs. The time series were preprocessed and analyzed by Fourier transform to determine the rhythm's frequencies, mesor, acrophase, and nadir. Results: In the body temperature the mesor was of 38.08±0.46ºC, we observed a gradual rise in average temperature, approximately of 1.1℃ during the first t wo weeks of the newborn's life. The Fourier analysis revealed that ultradian frequencies decreased along the days and at the same time the 24h frequency became the principal component of the pattern. For the locomotor activity, the average activity was of 50.53± 8.5 events per day, this average decreases with age to approximately 34.21 events per day in P15. The Fourier analysis revealed a similar tendency as the temperature, in which ultradian frequencies

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(1.5 and 2 hrs) of the locomotor activity pattern decreased with age, and from P9-P15 the 24 hrs frequency became the preeminent. Conclusions: The core body temperature and the locomotor activity patterns, showed similar variations related to age, in particular changes in the amplitude and in the frequencies of both rhythms. We observed that from postnatal day 9, newborn rabbits displayed a diurnal pattern with clear similarities to those previously described adult rabbits.

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Circadian and phototransduction components are present in chicken retinal ganglion cells

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Purpose: To study the transcriptional pattern of elements involved in the functioning of intrinsically photosensitive retinal ganglion cells (ipRGCs) in chicks by investigating the expression profiles of melanopsin genes (Opn4x and Opn4m), and of key enzymes involved in melatonin synthesis (AA-NAT, HIOMT). Methods: Disaggregated retinas of chick embryos, from day 4 to 18 (E4 to E18) and post hatching stages P5 and P10, were processed for RNA extraction, RT-PCR and quantitative RT-PCR. Primary cultures of chicken RGCs were obtained at E8 by anti-Thy-1 immunopanning. RGCs were kept under 12:12h LD cycles (cool bright light, 1200 lux) during 4 days, and at the 5th day were maintained in DD. AA-NAT activity was measured according to Garbarino et al, 2004, and immunocytochemistry performed with specific antibodies. Results: During retinal development, we observed early mRNA expression (E4) of transcription factors Pax6, Brn3b but not CRX, together with Gq, Opn4m, and of enzymes for chromophore regeneration (RPE 65, RGR, BCO). Expression of CRX, a-transducin G protein, rhodopsin and red opsin started later (E8-E12). In primary cultures of RGCs, Opn4x mRNA levels increased till day 5, then remained constant. HIOMT transcripts showed no changes across time. Immunopanned embryonic RGCs contained large numbers of Opn4x-immunopositive cells (over 15%). After 4 days of synchronization under a 12:12h LD cycle, cultures in DD showed, a circadian pattern of expression for both Opn4 isoforms, as well as for AA-NAT and HIOMT, however AA-NAT activity did not show significant variations along the day. A pulse of light at ZT8 in DD decreased AA-NAT and Opn4x expression, but increased HIOMT and Opn4m. Conclusions: In vivo and in vitro, the chicken retina shows differential expression patterns for melanopsin and melatonin genes indicating that their expression is modulated by both a retinal clock and the illumination conditions.

Influence of sleep homeostasis on clock related gene expression in the forebrain of SCN lesioned and intact mice

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Purpose: Sleep is regulated by two main processes: circadian (C) and homeostatic(S). Process C depends on a master pacemaker, the suprachiasmatic nucleus (SCN), and its rhythmic expression of several clock genes, including per1 and per2. Recent reports of sleep alterations in several different clock-gene KOs suggest an additional role in homeostasis. This is consistent with our observations that per1,2 expression in extra-SCN regions increase with wake and decrease with sleep. Methods: We used RT PCR and in situ hybridization to observe per1,2 expression in the brains of SCN lesioned (SCNx) and rhythmic (intact) mice. This was performed after a 6h sleep deprivation (SD) at light onset, after 2h sleep rebound, and in respective controls. We recorded sleep in SCNx mice to assess how the circadian process affects homeostasis. Results: Examinations verified the effects and size of each lesion. EEG analysis showed that sleep homeostasis is conserved in the absence of circadian drive as compared with control (sham lesion) mice. We also observed that SD elevated per1,2 cortical expression in SCNx and intact mice. Higher per1,2 baseline levels displayed in SCNx mice are also consistent with the higher day activity in these mice relative to controls. SD produced no change in expression of per1,2, within the SCN consistent with the primary role in regulating circadian timing. Conclusions: Our results show that sleep homeostasis is preserved in mice without a functional SCN and that clock-gene expression in extra-SCN brain regions are also driven by sleep homeostasis.

Non-circadian effects of light on mood in mice

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Purpose: Light stimulation plays a crucial role in the modulation of mood, partially by way of melanopsin. A great deal of research has been performed to demonstrate the effect of light on circadian rhythms and the relationships between circadian rhythms, alertness and depression. However, the direct and indirect mechanisms of light's influence on mood have yet to be determined. Our present study took an exploratory approach to test the hypothesis that light directly modulates mood in a non-circadian fashion. We compared the effect of an increased daily exposure to light on depression-like behaviors, in wild-type mice with mice in normal control conditions. Methods: The two photoperiod regimens were the standard 12L:12D as a control, and a skeleton-scotoperiod 12L:2D:8L:2D. After completion of the behavioral tests, mice were maintained for 16 days in DD followed by 32 days in 12L:12D to confirm that the circadian drive was still intact and entrainable by light. Behavior was evaluated by the Light-Dark Preference Test (LDPT) and the Forced Swim Test (FST). Results: We observed that that the parameters tested in the LDPT were not affected in the skeleton-scotoperiod contrary to the increased immobility time in the FST. Conclusions: These preliminary results suggest that light affects mood rather than anxiety in a direct, non-circadian fashion

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and will be further confirmed through a more extensive and comprehensive set of behavioral tests. The validation of our new experimental paradigm using excess light and causing a minimal effect on the circadian rhythm will be further explored in order to use it as a measure for addressing the direct influence of light on behavior.

The melanopsin-mediated direct effects of light on sleep and alertness interact with the circadian and homeostatic drive

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Purpose: Light influences sleep and alertness either indirectly through a well-characterized circadian pathway or directly through poorly understood mechanisms. Melanopsin (Opn4) is a retinal photopigment crucial for conveying non-visual light information to the brain. Methods: In various light-dark regimens (including 12h:12h light-dark (LD), single 1h L- or D-pulses during the 12h D- or 12h L-period, respectively; and one day under short LD1h:1h cycles), we analyzed sleep-wake time and the EEG in melanopsin-deficient (Opn4-/-) mice (n=10). Results: In contrast to wild type, single light pulse failed to induce sleep in Opn4-/- mice at this time of the day and the D-pulse-induced increase in EEG theta and gamma activity (EEG correlates of alertness and cognition) was delayed. Analysis of the LD1h:1h cycle revealed that only in Opn4-/- mice the light and dark effects greatly depended on circadian time. In addition to these acute light effects, Opn4-/- mice slept 1h less during the 12h L-phase. Despite this reduction in sleep time, EEG delta activity, a marker of sleep need, was decreased in Opn4-/- mice for most of the (subjective) D-period and the level of delta power reached after 6h sleep deprivation was significantly lower in Opn4–/– mice. This indicates that lack of melanopsin alters sleep homeostasis. Conclusions: The findings that melanopsin-mediated direct effects of light, the circadian drive and sleep homeostasis interact together to determine the timing and quality of sleep and waking. This calls for a re-evaluation of the role of light on human behavior and performance.

Light induces c-FOS expression in the SCN and the VLPO

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Purpose: Melanopsin, a photopigment involved in light absorption and irradiance level detection, is located in retinal ganglion cells (RGCs). Melanopsin is required, in particular, for the light-resetting of the biological clock through innervation to the suprachiasmatic nucleus (SCN). Conversely, the pathways by which these RGCs exert a direct influence on sleep and alertness remain poorly understood. The ventrolateral preoptic area (VLPO), a structure in the hypothalamus, where >50% of galanincontaining (GAL) neurons express c-fos during sleep, is targeted by melanopsinergic fibers. We hypothesize that these RGCs convey light information to “VLPO galanin sleep promoting neurons”, affecting sleep and waking independently of the circadian drive. Methods: We performed Galanin ISH and c-Fos immunostaining on brain sections in mice lacking melanopsin (Opn4-/-). The animals were sacrificed after a 1h light exposure. Results: Light induced c-FOS immunoreactivity in the SCN of both genotypes but was 2-fold smaller in Opn4-/- mice. This has been linked to a reduced ability to phase shift circadian rhythms in mice though does not rule out the possibility that reduced activation of SCN neurons could also contribute to the direct effects of light on sleep and waking. While overall c-FOS immunoreactivity in the VLPO area was not affected by the light pulse, the number of GAL neurons co-expressing c-FOS significantly increased compared to control conditions in Opn4+/+ mice. In Opn4-/- mice the induction of c-FOS in galaninergic neurons was absent. Conclusions: This suggests that melanopsin RGCs light in night-active species directly impinges on VLPO sleep-active neurons, resulting in an inhibition of arousal systems.

Suppression of SCN electrical activity during brief episodes of behavioural activity in mice.

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Purpose Behavioural activity influences the circadian pacemaker of the hypothalamic SCN. Here we investigate the relationship between type and intensity of spontaneous behavioural activity and SCN neuronal discharge levels in mice. Methods We performed in vivo electrical activity recordings in the SCN of freely moving mice and simultaneously measured the animal’s behavioural activity profile by passive infrared detection and infrared video camera recordings. Video images allowed us to discriminate between different types of behavioural activity, the most important ones being locomotor activity, resting, drinking, eating and grooming, and to distinguish three levels of intensity of the behaviour. Analyses of electrical activity of SCN subpopulations were performed offline. Results Our results indicate that brief episodes of behavioural activity are related to acutely decreased SCN neuronal activity levels. The neuronal suppressions show similar characteristics at different phases of the circadian cycle. Importantly, the magnitude of the suppression does not depend on the type or on the intensity of behaviour. Behavioural activity appeared to affect specific populations of neurons within the SCN. Conclusions We propose that the observed inhibitions reflect hyperpolarisations of SCN neurons, brought about by input from afferent pathways on subpopulations of the SCN. The amplitude of the SCN electrical activity is boosted by the presence of behavioural activity during the animal’s active phase. These find-

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ings may be relevant for reduced rhythm amplitudes in the elderly

3. SUPRACHIASMATIC NUCLEI, PACEMAKERS, CLOCKS AND PERIPHERAL OSCILLATORS

Modeling of the generalized photic perturbation

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Purpose: The suprachiasmatic nucleus (SCN) is the mammalian circadian center. The effect of the short-term light pulse in the SCN has been demonstrated in the phase response curve (PRC) and light exposure longer than 6 hours during the night also causes distinct phase-dependent phase responses (Comas et al. 2006). Further, an abrupt shift of LD cycle that causes a jet lag syndrome is associated with desynchronization of ventrolateral (VLSCN) and dorsomedial region of the SCN (DMSCN) (Nagano et al. 2003). In the present study, we propose a novel mathematical model that unifies the short- and long-term photic perturbation to the SCN. Method: We made a mathematical model that fits to the phase shift generated by both short-term and long-term light exposure. Assuming two different oscillators in VLSCN and DMSCN, we constructed the model of connected oscillators, of which parameters were determined by the phase responses after abrupt shifts of light:dark cycle, long-term or short-term light pulses. To examine the model, we irradiated the light to Wistar rats for three to twelve hours and assessed whether they produce the predicted phase responses. Result: The present mathematical model predicts the phase behavior of the circadian clock generated by short and long-term pulses. The validity of the model was confirmed in the experiments using rats. Conclusions: We constructed a model that offers a unified insight to the effects of light on the circadian center.

Lifelong consequences of early postnatal light environment

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Purpose: To examine whether early light experience has long-lasting effects on the brain master clock. Methods: Three groups of mice were reared under constant light (LL), constant darkness (DD) or light-dark cycles (LD) during the first 3 neonatal weeks. On postnatal day 21 (P21), all mice were weaned and transferred first to LL for 2 weeks, and then to LD until the end of the experiment. Locomotor activity was recorded throughout the experiment using infrared activity meters. At P50, brains were collected and immunohistochemistry was used to detect expression of arginine vasopressin (AVP), vasoactive intestinal polypeptide (VIP) and glial fibrillary acidic protein (GFAP) in the suprachiasmatic nuclei (SCN) of the hypothalamus. Results: In LL, LL-reared mice showed shorter free-running period and higher amplitude in the circadian rhythm of locomotor activity, compared to LD- and DD-reared mice. In LD, LL-reared mice also showed higher amplitude activity rhythm, with an earlier acrophase, and higher activity levels during the active phase. In the SCN, LL-reared mice had lower levels of AVP and VIP immunoreactivity, compared to LD- and DD-reared mice. Although GFAP-immunoreactivity was also lowest in the LL-reared group, the number of GFAP-positive cells was highest in this group, suggesting structural remodelling of SCN astrocytes by early light experience. Conclusions: Postnatal light environment has long-term effects on the expression of the mouse locomotor activity rhythm, and also on neuronal and glial cell populations of the SCN. We argue that these neurochemical and structural alterations may affect clock function, which may in turn modify animal's behaviour.

Heterogeneity in phase and period across the three dimensions of the SCN

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Purpose: The mammalian pacemaker in the suprachiasmatic nucleus (SCN) contains thousands of neural oscillators, with the majority of neurons capable of sustaining cell-autonomous rhythms of clock gene expression and electrical firing. A critical question for understanding pacemaker function is how individual oscillators are organized as a coherent tissue coordinating precise rhythms in behavior and physiology. Recent work suggests that the SCN is organized topographically into dorsal and ventral subregions that differ in phase and period, indicating functional heterogeneity across the oscillator population. However, in each study to date only a small proportion of the SCN has been examined and across studies discrepancies exist in SCN spatiotemporal organization. Methods: Here we undertake a comprehensive analysis of rhythmic gene expression (Per2:Luciferase) across the entire extent of the SCN using a newly developed Multi-position Automated Bioluminescence Imaging (MABI) approach. 44 coronal brain slices representing 4 rostro-caudal portions of the nucleus were recorded for 7 days, then analyzed to identify consistent patterns of phase and period in three dimensions Results: We find regional differences in phase and period across all three dimensions of the SCN. The consistency of these patterns across individuals and across planes of section suggests that the precise phasing of oscillators within the SCN is an important pacemaker feature. Conclusions: The present results underscore the sophistication of spatiotemporal organization within the pacemaker and suggest new directions for exploring its basis and function.

Histological organization and distribution of neuropeptides in the suprachiasmatic nucleus of the camel (Camelus dromedarius)

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Purpose: To study the histological organization and neuropeptides cartography of the suprachiasmatic nucleus (SCN) in the camel. Methods: Classical histology and immunofluorescence were used on hypothalamic specimens obtained from fixed brains of slaughtered dromedaries. Paraffin embedded sections (n=15) were stained with hematoxylin-eosine, toluidine blue and cresyl violet. Frozen sections (n=21) were processed by immunofluorescence for vasopressin (AVP), oxytocin (OT), vasoactive intestinal peptide (VIP), tyrosine-hydroxylase (TH), met-enkephalin (Met-enk), galanin (GAL), neuropeptide Y (NPY) and serotonin (5-HT). Results: The camel SCN extended behind the optic chiasm and could be divided into 3 parts: rostral (rSCN), mean body (mSCN) and retrochiasmatic (reSCN). The nucleus started rostrally with an extra-hypothalamic neuronal population surrounding the supraoptic recess of the 3rd ventricle. In addition to the majority of parvocellular neurons, some magnocellular ones were present in the ventral part. Very strongly immunoreactive (IR) TH neurons were present in the SCN: a parvocellular population dorsally or centrally located and a magnocellular population ventrally. OT-IR neurones occurred in the mSCN. AVP–IR neurons were located dorsally in the rSCN while in the mSCN, they first appeared ventrally and then moved in the dorsal part where they surrounded a VIP-IR neuronal population. VIP was also present in neurons of the rSCN, colocalized with TH. Moreover, few Met-enk-IR magnocellular neurons were present while no neurons IR for GAL, NPY nor 5-HT were observed. Finally, a high density of Met-enk and NPY-IR fibers was found. Conclusion: The anatomy and organization of the camel SCN are different from those of other mammalian species. The presence of OT and TH suggests specific roles in the modulation of the biological clock function in this species.

Rhythmic expression of clock genes can be driven by one meal in the liver but not in the central nervous system of goldfish (Carassius auratus)

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Purpose: The purpose of this work was to investigate the possible synchronization by meal time of certain central and peripheral biological clocks in the goldfish. Methods: Fish (~7 g bw) maintained under continuous light (24L) were divided into three groups (n=30/group). First and second groups of animals were daily fed at 10 a.m. and 10 p.m., respectively, and the third group was fed at random times during 30 days. Daily motor activity was registered throughout experiment. Fish were sacrificed during a 24 hours cycle (each 6 hours). Clock gene expression (per1, per2, per3, cry1, cry2 and cry3) in the optic tectum and liver was measured by Real Time PCR. Results: As expected, the locomotor activity of goldfish was clearly synchronized by feeding time in both groups with food delivered at 10 a.m. and 10 p.m., while randomly fed fish presented a continuous locomotor activity pattern throughout 24h. The expression of per1, per2, per3 and cry3 in the optic tectum, exhibited significant daily rhythms in the animals fed at 10 a.m. and 10 p.m., with a 12-h delay in the acrophase between them. No significant rhythms were detected in the optic tectum of randomly fed fish. However, in the liver most of the clock genes studied presented significant daily rhythms in phase in the three experimental groups (in relation to the time of the last meal). Conclusion: In the absence of the photoperiodic signal (24L), a constant feeding time is necessary and enough to maintain the rhythmic expression of clock genes in the optic tectum of goldfish, as well as the daily rhythms of motor activity. By contrast, such schedule feeding time is not necessary to maintain the rhythmic expression of clock genes in the liver, suggesting that the daily rhythm of clock genes in this organ only depends on the last meal time.

Rhythmic Swi/Snf chromatin remodelling cofactors underlie circadian transcription of the prolactin gene in a pituitary cell line

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Purpose : Individual mammotropes contain an endogenous timing system responsible for oscillations of the prolactin (PRL) gene, a clock-controlled gene (CCG) that nevertheless lacks the canonical E-box response elements necessary for direct circadian regulation. The present study was undertaken to better characterize the specific molecular processes underlying these oscillations. Methods: Stably transfected GH4C1 pituitary cells expressing the luciferase reporter gene under the control of the human PRL promoter were developed and the binding sites responsible for circadian oscillations were identified by mutagenesis. Their endogenous ligands were characterized by bioinformatic, DNA affinity capture followed by mass spectrometry (MS) or western blot, EMSA, and ChIP analysis. Results: Circadian oscillations in PRL promoter activity were dependent both on a non-canonical E Box and on one of the two Pit 1 binding sites present in this promoter (P2). The endogenous ligand of the E Box was identified as the helicase-like transcription factor (HLTF), a protein of the Swi/Snf chromatin remodelling complexes. HLTF controlled PRL promoter activity by regulating Pit 1 effects on P2. Neither HLTF nor Pit 1 exhibited a circadian expression pattern. Some of the major HLTF partners identified by MS (sfpq, RBM14, p54nrb and hnRNP U) displayed a rhythmic expression pattern in the mouse pituitary, with a peak preceeding by 2 hours that of PRL. Conclusions: The mechanism described here for PRL oscillations, which involves recruitment and assembly of a rhythmic cofactor allowing chromatin remodelling, could be used by the core oscillator to generate circadian pattern of CCGs lacking the canonical E-box response element.

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Ontogeny of central and peripheral circadian oscillators in chicken

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Purpose: The avian circadian system develops prenatally. The aim of our research was to analyze ontogeny of central and peripheral oscillators in chicken. Together with clock gene expression and melatonin levels, responsiveness to light pulses and persistance was monitored. Methods: Hatching eggs of broiler breeders were incubated in the light (L):dark (D) cycle 12:12 provided by the white fluorescent tube that produced illumination in the range of 40-80 lux at the level of the eggs. After hatching, chicks were kept in a brooder room with the same LD cycle as in the incubator. After RNA isolation, cDNA was synthesized and gene expression measured by real time PCR. Melatonin was measured by RIA. Results: In the embryonic pineal gland expression of Per2 and E4bp4 was rhythmic under LD conditions, but the rhythm of E4bp4 mRNA did not persist in constant darkness. Per2 expression and melatonin synthesis were light responsive during the subjective day. In the heart and liver expression of Per2 and Bmal1 was rhythmic in 4-day old chicks. Expression of Per2 in the liver was light inducible. Prenatally we did not observe daily rhythms in Per2 and Bmal1 expression in peripheral tissues. Expression of Per2 in the liver was not responsive to the light pulse. Conclusions: We suppose that rhythm in Per2 expression in the pineal gland is a consequence of synchronizing effect of the LD cycle sensed by the embryo. Absence of Per2 rhythmicity in peripheral tissues can reflect developmental stage of embryo or missing internal synchronizing cues. The central part of the circadian system is operating earlier than peripheral. Signal transduction between components of the circadian system is limited in chicken prenatally. Our data implicate an advanced stage of development of peripheral oscillators during the last third of incubation and prompt functioning of the circadian system after hatching.

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Does the extended amygdala contain autonomous circadian oscillators?

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Purpose: To determine the presence and circadian properties of autonomous circadian oscillators in nuclei of the extended amygdala complex. Methods: Coronal brain slice cultures were made from either the central nucleus of the amygdala (CeA), basolateral nucleus of the amygdala (BLA) or oval nucleus of the bed nucleus of the stria terminalis (ov-BNST) from adult male mPer2::luc mice. PER2 driven luminescence expression was either tracked using photomultiplier tube assemblies (PMTs) or visualised on a highly sensitive Olympus LV200 luminescence microscope using photovideomicroscopy. Results: Brain slice cultures from the CeA expressed a single peak in PER2 expression which diminished to background levels after approximately 24h in vitro. Once damped, PER2 expression in the CeA could not be induced by treatment with corticosterone or dexamethasone, potent activators of glucocorticoid receptors. Similarly, forskolin, an activator of adenylate cyclase signalling failed to restart damped PER2 expression. No endogenously driven expression of PER2 was detected in isolated cultures of either ov-BNST or BLA, and PER2 could not be induced in cultures from either area with dexamethasone, corticosterone or forskolin. Conclusions: A number of recent in vivo studies have reported rhythmic expression of the core molecular clock component PER2 in the CeA, BLA and ov-BNST, raising the intriguing possibility that these areas contain circadian oscillators. In this study we isolated cultured samples of these amygdala nuclei from extraneous inputs to test for autonomous circadian function. We demonstrate that none of these nuclei of the extended amygdala contain autonomous circadian oscillators and conclude that rhythmic activity in vivo must be wholly driven by inputs from other brain areas or systemic signals.

Experimental design for the analysis of retina clock in explants from Per1-luciferase rats: analysis of entrainment and clock localization

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Purpose: to optimize culture conditions for retinal explants, to monitor real-time Per1 oscillations and to use this experimental design for the study of clock localization and entrainment. Methods: retinas were dissected from Per1-luc rats housed under a daily LD12/12 cycle, and mounted on tissue culture filters around ZT12 to be cultured during 24h on Neurobasal-A medium (37℃, 5% CO2). At CT12 of the second day explants were transferred onto 199 medium in sealed dishes for another 24h (36℃). 199 medium was renewed 24h later (CT12) and Beetle luciferin (0.1mM) added before starting 5-7 day recordings in the LumiCycle (36℃). Effect of the light/dark cycle was assessed by superimposing 48h of LD 12/12 on the first steps of this protocol. Effects of cAMP pathway stimulation was analysed by adding a 1-h incubation with 10µM forskolin immediately before the start of luminescence recordings. As a first step towards the localization of the clock, retinas were cut horizontally inside the outer plexiform layer by vibratome slicing, and photoreceptor layers and inner retinas were cultured as previously in the LumiCycle. Results: Our Neurobasal A/199 medium-based protocol was found the only condition in which sustained luminescence oscillations could be observed in whole retina explants. Exposure of explants to a LD 12/12 cycle faintly synchronized the cultures, whereas forskolin treatment induced strong synchronization with a significant reduction of the period. Horizontal slices of the retina displayed robust oscillations in case of the inner retina but none in case of photoreceptor layers. Conclusion: we designed a powerful method to analyze the retina clock in Per1-luc rats that will prove useful for further understanding of its localization and dissection of entrainment mechanisms.

Clock gene expression in the mouse hippocampus is altered in Per1-/- mice

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Purpose: Core clock genes, namely mPer1/PER1, mPer2/PER2, mCry1, mCry2/CRY2, mClock/CLOCK, and mBmal1/BMAL1 were analyzed for spatial and temporal diurnal dynamics in the hippocampus of WT and Per1-/- mice. Methods: WT and Per1-/- mice were housed under a 12-hour light/dark cycle. For mRNA studies total mRNA was isolated from the whole hippocampus and processed for real-time PCR using clock gene specific primer. For protein studies, brains were processed for immunohistochemistry and immunofluorescence using clock gene specific and cell type specific antibodies. Results: The mRNA of investigated core clock genes, as detected by real-time PCR in hippocampal extracts, exhibit a rhythmic expression in a time-locked fashion in WT and Per1-/- mice over a diurnal day-night cycle. All investigated core clock genes protein products could be observed in the cell nuclei of the Stratum pyramidale and the Stratum granulosum of the hippocampal formation of WT and of Per1-/- mice. Immunohistochemical co-labeling investigations revealed clock gene protein expression exclusively in hippocampal neurons, including parvalbumine positive interneurons, but not in glia cells. In the hippocampus of WT mice, a diurnal rhythm in protein expression was detected. In Per1-/- mice, protein rhythms of PER2, CRY2, CLOCK and BMAL1 were greatly altered. Conclusions: Here presented data show a time-of-day-dependent rhythmic expression of clock genes and their corresponding protein products in hippocampal neurons of WT mice. The loss of Per1 severely affects the coordinated clock gene expression in the hippocampus, implicating a trigger function for this clock gene in time-dependent mnemonic processes.

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Role of Per1 in synaptic plasticity and memory formation in the hippocampus of mice

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Purpose: We wished to analyze the impact of the Per1 gene for hippocampal learning and memory formation and to quest for molecules of time-dependent mnemonic processes that are affected in Per1-deficient (Per1-/-) animals. Methods: WT and Per1-/- mice were housed under a 12-hour light/dark cycle. Mice were tested in an eight-arm radial arm maze test and subsequently scarified 2 and 4 h after end of trial on day 1 and day 5 in order to analyse hippocampal expression of learning associated proteins (ARC, Synaptopodin, PKCZeta, pHistonH3). Results: During the first 2 days of habituation, both, Per1-/- and WT mice showed similar performance. During consecutive trials (days 3-5), Per1-/- mice exhibit a higher number of repeated arm re-entries (errors), compared to WT mice. On day 1 and day 5 of the spatial learning test, in both, Per1-/- and WT mice, Western blot analyses of hippocampal extracts showed an increase in ARC and PKCZeta protein amount 2 hours after the eight-arm radial arm maze test. In contrast, 2 h after the eight-arm radial arm maze test on day 1 and day 5 the amount of Synaptopodin protein was significantly reduced in the hippocampus of Per1-/- mice, whereas a constitutively high amount of Synaptopodin protein was present in the hippocampus of WT mice. Conclusions: In the eight-arm radial arm maze test Per1-/- mice show an affected food rewarded spatial learning behaviour as compared to WT siblings. The also observed differences in hippocampal Synaptopodin protein amount after the spatial learning task in Per1-/- mice as compared to WT suggests an involvement of this clock gene in cytoskeleton-associated synaptic plasticity.

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Evidence for a testicular clock in Djungarian hamsters (Phodopus sungorus)

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Purpose: To investigate the duration of the cycle of the seminiferous epithelium in hamsters under photoperiods with shortened of prolonged day lengths. Methods: Adult male Djungarian hamsters (n = 20 per group) were exposed to day lengths of 24 hrs (16L:8D), 23 hrs (16L:7D), or 25 hrs (16L:9D), for 43 days. Entrainment to the photoperiods was verified individually by passive infrared detectors. After a single injection of bromodeoxyuridin (BrdU) on experimental day 35, subgroups of hamsters (n = 10) were sacrificed 3 hrs, and 8 days 3 hrs after injection. Immunocytochemical localization of BrdU-labeled cells and microscopical identification of stages of the testicular cells in the seminiferous tubuli was performed to calculate the duration of the cell cycle. In addition, another experiment was performed to investigate the diurnal variations of expression of haBmal1 and haPer1 in testes. To this end, hamsters (n = 4 per group) were sacrificed every 3 hrs during 24 hrs. Results: The vast majority (15) of animals were entrained to the 23 hrs day lengths, but only 6 animals of the 25 hrs group. The duration of the cycle of the seminiferous epithelium was not different between the groups despite small variations within the groups. The expression patterns of haPer1 and haBmal1 showed significant diurnal variations. Conclusions: The well known precision and very low variability of the duration of the cycle of the seminiferous epithelium is not dependent on the exogenous day length and probably a result of diurnal expression patterns of clock genes, indicating the existence of a testicular clock with widespread implications for the understanding of testicular cell maturation.

Electrophysiological heterogeneity of glycine responses in neurons of the mouse suprachiasmatic nucleus

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Purpose: To analyze the role of glycine in resetting the

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mammalian circadian clock. Methods: Organotypic hypothalamic slices from wild type (C57bl/6) and knock-out mice lacking one subunit of glycine receptor (GlyRa1-/-, GlyRa2-/- and GlyRa3-/-) were prepared and cultured for several weeks on Millicell-CM culture inserts before extracellular recordings of SCN neuronal activity were performed using high density multimicroelectrode arrays (MEA). Glycine and/or strychnine, a selective blocker of glycine receptor, were applied using an autosampler and their short-term effects on firing rate as well as on the phase of the rhythm were determined. In addition, voltage-clamp recordings were performed on acute SCN slices from 3 weeks old wild-type and knock-out mice in order to characterize the effects of glycine and its agonists, taurine and ß-alanine on membrane currents. Results: Patch clamp recordings revealed that glycine evokes dose-dependently an outward current at a holding potential of 0 mV. In many SCN neurons this current can be fully blocked by the glycine receptor antagonist strychnine. MEA recordings showed that glycine can also enhance the firing rate of SCN neurons by 30 %, an action which could be attributable to the binding of glycine on NMDA receptors. Long term recordings revealed that glycine can phase-shift the circadian rhythm in firing rate of SCN neurons depending on the time of application, i.e. a phase advance during subjective day, and a delay during the early subjective night (CT 16). Conclusions: Our results suggest that glycine modulates SCN neuronal activity by two pathways, a potentiation of NMDA receptor activity, as well as a binding to its specific glycine receptors.

Orexin A modulates neuronal activity in the SCN in vitro

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Purpose: To examine the involvement of orexin in the regulation of the mammalian circadian clock. Methods: Voltage clamp recordings were performed on acute SCN slices from 3 weeks old rats and effects of different concentrations of orexin A on the frequency of spontaneous inhibitory postsynaptic currents (IPSCs) were analysed in the presence or absence of tetrodotoxin (TTX). Additionally, long-term recordings of the firing rate in primary cell cultures of the rat SCN and in organotypic SCN slices prepared from 2 to 6 days old C57BL/6 mice were carried out using multielectrode arrays (MEA). Results: Whole-cell patch clamp experiments revealed that orexin A caused significant changes in IPSCs frequency in a reversible manner without showing effects on the mean amplitude or decay time constant. Different concentrations of orexin A altered the frequency of IPSCs in up to 67% of SCN cells recorded. Low concentrations of orexin evoked an increase of sIPSCs, whereas the highest concentration (250 nM) caused predominantly a decrease of sIPSCs. The effects of orexin A on IPSCs were prevented by the orexin -1 receptor antagonist SB 334867 and also reduced in the presence of TTX. Multielectrode array (MEA) recordings from dispersed SCN neurons revealed that orexin A dose-dependently enhanced extracellularly recorded neuronal activity of many neurons (38%), while other neurons were inhibited (28%). Long-term recordings of extracellular activity in organotypic slices gave evidence of a phase shifting effect of orexin A. When applied just before the daily peak of spike rate, orexin A induced a phase-advance of the circadian rhythm. Conclusion: Our data show that orexin A may have a direct effect on SCN neurons and modulates inhibitory synaptic transmission between SCN neurons.

Rhythmic intestinal drug elimination via ABC transporters: a potential determinant of anti-cancer drugs chronopharmacology

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Purpose: In order to determine mRNA expression rhythm of ATP-binding cassette (ABC) transporters in the intestinal segments in mice. Methods: Twenty-four hour rhythms were investigated for the mRNA gene expression of P-gp (abcb1a, abcb1b), mrp1 (abcc1) and mrp2 (abcc2) in colon and ileum mucosae of ?B6D2F1 mice, synchronized to standard lighting conditions (LD12:12). Gene expression was determined with quantitative PCR at 6 circadian times, with ZT0 and ZT12 corresponding to light and dark onsets respectively. Results: Abcc1 varied ~2.5 fold in ileum ($p<0.02$) and colon ($p<0.01$) along the 24 h, with coincident maxima near ZT20, according to cosinor analysis. Abcb1a varied ~3-fold in ileum ($p=0.02$) and ~10-fold in colon ($p<0.001$) along the 24 h, with coincident maxima near ZT15. Abcc2 transcription was also largely rhythmic in ileum, with a ~3-fold circadian variation ($p = 0.003$), but this was not the case in colon. Conversely, Abcb1b was rhythmic in colon ($p=0.02$), but not in ileum. Conclusions: ABC drug transporters display large rhythmic changes in intestinal segments, with tissue specificity. Rhythms in ABC transporters could account for 24-h changes in metabolism and intestinal toxicities of anticancer drugs. The relevance of these findings needs further assessment with correlative functional studies.

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Analysis of subregion-specific hippocampal gene expression using laser microdissection

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Purpose: To further dissect gene expression in hippocampal subregions of the hippocampus of WT and Per1-/- mice, laser microdissection (LMD) and quantitative real-time PCR was introduced. Methods: WT and Per1-/- mice were housed under a 12-hour light/dark cycle. In cryostat-cut hippocampal slices (12µm) the individual hippocampal

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subregions CA1, CA3, and Gyrus dentatus were separated using a LMD system. Total mRNA, extracted from whole hippocampus and form individual subregions was isolated and processed for real-time PCR, using validated gene specific primer pairs. Results: High quality mRNA could be extracted form pooled tissue pieces of individual subfields. The amount of real-time PCR amplified cDNA for clock genes Per1, Per2, Cry1, Cry2, Clock, and Bmal1 did not differ between hippocampal subfields. This validated technique is now applied to a comparative analysis of various plasticity-relevant genes that were detected to be differentially expressed in the hippocampus of WT and Per1-/- mice in a microarray analysis. Conclusions: Presented data demonstrate that the combination of LMD with subsequent real-time PCR can be reliably used to investigate endogenous or induced dynamics in (clock-) gene expression in hippocampal subfields. Results show further that a prior toluidine blue staining for topographical orientation during the LMD process does not affect mRNA quality. Furthermore, we were able to confirm that the quantity of the isolated RNA is not affected by the LMD procedure and that the amount of the received RNA is linearly related to the size of the dissected tissue. Our preliminary results provide the basis for further analyses of dynamics in quantity of molecules, relevant for hippocampal plasticity. In particular, the technique allows deciphering dynamics in clock gene expression in hippocampal subfields.

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Phasing of circadian rhythms along the rat gut

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Purpose: Recently, circadian clocks in the rat and mice digestive system were discovered. The aim of the study was to ascertain whether circadian clocks within the individual parts of the gut are synchronized, or whether there is a difference in their circadian phases. Next aim was to determine the circadian phases in the expression of the cell cycle regulator Wee1 in the duodenum and the distal colon. Methods: Adult rats were maintained under regime with 12 h of light and 12 h of darkness (LD 12:12). On the day of the experiment, rats were released into constant darkness and sampled every 4 h throughout the whole circadian cycle. In a separate experiment, rats were sampled every 1 h during the light phase of the LD cycle. Daily expression profiles of clock genes Per1, Per2, Bmal1, Rev-erb-a, Clock, Cry1 and clock-controlled gene Wee1 were examined by real-time RT-PCR quantification within the epithelium of the duodenum, jejunum, ileum and distal colon. Results: Per1, Per2, Bmal1, Rev-erb-a genes were expressed rhythmically within the epithelium in all studied parts of the gut. Comparison of clock gene expression rhythms revealed differences in their phases, such that the rhythms in the duodenum were phase-advanced to those in the distal colon. Also, Wee1 expression exhibited circadian rhythm within the duodenum and distal colon and showed similar cranio-caudal gradient in phasing of its rhythmical expression. Conclusions: Our data demonstrate that individual parts of the gastrointestinal tract have their own circadian clock and these clocks are synchronized with a phase-delay along the cranio-caudal axis. Moreover, they support the view that the individual circadian clocks may control the timing of cell division cycle within different regions of the gut.

VIP-VPAC2 signalling is not essential for circadian rhythms in behaviour

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Purpose: To determine the combined effect of genetic deletion of vasoactive intestinal polypeptide and its receptor, VPAC2, (VIP-/- x Vipr2-/-) on mouse behavioural rhythms. Methods: Wild-type control (WT) and VIP-/- x Vipr2-/- mice were housed in running-wheel equipped cages and locomotor and drinking rhythms under 12h:12h light-dark conditions assessed before transfer into constant darkness (DD1). After 14 days in DD1, wheels were locked for 18h/day, thereby restricting voluntary exercise to a scheduled 6h/day for 21 days. Subsequently, the wheel was unlocked and the free-running rhythms determined over the next 14-day period (DD2). Results: In light-dark conditions, all mice showed apparent 24h rhythms in wheel-running and drinking behaviour. In DD1, all 12 WT mice were strongly rhythmic period (~23.7h), whereas VIP -/- x Vipr2-/- showed a range of behavioural rhythms with 12/18 showing a single rhythmic component (~22.3h) of modest power and 6/18 weakly rhythmic with multiple low power components. Under scheduled voluntary exercise, the drinking rhythm of WT mice did not entrain to the opportunity to exercise, whereas within 7 days, 80% of the VIP-/- x Vipr2-/- mice synchronized drinking activity to the daily unlocking of the wheel. Following the wheel-locking regime, in DD2, all WT free-ran with a similar period as seen in DD1. VIP-/- x Vipr2-/- mice began their free-running locomotor activity from the time of wheel unlocking and now 15/18 animals were rhythmic with a significantly longer period than that seen in DD1 (24.1h vs 22.3h). Conclusion: These data demonstrate that in the absence of VIP-VPAC2 signalling, mice can sustain ~22.3h rhythms that are readily altered by a 24h non-photic Zeitgeber. Neither VIP nor or its receptor are essential for the expression of circadian rhythms in behaviour, but their absence enhances responsiveness to Zeitgebers such as scheduled voluntary exercise.

Is clock gene Period1 involved in NOGO-mediated plasticity in the mouse hippocampus?

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Purpose: The temporal gating of hippocampal mnemonic processes is severely affected by deletion of the clock gene Period1 (Per1). Hippocampal plasticity is associated with the differential expression of genes, involved in structural re-organisation of neurons and neuronal connectivity.

Methods: Using comparative microarray analyses of cDNA, extracted from whole hippocampus of wildtype (WT) and Per1-/- mice, we detected a dramatic expression difference in NOGO (RETICULON4), namely a 9-fold up-regulation in the knockout animals. Expression profile of NOGO was compared in hippocampal extracts and in pri-

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mary hippocampal neuronal cultures derived from WT and Per1-/- mice, using immunoblot and immunohistochemical analyses. Results: Western blot analyses extend the presence of NOGO mRNA in the hippocampus of WT animals as found in the microarray to the protein level. In primary hippocampal neurons of Per1-/- mice, NOGO protein abundance is constitutively elevated as compared to WT animals. Stimulation of primary hippocampal neurons with BDNF, a central plasticity-related neurotransmitter, or the enhancer of intracellular cAMP, Forskolin, or with Rolipram, a phosphodiesterase inhibitor that prevents cAMP degradation, resulted in a NOGO up-regulation in WT cultures only. Conclusion: NOGO, expressed in oligodendrocytes and in neurons, functions as an inhibitor of the growth of axons and dendrites of neurons. We suggest that the elevated expression of NOGO in Per1-/- mice is a possible mechanism that is associated with the observed learning deficits in these knockout animals.

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4. The multi-oscillatory nature of the circadian network

Characterization of circadian clock genes expression in the anterior pituitary gland

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Purpose: To characterize the circadian clock of the pituitary gland and explore its regulation by light and feeding cues. Methods: Mice were kept under three different photoperiods (12L:12D, 8L:16D or 16L:8D). Pituitary glands were then collected at various time points and processed for real-time quantitative PCR or immunohistofluorescence. Results: All the main circadian clock genes are expressed in the gland. Under 12L:12D, the daily profiles of expression in the gland are identical to the temporal patterns observed in the liver from the same mice. Double detection by immunofluorescence of the PER2 protein and pituitary secretory products reveals that clock cells of the gland are all synchronized, whatever their hormonal content. Interestingly, the amplitude of the PER2 rhythm is larger in ACTH-containing cells than in other endocrine pituitary cells. Under long and short photoperiods, the amplitude of Per2 and Bmal1 expression is severely reduced in the pituitary gland. In contrast, no change in amplitude is noted in the liver, but both rhythms appear phase-locked to the light-dark transition, suggesting that the onset of feeding activity is the main zeitgeber of the liver clock under these conditions, whereas light and feeding may act as competing cues in the pituitary. To address this question, we imposed daytime or night time restricted feeding (DRF and NRF, respectively) to mice under 12L:12D. NRF had no impact on the daily expression of clock genes in the pituitary gland, as compared to mice fed ad libitum, whereas DRF induced a dramatic decrease in the amplitude of the Bmal1 and Per2 rhythms. Importantly, these almost flat profiles contrast with the inverted rhythms reported for other peripheral organs under DRF conditions. Conclusions: The expression of circadian clock genes in endocrine pituitary cells is under the combined influence of light cycle and food intake. The gland may thus be an integrating centre of the circadian system.

Rhythm of proton pump activity in Drosophila melanogaster male reproductive system

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Purpose: Characterization of H+-ATPase expression and function in the male reproductive tract of fruit fly. Methods: Expression level of gene coding B subunit of H+-ATPase was analyzed by qRT-PCR and changes of corresponding B protein were determined by Western blot. To characterize functions of the peripheral oscillator in proton pump regulation the seminal vesicles (SV) from wild type flies (CS) and clock mutant flies per0 were cultured in vitro and daily profile of the culture medium pH was determined using calorimetric assay. Results: No rhythmic changes of B subunit transcript level in seminal vesicles of CS flies were observed. However the B protein expression analysis revealed robust rhythm with peak during light and through during dark phases respectively. In per0 mutant flies both: transcript and protein levels changed daily. The intraluminal pH of seminal vesicles from CS males in LD conditions reaches highest level during dark phase and lowest at night. Similar pattern was observed when insects were kept for 6 days in constant darkness and described rhythm was completely abolished when flies were exposed to continuous light. Conclusions: Our data provide new insights into the function of peripheral clocks present in male reproductive tract of fruit fly which influences seminal vesicles milieu probably by regulation of H+-ATPase expression and activity.

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Differential effects of a restricted feeding schedule on clock gene expression in the hypothalamus of the rat

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Purpose: To determine whether restricted feeding schedules (RFS) affect the hypothalamic daily rhythm of clock genes. Methods: Wistar rats kept in a light/dark cycle (12:12 h), were fed ad libitum (C) or entrained to RFS with food available for 2 h daily (ZT6-ZT8) during three weeks. Rats were decapitated every 3h starting at ZT0 (lights on) and were processed with in situ hybridization. Results: For Per1, RFS induce a phase advance in the suprachiasmatic nucleus (SCN) and induced a rhythm in the arcuate (ARC) and dorsomedial (DMH) nuclei anticipating food access (ZT6) while it remained unaffected in the ventromedial (VMH) and paraventricular (PVN) nuclei. The Per2 daily rhythm was not affected by RFS in the SCN and ARC nuclei whereas it was down-regulated in the DMH and VMH. In the PVN, a peak of Per2 expression

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was induced 6h after food access. The daily expression in hypothalamic nuclei of Bmal1 was not significantly modified by RFS. Conclusions: Present data indicate that RFS affect differentially clock gene response in hypothalamic nuclei and highlight the SCN, ARC and DMH as relevant structures for anticipation and the PVN as a responding structure to meal time.

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Phase resetting relies on rapid shifts of a small sub-population of ventral SCN neurons

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Purpose: We provide a quantitative analysis of the bimodal electrical activity pattern observed in electrical activity recordings following a 6 hour delay of the light-dark cycle. Methods: Wildtype Wistar rats were exposed to a 6 h delay of the light cycle. Next, hypothalamic slices were prepared, and recordings of electrical impulse frequency with stationary electrodes were performed. The resulting bimodal activity records were analyzed using different computational methods, including curve fitting analysis, subpopulation analysis and simulation studies. Results: About 60% of the recordings showed a bimodal pattern. Analysis of the bimodal activity records shows that the unshifted component is relatively broad (6.1 ± 1.6 h) and the shifted component more narrow (2.3 ± 1.3 h). Curve fitting analysis showed that the number of action potentials that contribute to the shifted component is about 20-30 % of those that contribute to the unshifted component. Subpopulation analysis confirmed these findings, and showed strong synchronization in peak phase in the shifted component but not in the unshifted component. Conclusions: Phase resetting is brought about by an instant shift of a surprisingly small number of SCN neurons. The phase of these neurons becomes highly synchronized to the new light-dark cycle.

Quantitative methods for circadian system analysis based on data mining and novel robustness measures

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Purpose: The development of new quantitative methods for studying circadian rhythms through the application of data mining techniques and novel robustness measures and the validation of the methods using time series data from Neurospora crassa experiments. Methods: Feature extraction, a dimensionality reduction technique in data mining, can be used to analyze circadian time series data based on extracted features such as period, phase and amplitude. The extracted features are then used as parameters for the functions measuring properties such as similarity of circadian behavior of different strains. The novel robustness measures use the general approach to biological robustness of H. Kitano, which attempts to quantify system robustness in terms of perturbations and their probabilities. Results: Data from an extensive set of experiments on Neurospora crassa where three variables are changed systematically: (1) cycle length, (2) photoperiod and (3) free-running period (FRP), was used to study various properties of circadian rhythms. Feature Sequence Analysis, which studies variations from one cycle to the next, provided particularly interesting results. The robustness measure, which used specific perturbations on cycle length and photoperiod, reflected strain-specific patterns as the length of day was varied. Conclusions: Quantitative methods based on data mining techniques such as Feature Sequence Analysis and the stated novel measure of robustness are suitable methods for analyzing circadian rhythms. Validation using the N. crassa time series data show biologically relevant results and interesting circadian properties.

Phase shifting capacity of the circadian pacemaker determined by the SCN neuronal network organization

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Purpose: The amplitude of phase shifts depends strongly on the photoperiod to which animals are exposed. The purpose of this study was to provide an explanation for this phenomenon. Methods: We entrained C57 mice to long and short days and recorded running wheel activity. After at least 30 days in the photoperiod the animals were transferred to darkness and on day 4 they were used for behavioral and electrophysiological experiments. Light pulses were given to construct a Phase Response Curve for long and short day length. NMDA was applied to investigate phase responsiveness in SCN slices. Result: We observed large behavioral phase shifts in animals from short day length and small phase shifts in animals from long day length. We further investigated whether these responses were intrinsic to the SCN by recordings of brain slices. Application of NMDA induced an increment in electrical activity that was not significantly different in the slices from long and short photoperiods. However, these responses led to large phase shifts in slices from short days and small phase shifts in slices from long days. Analysis of neuronal subpopulation activity revealed that in short days the amplitude of the rhythm was larger than in long days. Conclusions: The data indicate that the photoperiodic dependent phase responses are intrinsic to the SCN. We observed large phase shifting responses in high amplitude rhythms in slices from short days, and small shifts in low amplitude rhythms in slices from long days, in contrast to earlier predictions from limit cycle theory. We propose that synchronization among SCN neurons determines the phase shifting capacity of the circadian system.

5. Output pathways of the main circadian clock

The rat fetal heart is a peripheral circadian clock

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In altricial species, like rats, overt circadian rhythms of several physiological variables start postnatally. In the fetal rat, SCN neurogenesis is completed close to birth and oscillatory expression of clock genes and metabolic activity is present at 20 days of gestation. However recent evidence supports the presence of circadian rhythms in the rat fetus at early gestational ages. Indeed we found that the rat fetal heart presents rhythmic expression of clock genes in vivo at 18 days of gestational age. Purpose: To explore the intrinsic oscillation of Per2 and Bmal1 in the fetal heart in vitro. Methods: Pregnant rats (n=12) were euthanized at 20-24h at 18 days of gestational age by thiopental overdose. Fetal hearts were dissected, pooled in 30 ml DMEM-F12 and pre-incubated by 6h at 37°C. Then 3 hearts per well were incubated i n triplicate for 27hrs. Hearts were collected every 4 hrs for 27 hrs, starting at 0800h. RNA was extracted using a commercial kit and the expression of the clock genes Per2 and Bmal1, Mt1 melatonin receptor and glucocorticoid receptor (GR) were measured by real time-PCR. Results: We found in fetal heart in culture an oscillatory expression of Bmal1 and Per1 with acrophases at 2000h and at 0400h, respectively. Expression of Mt1 and glucocorticoid receptors was maintained without significant oscillation.

Conclusions: Our results showed an intrinsic oscillation of clock gene expression in fetal heart in vitro. Our data support that fetal heart is a functional peripheral clock, independent of the fetal SCN oscillation. The expression of MT1 and glucocorticoid receptors suggests that in vivo the fetal heart circadian rhythm of clock gene expression is synchronized by maternal melatonin or fetal glucocorticoids.

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Early circadian expression of Mt1 melatonin receptor and clock genes in fetal rat organs

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Purpose: To study the ontogeny of circadian rhythms in fetal rat organs. Methods: Pregnant females (n=3-4/clock time) were euthanized at 16 and 18 days of pregnancy by thiopental overdose at 8-12-16-20-24-04 hrs. A maternal blood sample was collected to measure melatonin by RIA. Fetuses (n=9/clock time) were delivered by histerotomy. At 16 days, the head (whole fetal central nervous system) and the body (peripheral tissues) were separated. At 18 days, the size of the fetus allowed dissection of liver, heart, adrenal and placenta. Tissues were preserved in Trizol until RNA extraction using a commercial kit (Promega). In each fetal compartment/tissue we measured expression of the clock genes Per2 and Bmal1 and Mt1 melatonin receptor by real time PCR. Results: Plasma maternal melatonin presented a circadian rhythm that did not vary with the gestational age with maximal plasma concentration between 2000-0400 h. At 16 days, the clock genes Bmal1 and Per2 showed an oscillatory expression in head and body, with a maximal expression (acrophase) for Bmal1 at 04-08 hrs and for Per2 at 16-20h. At 18 days we found that heart, adrenal and placenta showed oscillatory expression of Bmal1 (acrophases between 20-04) and Per2 (acrophases between 08-12h). Mt1 melatonin receptor oscillated at both gestational ages. At 18 days of gestation, the acrophases occur at day time (08-16), opposite to the maternal plasma melatonin rhythm. Clock genes and Mt1 were lower in the liver than in other tissues, and as reported by others we did not detect 24-h oscillation. Conclusions: These data suggest an early coordinated clock gene oscillation in fetal rat tissues before the fetal SCN starts to oscillate. The presence of a phase delay of the clock genes in the fetus respect to the data available in adult SCN as well as early oscillatory expression of MT1 is consistent with the hypothesis that fetal organs may be peripheral clocks to the dam SCN.

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6. Diurnality versus nocturnality

Spawning rhythms in fish: is the time of day important?

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Purpose: To investigate the existence, endogenous control and main synchronizers of daily spawning rhythms in four teleost species (Senegal sole, Solea senegalensis, Gilthead sea bream, Sparus aurata, European sea bass, Dicentrarchus labrax, and Zebrafish, Danio rerio). Methods: Eggs were collected hourly using an autonomous, programmable egg-collector to identify the time of day each species preferred to spawn. Furthermore, shifted photoperiods and constant lighting conditions were applied to study the resynchronization of the rhythm and its endogenous origin, respectively. Results: All four species presented marked daily spawning rhythms, with different times of spawning depending on each pattern of behavior. Nocturnal fish such as Senegal sole and European sea bass spawned at night, sole during the first dark hours and sea bass slightly later. Dual and diurnal species, such as gilthead sea bream started to spawn in the late afternoon, while zebrafish spawned early in the morning. After shifting the LD cycle, both Senegal sole and gilthead sea bream gradually resynchronized their daily spawning rhythms. When fish were subjected to LL conditions, the spawning rhythm persisted during 2 days in Senegal sole and free-run (t = 22.3 h) during several days in zebrafish. Conclusions: Fish did not spawn equally at any time of day: according to each species's behavior, fish spawned during daytime or at night. Such a daily spawning rhythms was strongly entrained by light, but had an endogenous control.

Internal temporal order in a dual phasing rodent, the Octodon degus

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Purpose: Octodon degus is a dual phasing rodent which has the ability to switch from diurnal to nocturnal activity under laboratory conditions in response to wheel running availability. Daily rhythms on different physiological variables have been widely described; however, no studies have been conducted in dual phasing species such as degus. This species can help us to know whether a complete temporal order inversion, in parallel to that observed in activity pattern, occurs. The present study aims to determine day-night differences in 27 variables depending on degus chronotypes, either diurnal or nocturnal. Methods: A total of 28 males degus (26-32 months old), 17 diurnal and 11 nocturnal, were individually housed with free wheel running access under LD 12:12 cycle. Wheel running activity and body temperature rhythms were recorded throughout the experiment. Melatonin, total antioxidant capacity, haematological and biochemical variables were determined in blood samples obtained every six hours (ZT 1, ZT 7, ZT 13 and ZT 19). Results: Although wheel running activity and temperature rhythms switch to a nocturnal chronotype, most variables remained unchanged when compared to diurnal chronotype. Differences between chronotypes were only found in melatonin rhythm, urea blood levels and lymphocyte number at mid-light. Conclusions: It has been demonstrated that the internal temporal order of a dual phasing mammal does not show a complete inversion in accordance to its activity and body temperature pattern. Thus, switching mechanism seems to be located downstream from the pacemaker.

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Nitrogen and protein content analysis of human milk, diurnality vs. nocturnality

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Purpose: To determine the effect of diurnality or nocturnality on nitrogen and protein content of the breast milk. Methods: We collected human milk samples from health mothers living throughout Community of Extremadura (Spain) from January 2008 to December 2008 with less than two months of lactation. We divided the samples in three groups: calostral group (1-5 days postpartum), transitional group (6-15 days postpartum) and mature group (>15 days postpartum). All samples were stored in a freezer at -80ºC. We considered as day period between 08:00-20:00h and night period 20:00-08:00h. Analysis of the human milk samples was based on the Kjeldahl method. Protein contents were calculated from total nitrogen x 6.38. The statistical analysis of the data was descriptive (mean ± standard deviation) and inferential (T-Student test) Results: No differences (P>0.05) were found to exist among the contents of individual human milk samples. The mean contents of each component were as follows: Total nitrogen of calostral, transitional and mature group was 0,30 ± 0,06 g/dl (night period), 0,29 ± 0,05 g/dl (day period); 0,26 ± 0,04 g/dl (night period), 0,25 ± 0,04 g/dl (day period); 0,21 ± 0,04 g/dl (night period), 0,19 ± 0,03 g/dl (day period) respectively. Protein content of calostral, transitional and mature group was 1,92 ± 0,4 g/dl (night period), 1,84 ± 0,3 g/dl (day period); 1,65 ± 0,4 g/dl (night period), 1,62 ± 0,4 g/dl (day period); 1,34 ± 0,3 g/dl (night period), 1,26 ± 0,2 g/dl (day period) respectively. Conclusion: Although we observed differences in the nitrogen and protein content during the individual stages of lactation, in this population of lactating women, none of the components analyzed varied significantly between day and night.

Nocturnalism induced by high environmental temperature during the photophase in the diurnal Octodon degus

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Purpose: Octodon degus is a Chilean dual phasing rodent that inverts its phase preference due to the availability of a wheel running in its cage. It has been hypothesized that this inversion is owed to thermoregulatory constrains induced by vigorous physical exercise, thus activity is moved to the night with lower environmental temperatures. Thus, the aim of this work is to verify whether avoiding overheating is responsible for nocturnal degu chronotype. Methods: Sixteen male degus, individually housed, with free access to a wheel running and a thermal heater located under each plastic cage, were subjected to four experimental situations: 12:12 LD (control phase), 12:12 LD with 12h of high temperature during the photophase, DD maintaining the previous 12h of high temperature, and, finally, DD with normal temperature. Wheel running activity and body temperature were continuously recorded. Results: A 100% of nocturnal chronotypes were obtained with high temperatures during the photophase. Nocturnal chronotype acquisition and maintenance seems to imply both the pacemaker and negative masking effects by light and high temperature. Conclusions: High temperatures during the day are clearly responsible for activity inversion, indicating thermoregulatory constrains in this inversion, however temperature and light interacts to generate and maintain nocturnal chronotype in this species.

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7. Pineal cell biology

Role of mPER1 in melatonin synthesis in the mouse pineal gland

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Purpose: Nocturnal increase in melatonin synthesis in the mouse pineal gland requires transcriptional activation of the Aanat gene encoding for the penultimate enzyme in melatonin synthesis. It is known that the Aanat promoter contains an E-box element which can be controlled by clock gene proteins. The transcriptional repressor mPER1 has been shown to be involved in the regulation of rhythmic gene expression in a variety of tissues and PER1 protein levels are increased in the pineal gland during night time. To investigate the role of mPER1 in the regulation of melatonin synthesis we compared mPER1-deficient and WT mice. Methods: The diurnal changes in Aanat mRNA expression, AANAT enzyme activity and melatonin concentrations in the pineal gland (and blood-serum) were analyzed by QPCR, activity test, and RIA, respectively. In addition, Aanat mRNA expression and melatonin concentrations were analyzed in cultured pineal glands after stimulation with noradrenalin (NA). Results: Aanat expression, AANAT enzyme activity and levels of melatonin during late night were significantly elevated in the pineal of mPER1 -/- mice compared to WT. In cultured pineal glands of PER1 -/- mice the basal levels of Aanat expression and melatonin concentration were significantly elevated as compared to WT mice and could not be further elevated by stimulation with NA. Conclusion: The data suggests an important role of mPER1 in the control of the amplitude in Aanat mRNA expression.

Endogenous glucocorticoids regulates the rhythmic expression of nuclear factor kappa B (NFkB) in pineal gland of Syrian hamster

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Purpose: Considering the role for NFkB in the control of Aa-nat gene in rat pineals we addressed its expression and regulation in the Syrian hamster pineals. Methods: NFkB-DNA complexes were analyzed by EMSA in pineal nuclear extracts of Syrian hamster (Mesodricetus auratus) under 14:10h light/dark cycle (LD). Results: Three NFkB-DNA complexes were visualized being its specificity confirmed by competition with specific and non-specific unlabeled oligonucleotides, as well as by the in vivo treatment with the inhibitor PDTC (50-200 mg/kg, ip, ZT9), which significantly inhibited the complexes in a dose-dependent manner. A rhythmic constitutive variation of nuclear pineal NFkB from animals sacrificed along 24h was found with a peak of activity at ZT11 and the lowest levels at ZT21. The effect of light on NFkB translocation was addressed in animals kept in constant light condition (LL) the night before the sacrifice, showing a decrease in the peak of nuclear translocation as compared to animals in LD. A parallel increase in the plasma levels of corticosterone (CORT) at ZT11 was observed (1.56?0.20 vs 6.20?2.14 ng/ml; n=3; p<0.05). The modulatory effect of endogenous CORT on the nuclear translocation of NFKB was evidenced by the blockage of the glucocorticoid receptors (GR). In animals under LL, the GR antagonist RU486 (50 mg/kg, ip, ZT14) restored the peak of NFkB nuclear translocation as compared with non-treated animals (n=5). Conclusion: These results show that the NFkB-DNA complexes, which are rhythmically expressed in Syrian hamster pineal glands, have its activity inhibited in LL. Endogenous CORT, through GR activation, has an important role in the physiological control of NFkB translocation. These data disclose an additional molecular pathway to investigate the regulation of Aa-nat transcription in the Syrian hamster.

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Insulin temporal sensitivity and its signaling pathway on rat pineal gland

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Purpose: Due to the demonstrated potentiating effects of insulin on norepinephrine-mediated melatonin synthesis by the pineal gland, the aims of the present study were to investigate the existence of differential sensitivity periods to insulin as well as the protein components of insulin signaling pathway (ISP) presence and the possible participation of MAPK and PI3K on melatonin synthesis. Methods: Melatonin contents of in vitro and from pineal-microdyalised animals were assayed by HPLC. The total protein content and the phosphorylation state of some ISP proteins were stablished by immunoblotting and immunoprecipitation in norepinephrine-synchronized pineal gland insulin-stimulated cultures. Results: According to the temporal insulin susceptibility sensitivity protocol, two windows of differential sensitivities were observed at beginning and at the end of the norepinephrine-induced night. The major ISP componentes as IRß, IGF-1R, IRS-1, IRS-2 and PI3K were shown to be highly expressed in the pineal gland, in addition to the basal phosphorylation levels of pp85 and pp185. Insulin is apparently not involved on 14-3-3:AANAT complex formation, nor on 14-3-3 phosphorylation. NE stimulation by itself caused an increase on pMAPK 1 and 2 levels that was further stimulated by the association of insulin. PI3K activation blockage by Wortmannin revealed a partial participation of PI3K on melatonin synthesis potentiation by insulin. Conclusions: The present study has characterized periods of differential insulin-sensitivity in pineal gland on NE-induced night, modulating melatonin synthesis, as well as commonly expressed and recruited proteins by the ISP including: IRß, IGF-1R, IRS-1and 2, PI3K, MAPK.

The roles of salt-inducible kinase 1 (SIK1) and transducer of regulated CREB activity 2 (TORC2) in regulating arylalkylamine-N-acetyltransferase (Aa-nat) transcription in the rat pineal gland

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Purpose: To determine the roles of SIK1 and TORC2 on Aa-nat transcription and the function of the phosphorylation sites Ser577 (PKA-site) of SIK1, and Ser171 (SIK1-site) of TORC2, respectively, in the rat pineal gland. Methods: Rat pineal glands were collected during the light and dark periods and mRNA levels of Sik1 and Torc2 were determined (real-time RT PCR). In cell culture studies, pinealocytes were transfected with adenovirus expressing

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short hairpin targeting Sik1, wild type Sik1 or Torc2, the mutated Sik1 Ser577Ala or Torc2 Ser171Ala before stimulation with norepinephrine (NE, 3 µM). Aa-nat expression was determined by measuring the mRNA and protein (immunoblot) levels, and enzymatic activity (radio-enzymatic assay). Results: Although there was a nocturnal induction of Sik1, Torc2 had no day/night rhythm. Overexpressing wild type Sik1 inhibited Aa-nat transcription and enzymatic activity after 4 h of NE stimulation. Transfection of pinealocytes with the mutated Sik1 resulted in a more potent inhibition on Aa-nat transcription compared with the wild type. In contrast, overexpression of wild type Torc2 increased Aa-nat transcription and enzymatic activity after 12 h of NE stimulation. Transfection of pinealocytes with the mutated Torc2 had a more potent effect on Aa-nat transcription compared with the wild type. Moreover, overexpression of Sik1 was not able to suppress the enhancing effect of mutated Torc2 on Aa-nat mRNA. Similar results were obtained on other cAMP-regulated genes including 5'deiodinase, cAMP inducible early repressor, and mitogen-activated protein phophatase-1. Conclusion: The importance of the phosphorylation sites of SIK1 and TORC2 in their function as an endogenous repressor and transcriptional coactivator respectively are confirmed. This SIK1/TORC2 mechanism may regulate the temporal expression profile of Aa-nat and other cAMP-regulated genes in the rat pineal gland.

Mechanisms controlling melatonin secretion in the turkey pineal gland – studies in vitro

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Purpose: To describe mechanisms regulating the secretion of melatonin (MLT) in the turkey pineal. Methods: The explants of the turkey pineals were kept in the superfusion culture under light cycles delayed, advanced and reversed to those acquired in vivo as well as in continuous light or darkness. They were exposed to light-impulses in continuous darkness and during dark phases, treated with 8-bromo-cAMP, inhibitors of transcription, translation and proteolysis as well as agonists of adrenergic receptors. Concentration of MLT in the culture medium was measurred by RIA. Results: 1) MLT secretion in the turkey pineal gland adapted easily and accurately to different light conditions; 2) the turkey pineal glands cultured in continuous light and continuous darkness secreted MLT in circadian fashion with rises at subjective nights; 3) light treatment during scotophase inhibits MLT secretion and delayed or advance the rhythm; 4) 8-bromo-cAMP strongly stimulated the MLT release from the turkey pinealocytes; 5) MG-132 diminished or inhibited the effect of light on the MLT secretion; 6) inhibitors of transcription and translation blocked the night-time increase in the MLT release; 7) agonists of adrenergic receptors strongly decreased the MLT secretion. Conclusions: The MLT secretion in the turkey pineal is regulated by an environmental light acting on local photosensitive cells, a circadian oscillator located in these cells and by the adrenergic innervation. Activity of turkey pinealocytes entrains quickly and accurately to changes in light conditions. Therefore they are good models for chronobiological studies. Night-time increase in the MLT secretion is dependent of protein biosynthesis. The decrease of MLT secretion under the light exposure is the effect of the proteosomal proteolysis. cAMP plays a key role in the regulation of MLT secretion. The norepinephrine and alfa2 -agonists strongly inhibited the pineal hormone secretion.

The effect of norepinephrine and phorbol 12-myristate-13-acetate on melatonin secretion form bovine pineal explants in superfusion culture

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Purpose: To characterize: 1) kinetics of changes in the secretion of melatonin (MLT) from bovine pinealocytes in response to the treatment with adrenergic agonists and phorbol 12-myristate-13-acetate (PMA); 2) role of transcription and translation in the increase in MLT secretion evoked by norepinephrine (NE), isoproterenol (ISO) and PMA. Methods: The explants prepared from the pineals of 4-month-old bulls were incubated in the medium superfusion for 15 hrs. They were treated with adrenergic agonists alone and together with actinomycin D (Act) or cycloheximide (Clx). Act and Clx were introduced to medium 1 hr before and during adrenergic stimulation. In some experiments PMA was used instead of adrenergic agonists. Results: The treatment of the pineal explants with NE and ISO caused the biphasic, concentration-dependent, increase in the MLT secretion. During the first, rapid phase the MLT secretion raised 8 – 9 folds (NE at 10 microM) within 30 minutes. Then, the pineal hormone release increased slowly during next 120 - 180 minutes to reach the maximal level (NE at 10 microM - ca 12 times over the basal secretion). The treatment with Act did not influence the increase in the MLT secretion caused by the NE. The incubation in presence of Clx eliminated the slow phase, although it did not change the quick phase of the NE-induced increase in MLT secretion. During incubation in the presence of PMA a slow, but consecutive increase in MLT secretion was noted. The maximal level (at 10 microM - ca 4 times higher than before treatment) was noted at the end of incubation. The response to PMA was largely decreased by Act and abolished by Clx. Conclusions: 1) The increase in MLT secretion in bovine pinealocytes in response to adrenergic stimulation involves both protein-synthesis dependent and independent phenomena. 2) PMA stimulates MLT secretion via a pathway involving protein synthesis.

Effect of peritoneal leukocytes on AA-NAT activity in the chicken (Gallus domesticus) and Siberian hamster (Phodopus sungorus) pineal glands cultured in vitro

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Purpose: To characterize the effect of immune cells on AA-NAT activity in pineal glands cultured in vitro. Methods: The experiments were performed on 3-week-old chickens kept in L:D = 12:12 and adult Siberian hamsters kept in SD = 8:16 and LD = 16:8 photoperiods. Isolated at midday or midnight pineal glands were cultured in vitro for 24 - 30h in 37℃ (hamsters) or 41℃ (chickens), 5% CO_2 atmosphe re.

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Control chicken pineal glands were cultured in medium (RPMI) only, and those isolated from hamsters were additionally stimulated with neuropeptide Y (NPY). Experimental pineal glands were cultured in the presence of different concentrations of peritoneal leukocytes. In cultures of Siberian hamster pineal glands part of peritoneal leukocytes was stimulated with lipopolysaccharide (LPS). AA-NAT activity was measured by the liquid biphasic diffusion method. Results: Presence of peritoneal leukocytes in culture modified significantly the AA-NAT activity in pineal glands. In Siberian hamsters kept in LD we observed a decrease in AA-NAT activity. This decrease was more significant when leukocytes were stimulated with LPS. The decrease in AA-NAT activity in pineal glands isolated from hamsters kept in SD was observed only in the presence of LPS-stimulated leukocytes. On the other hand, the AA-NAT activity in chicken pineal glands increased significantly in the presence of peritoneal leukocytes and was cell-concentration dependent. Conclusion: The in vitro activity of pineal gland measured by AA-NAT activity is directly modulated by lymphoid cells, presumably through the immune mediators – cytokines. However this effect is species dependent

Genetics of the zebrafish shadow response

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Purpose: To characterise the shadow response in wild type and visual mutant zebrafish larvae. Shadow responses are known to be evoked through the pineal of larvae of the blind cavefish (Astyanax mexicanus) and the frog (Xenopus laevis). Methods: Zebrafish larvae are raised under normal light:dark conditions until 5 dpf. Mutant larvae with visual defects are identified by OKR. On the afternoon of the fifth day, larvae are placed in a 96-well plate in the Viewpoint “Zebralab” system. The lights are maintained ON for 2 hours, then 3 repeats of lights OFF for 30 min followed by lights ON for 30 min. The “per second” movement of each larva is recorded by video camera and analyzed by Zebralab software and graphed. Results: Wild type larval zebrafish exhibit distinct responses to a sudden increase or decrease in local illumination. The visual zebrafish mutants exhibit distinctively defective shadow responses. Conclusion: We have identified 4 genes required for a normal shadow response in zebrafish.

Evidence for differential photic regulation of pineal melatonin synthesis in teleosts

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Purpose: The aim of this study was to first compare the circadian control of melatonin production and second determine sensitivity of the pineal gland in a range of teleosts to demonstrate evolution of this core system within a single vertebrate class. Methods: The impact of opthalectomy on diel cycles of plasma melatonin was measured in six different teleost species (Atlantic salmon, rainbow trout, European seabass, Atlantic cod, Nile tilapia and African catfish). Furthermore, the ex-vivo pineal gland sensitivity to light was compared in salmon, cod and sea bass. Results: The plasma melatonin response to ophthalmectomy was species specific. Results could be summarised as demonstrating the presence of three forms of circadian organisation. First, salmonids presented a decentralized system in which the pineal gland responds directly to light independently of the eyes. Then, in seabass and cod both the eyes and the pineal gland are required to sustain full night-time melatonin production. Finally, a third type of circadian control of melatonin production is proposed in tilapia and catfish in which the pineal gland would not be light sensitive (or only slightly) and required the eyes to perceive light and inhibit melatonin synthesis. The pineal gland ex-vivo sensitivities to light intensity were variable with there being up to a 10,000 fold difference in sensitivity between species. Differences in spectral sensitivity were also apparent. Conclusions: Results demonstrate that the circadian control of melatonin production has dramatically changed with at least three different systems being present in teleosts. This, in conjunction with clear differences in pineal light sensitivity, would suggest that mechanisms involved in the perception of light and the transduction of this signal through the circadian axis has changed in teleosts possibly as a reflection of the photic environment in which they have evolved in.

The pineal oscillators in chicken embryos need no LD cycles to start

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Purpose: To characterize the 24-hour pattern of clk and cry1 mRNA expression in the pineal gland of chicken before hatching. Methods: Fertilized chicken eggs were incubated under constant darkness (DD). Other environmental factors such as temperature, humidity and egg rocking were kept constant. Pineal glands were collected between the 13th and 19th embryonic days (ED13-19) for semi-quantitative RT-PCR using primers for chicken clk, cry1 and ?-actin mRNAs. For in vivo studies, glands were sampled in 4 hour intervals within experiments of various lengths. For in vitro studies, glands were placed in a multi-channel flow-through system for 3 days under DD. Sampling every 4 hours from the channels started on the second day. Results: Between ED13–16, clk mRNA levels showed a steady increase while cry1 showed a continuous decrease in vivo. In vitro, episodic changes in the levels of clk and cry1 mRNAs were seen with no circadian rhythm between ED13-16. By ED17, the in vivo expression of both clk and cry1 increased. On ED18 and ED19, a tendency of decrease (minimum at 6:00 h) followed by an increase (maximum at 18:00 h) was detectable in the levels of both clk and cry1 mRNAs within one day, both in vivo and in vitro. The patterns of embryonic expression of clock genes under DD showed similar circadian changes from ED17 to that seen in adult chickens kept under light/dark (LD) cycles in vivo, however, with lower amplitude (50%). Conclusions: For the generation of synchronized patterns of clock gene expression at a certain point of pineal development, rhythmic environmental factors are not essential in chicken.

Pinealocytes in Common gull – ultrastructural study

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Purpose: To characterize of pinealocytes in the pineal gland of Common gull, Larus canus. Methods: The pineals of 6 young juvenile common gulls living in natural conditions, which had been untreatably injured during strong storms and qualified for euthanasia were used. The birds were killed by administration of the lethal dose of pentobarbital, the pineals were immediately removed and prepared for ultrastructural examine. Results: The rudimentary-receptor pinealocytes and secretory pinealocytes were identified. Rudimentary-receptor pinealocytes differed with each other in size and shape; therefore we divided these cells into two types, called A and B. The cells of the A type were elongated and showed regular stratified distribution of organelles which create zones. Pinealocytes type B were much shorter than A, showed polarity of their internal structure, but the regular distribution of all organelles in form of zones was not observed. Cilia without central pair of microtubules outgrew from the apical parts both types of cells. In the basal processes of cells microtubules as well as clear and dense vesicles were observed. Secretory pinealocytes with nucleus located centrally in the cell formed rather uniform population and contained irregularly dispersed organelles. Common gull - specific feature of pinealocytes was the presence of intracellular intrusions in form crystals with regular inner structure and areas of glycogen particles. It was possible to follow the successive phases of glycogen accumulation in pinealocytes started from one or more small foci of densely packed glycogen particles, next formed the “glycogen lake”, which increased in size and filled almost whole cytoplasmic compartment. Conclusion: Two rudimentary-receptor pinealocyte types and secretory pinealocytes have been identified in gland. These cells show species-specific features - presence of intracellular intrusions in form of crystals and glycogen storage.

Daily rhythms in plasma and pituitary vasotocin content: involvement in melatonin synthesis in the pineal organ of rainbow trout

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Introduction: Arginine vasotocin (AVT) is a neurohypophysial peptide hormone analogue of mammalian vasopressin (VP). Both hormones have been implicated in the regulation of ionosmostic and water balance, but they could have also a role in the regulation of melatonin rhythms. As respect to AVT, previous studies in fish have shown daily variations in plasma AVT levels, and a temporary relation between AVT and melatonin has been proposed. Purpose: First, to characterize the daily rhythm of pituitary AVT secretion in trout and its association with environmental lighting cycle and second, to study the effects of AVT on melatonin secretion in trout pineal organ cultures. Methods: In a first experiment, trout were kept under natural photoperiod and in constant darkness, and sampled at different times. The plasmatic and the pituitary AVT contents were measured by gradient HPLC with fluorescence detection. In a second experiment, the effect of different concentrations of AVT on trout melatonin production was studied in cultured pineal organs kept under several lighting conditions. Results: The results showed a clear rhythm in the pituitary and plasma AVT levels with peaks during the day-night transition phase. Under constant darkness, both pituitary and plasma AVT rhythms remain unaltered with similar daily profiles than those observed under natural photoperiod. In addition, we found that treatment with AVT at concentrations from 10-9 and 10-11 M increased melatonin production in pineal organs in vitro, particularly when assays were carried out under light of low intensity. Conclusions: The results suggest the existence of an endogenous rhythm of AVT production that might play a role as a diurnal signal modulating melatonin synthesis in fish pineal organ.

Postembryonic changes in the circadian rhythm of the chicken pineal gland biosynthetic activity

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Purpose: To find interrelationships between circadian rhythm of the activity of two enzymes in the melatonin biosynthetic pathway (serotonin N-acetyltransferase AA-NAT and hydroxyindole-O-methyltransferase HIOMT) and the level of precursors (tryptophan TRY and serotonin 5-HT) and a product (melatonin MEL) in the pineal gland of the early post-hatch male chickens. Methods: Pineal glands from 2-, 9- and 16-day old birds kept from the day of hatch in L:D 12:12 conditions were isolated under dim red light every 2 hs over a 24 hs period, immediately frozen and subsequently used for analysis of: AA-NAT and HIOMT activity (liquid biphasic diffusion method), and TRY, 5-HT and MEL content (ELISA). Results: Circadian rhythm of the pineal AA-NAT activity, with a well pronounced nocturnal peak, was observed already in 2-day-old birds and this pattern did not change with age while the amplitude increased between 2- and 9-day of age. Similarly, MEL content in the pineal gland changed rhythmically since 2-day of age, but neither the level nor amplitude exhibited age-related differences. On the contrary – HIOMT activity changes were age-dependent: in 2-day-old chickens the activity did not exhibit rhythmicity while in 9- and 16-day-old a bimodal rhythm appeared, and, moreover, the level and amplitude increased with age. The diurnal changes in the MEL precursors were not rhythmical and their content did not increase between 9- and 16-day of age. Conclusions: Rhythmical biosynthetic function of the chicken pineal gland undergoes the post-embryonic modifications, exhibited by an increase in the nocturnal peak of AA-NAT activity and MEL content during the first week of the development. Lack of their further increase does not seem to depend on the availability of TRY, being several times more abundant than MEL but the developmental changes in 5-HT content seem to have some limiting impact.

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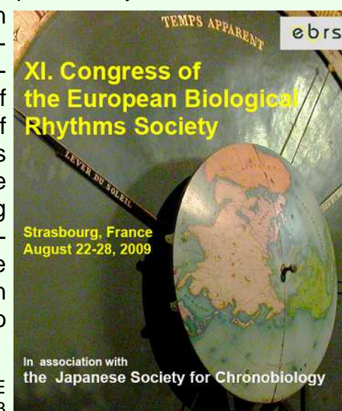


Society for Research on Biological Rhythms

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

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/

Ría de Vigo

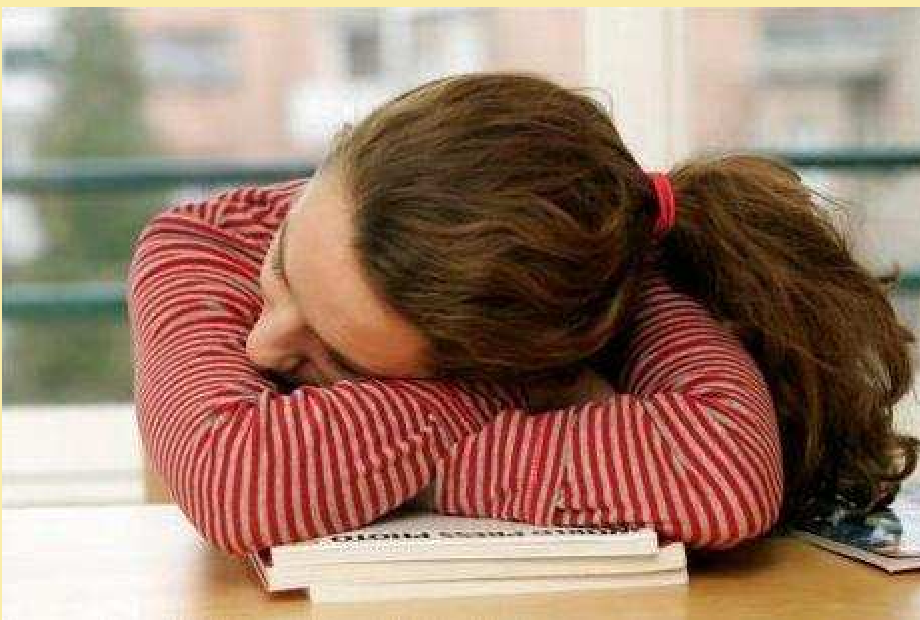
Différencier retard de phase et retard d'endormissement chez l'adolescent

Résumé établi par le Dr Mathilde Ferry (Le Quotidien du Médecin, 4/12/2009), d'après la conférence de Patricia Franco (Unité de sommeil pédiatrique, Lyon), à une session du congrès de la SFRMS parrainée par la SFC

Parmi les nombreux adolescents en privation chronique de sommeil, les vrais syndromes de retard de phase sont rares. Du fait des conséquences cognitives et sociales importantes, la prévention de l'insomnie est indispensable et passe par le contrôle des facteurs d'environnement.

Des modifications du sommeil se produisent de l'enfance à l'adolescence, sous l'effet de facteurs physiologiques, environnementaux, psychologiques et parfois psychopathologiques. Des processus intrinsèques agissent sur les systèmes homéostasique et circadien, permettant la maturation du sommeil. Il se produit, dès l'âge de 12 ans, une diminution de 40 % du sommeil lent profond avec réduction de 60 % des ondes delta, du fait d'une diminution du nombre des connexions synaptiques corticales et de l'activité métabolique cérébrale. Une augmentation du sommeil lent léger permet de la compenser, d'où une stabilité de la durée totale de sommeil (9,25 heures). L'adolescent présente alors une somnolence diurne accrue et une évolution vers une typologie vespérale plutôt que matinale. Avec la maturation pubertaire, l'heure d'endormissement est plus tardive et la sécrétion de mélatonine est retardée.

Plusieurs hypothèses ont été émises :



une augmentation de la durée de la période intrinsèque du cycle circadien chez l'adolescent (24 h 27 min) par rapport à l'adulte (24 h 12 min)

une augmentation de sensibilité au retard de phase induit par l'exposition à la lumière.

Ainsi, l'évolution vers le retard de phase se fait entre 10 et 20 ans, plus précocement chez la fille que chez le garçon, avec un effet inverse à partir de 20 ans. Il s'agit d'un retard de phase physiologique. Des études ont mis en évidence le rôle des hormones sexuelles sur les régulations circadienne et homéostasique. Avec la maturation, les adolescents se couchent plus tard (surtout le week-end) et donc se lèvent plus tard, d'où une diminution du sommeil total, notamment les jours scolaires : des résultats qui ont été retrouvés dans seize pays différents. Aux processus intrinsèques viennent s'ajouter des facteurs extrinsèques : diminution de l'influence parentale, augmentation de la pression scolaire (horaires scolaires), de la pression sociale, de l'usage des technologies d'information et de communication (TV, ordinateur, téléphone portable), mais aussi de la consommation de caféine, d'alcool et de tabac. Enfin, des comorbidités psychiatriques peuvent s'y associer (dépression, troubles bipolaires, troubles de la personnalité…).

La prévalence des difficultés d'endormissement de l'adolescent est d'environ 17 %.Il peut s'agir d'une insomnie d'initiation du sommeil ou d'un syndrome de retard de phase (SRPS). La prévalence de l'insomnie de l'adolescent varie entre 4 et 10 %. Elle début dès l'âge de 11 ans, d'abord chez les garçons, puis prédomine chez les filles (x 2,75) après l'apparition des menstruations. Il y a plus d'insomnie d'initiation que de sommeil de mauvaise qualité et que d'insomnie de maintien du sommeil. Il est à noter que plus de 50 % des insomnies sont associées à des troubles psychiatriques. Quant au SRPS, il concerne 7 à 10 % des consultations d'adultes insomniaques. Il apparaît à l'adolescence, plus souvent chez ceux qui présentent déjà une insomnie. En Europe, son taux est de 0,4 % chez

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les 15-18 ans. Il convient de faire la distinction entre un SRPS et un aspect normal durant l'adolescence, exacerbé par un comportement voulu (retard motivé de la phase de sommeil). En effet, le vrai SRPS est rare. Néanmoins, plus de 50 % des adolescents avec un SRPS présentent des signes cliniques de dépression. D'où l'importance de toujours penser à un facteur dépressif sous-jacent en cas de phobie scolaire. Un agenda du sommeil (périodes scolaires et non scolaires), une actimétrie, un dosage de mélatonine salivaire ou urinaire, et éventuellement une polysomnographie permettront d'établir le diagnostic. La distinction entre SRPS et insomnie sera portée sur l'actimétrie pendant les jours non scolaires. L'insomnie d'endormissement et le SRPS ayant tous les deux un rythme de température centrale et une sécrétion de mélatonine retardés, le dosage de mélatonine et les courbes de température ont peu d'intérêt.

Vingt-cinq à 87 % des adolescents pensent qu'ils ne dorment pas assez. Trente à 60 % se plaignent de somnolence diurne. De nombreux adolescents sont en privation chronique de sommeil avec des conséquences neuropsychologiques non seulement sur leur développement et leur comportement, mais également sur leur métabolisme (ceux qui dorment peu ont 58 % de risque de surpoids et d'obésité). En outre, les accidents de la route et les accidents domestiques augmentent.

La prise en charge de l'insomnie de l'adolescent porte principalement sur l'hygiène de vie et de sommeil : suppression des activités trop stimulantes avec le coucher. La chronothérapie et la luminothérapie peuvent être utiles. La mélatonine est efficace au niveau circadien. Enfin, une psychothérapie est nécessaire en cas de dépression et de phobie scolaire.

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Deadline for Abstract Submission:

February 15, 2010

Chronobiologistes…

encore un effort pour vos contributions à Rythmes.

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

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 !

Vous devez envoyer vos contributions en document attaché. Les fichiers seront préférentiellement sauvegardés au format ∗.doc, ∗.rtf, ou ∗.txt après avoir été produits par un traitement de texte standard. Pour tout autre format que ces formats répandus, nous consulter.

Il est impératif de nous faire parvenir un fichier texte sans retours à la ligne multiples, tout en conservant l'accentuation. De même, ne mettez pas de lignes blanches pour marquer les paragraphes ni mises en page complexes, que nous devrons de toutes façons changer pour rester dans le style du journal.

Les images pourront être en tiff, bmp, gif, jpeg, jpg ou png. Rythmes est mis en page sur un PC, donc les formats PC sont préférés, car cela évite des manipulations.

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.

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Les articles publiés dans ce bulletin reflètent l'opinion de leurs auteurs, et en aucun cas celle de la Société Francophone de Chronobiologie.

Ont contribué à ce numéro

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