Lettre de la Société Francophone de Chronobiologie

RYTHMES

Bulletin du Groupe d'Étude des Rythmes Biologiques

Tome 40 - Numéro 3

Sept 2009

http://www.sf-chronobiologie.org

Sommaire

Éditorial

Le XIème congrès de la Société Européenne des Rythmes Biologiques s'est tenue à Strasbourg du 22 au 28 Août 2009, en association avec la Société Japonaise de Chronobiologie. Il était organisé par Paul Pévet et son équipe. Pour la première fois, sa durée a été allongée à 6 jours pleins, avec 2 sessions simultanées de Symposia. C'est dire l'extrême richesse du programme qui nous était offert, témoin de l'importance prise par la dimension temporelle dans les recherches biologiques, tant dans les domaines fondamentaux qu'appliqués. Au cours de ce congrès, nous avons perçu la pertinence de la volonté manifestée par certains il y a quelques années, pour faire évoluer la Société Européenne dédiée à la glande pinéale en une société élargie aux rythmes dans leur ensemble. Au congrès de Strasbourg, seuls 3 symposiums sur 19 étaient consacrés à la mélatonine. Soyez rassurés, cette hormone conserve tout son intérêt, en particulier comme marqueur de l'activité de l'horloge circadienne et des travaux intéressants impliquant la mélatonine ont été rapportés dans l'Alzheimer, tant au plan fondamental qu'appliqué par les groupes de James Olcese et d'Eus Van Someren, respectivement.

Ce congrès a grandement contribué aux échanges scientifiques, avec 12 conférences, 92 communications orales et 234 communications affichées exposées en permanence et largement visitées au cours de sessions spécifiques. Trois lectures plénières ont été consacrées aux grands scientifiques ayant influencé notre discipline, Johannes Ariens-Kapper, Julius Axelrod et Eberhard Gwinner et nous ont rappelé que la Science se doit de conserver une mémoire.

Malgré la période choisie (fin des vacances d'été) et la crise économique, la fréquentation a été importante : Environ 450 inscrits dont une centaine de participants issus des laboratoires français et une vingtaine issue de laboratoires francophones. Les nombreuses bourses accordées et le faible coût d'inscription y ont contribué. Nous espérons que ces acquis et cet élan seront conservés lors du prochain congrès de la Société Francophone organisé par Franck Delaunay et son équipe en septembre 2010. Le prix de notre Société a été attribué avant le congrès, ce qui a permis au lauréat, Jorge Mendoza, de présenter ses travaux lors d'une session intégrant les lauréats des autres Sociétés organisatrices. Cette procédure d'évaluation des prétendants à la bourse, anticipant le congrès, pourrait être maintenue lors des futures réunions francophones.

Malgré la fatigue liée à notre participation indéfectible aux sessions, nous avons

(Suite page 66)

pu profiter de la qualité de vie strasbourgeoise, en particulier en soirée. La parfaite organisation et la qualité de l'accueil de **l'équipe de Paul Pévet**, à la fois amical et d'une grande disponibilité, ont contribué à renforcer le succès de la manifestation.

Pour terminer, je mentionnerai que cette manifestation européenne, bien que riche de discussions amplifiées par des microphones largement baladeurs et d'échanges conviviaux, n'a pas à ma connaissance contribué à la dissémination du virus A H1N1, en dépit de certaines toux suspectes d'origine insulaire.

Bruno Claustrat

Président

In Memoriam Jean De Prins 1932 – 2009

Après l'Athénée d'Ixelles il poursuit, à l'Université Libre de Bruxelles (ULB), des études de physique et de mathématiques. Il travaille ensuite au Laboratoire Suisse de Recherches Horlogères à Neuchâtel. Il présente sa thèse de doctorat à l'Université de Neuchâtel en 1961.

Il est rappelé à l'ULB pour créer un laboratoire et enseigner la physique en néerlandais et en français ainsi que l' « Histoire des Sciences ». Il crée plusieurs cours d'électronique. Au cours de sa carrière il dirige de nombreux mémoires de licence et thèses de doctorat.

Il fonde un laboratoire des étalons de fréquence, puis son activité scientifique se déplace progressivement vers les problèmes délicats posés par le traitement des données expérimentales sur les phénomènes complexes dépendant du temps comme le « bruit en 1/f », les crues des grands fleuves, l'acoustique, puis comme préoccupation essentielle, la chronobiologie. Dans ce domaine Jean De Prins a de nombreuses collaborations internationales avec des médecins et des biologistes ; il est invité à donner des cours à l'étranger et participe à la mise au point d'un logiciel convivial pour le dépouillement des données de chronobiologie.

A deux reprises il a été président de notre société.

Il est décédé le 15 mai 2009.

Thérèse Vanden Driessche

Vos coordonnées accessibles sur le site de la SFC

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Pensez à actualiser vos données

Utilisez ce formulaire pour une première inscription ;

Modifiez vos données en ligne si nécessaire (voir page 67).

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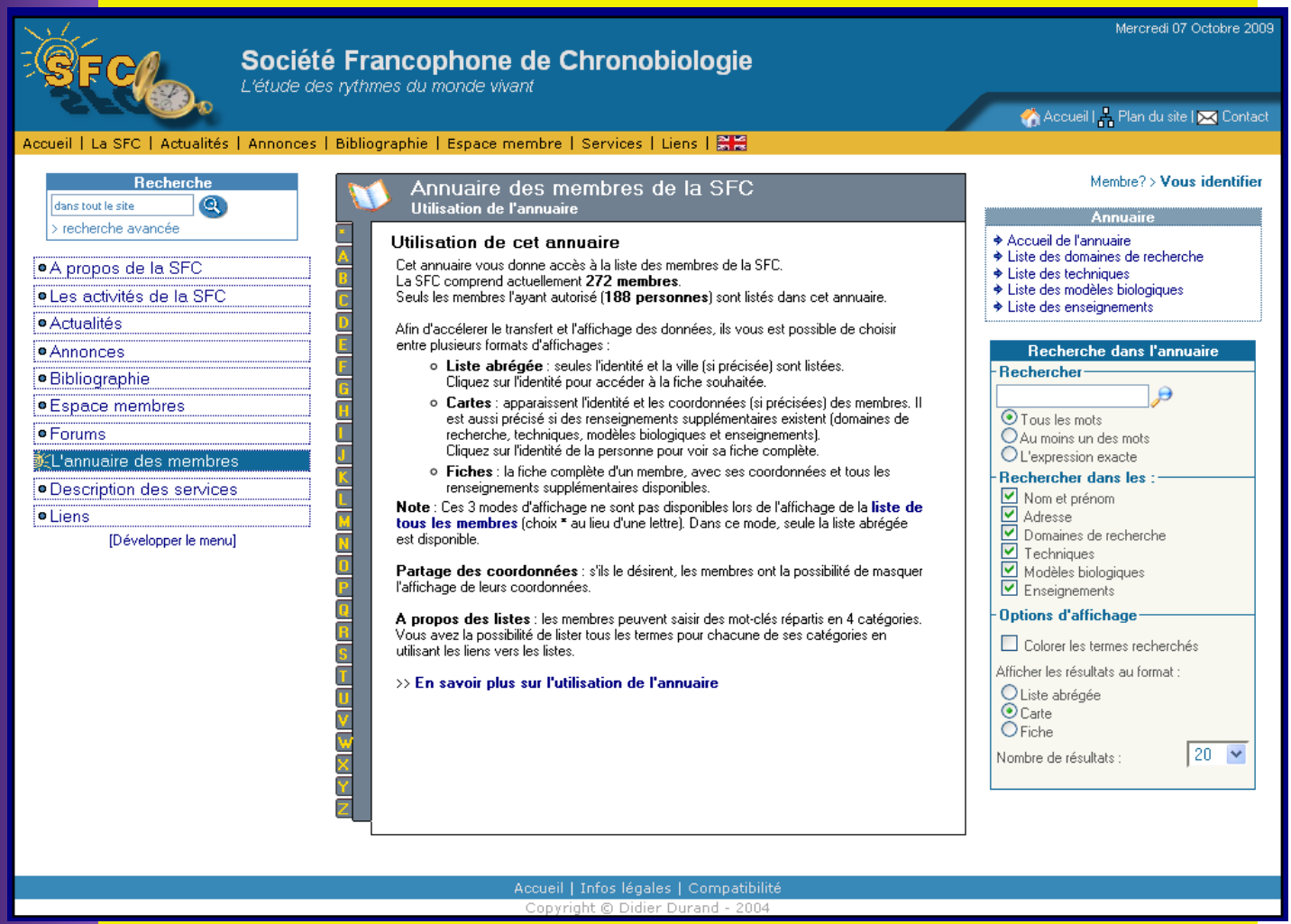
Visitez régulièrement le site Web de la SFC

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

http://www.sf-chronobiologie.org

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

Sophie LUMINEAU



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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.

Compte-rendu de l'assemblée générale de la SFC
24 août 2009, Strasbourg

Membres excusés pour la réunion du conseil d'administration du 23/08/09: Bernard BRUGUEROLLE, René CLARISSE, Sophie LUMINEAU (secrétaire adjointe), Isabelle MENEY-ESSABER et Berthe VIVIEN-ROELS (trésorière adjointe).

Membres présents à la réunion du conseil d'administration du 23/08/09: Fabienne AUJARD (trésorière), Olivier BOSLER, Etienne CHALLET (secrétaire général), Bruno CLAUSTRAT (président), Howard COOPER (vice-président), Franck DELAUNAY, Ouria DKHISSI-BENYAHA, Albert GOLDBETER, Francis LÉVI et Benoît MALPAUX.

Ouverture de séance à **19 h par Bruno CLAUSTRAT, président, en présence de 37 membres.**

En premier lieu, l'assemblée vote à l'unanimité l'approbation du compte-rendu de l'assemblée générale de la SFC tenue en 2008 à Caen.

1. Compte-rendu moral du président

Dans son allocution introductive, Bruno CLAUSTRAT évoque la bonne visibilité nationale et internationale de notre société. Parmi les actions-phares de cette année, figure la session «Chronobiologie, performances et sommeil » co-parrainée par la SFC et la Société Française de Recherche et Médecine du Sommeil (SFRMS) lors du congrès du sommeil (du 19 au 21 novembre 2009 à Marseille ; http://www.lecongresdusommeil.com/). Notre président souligne l'intérêt qu'il y aurait à disposer d'une plaquette décrivant la SFC.

Par ailleurs, le rôle du vice-président pourrait concerner plus spécifiquement les relations internationales. Howard COOPER mettra à jour le recensement des enseignements francophones de chronobiologie qui avait déjà été amorcé. Ceci pourrait déboucher sur un enseignement national dans le meilleur des cas.

2. Bilan financier par la trésorière

Au cours de l'année écoulée, les dépenses se sont élevées à 7 080,85 € alors que les crédits ont été de 6 466,65 €.

A la date du 20 août 2009, le CCP est crédité de la somme de 4 591,55 € et le livret de caisse d'Epargne de 12 458,15 €. La société a donc un avoir total de 17 049,70 €. L'assemblée félicite la trésorière de la bonne tenue des comptes de la SFC et accorde le quitus à l'unanimité.

3. Bilan des adhérents et cotisations 2010

La société compte 178 adhérents à jour de leur cotisation, alors qu'ils étaient 130 en juin 2008.

Depuis cette date, 14 nouvelles personnes (dont 7 doctorants) souhaitent adhérer à la SFC :

1. SIFFROI-FERNANDEZ Sandrine, chercheuse post-doctorante, Nice
2. RIBOULEAU-VAUZELLE Marie-Claude, Dr pharmacien
3. NAJJAR Raymond, doctorant, Lyon
4. BURCKEL André, biologiste
5. LAURENT-GYDE Virginie, maître de conférences, Strasbourg
6. PICASSO Sylviane, Dr nutritionniste
7. BERTHOMIER Cédric, diététicien
8. PIFFERI Fabien, chercheur post-doctorant, Brunoy
9. DEVAVRY Séverine, doctorante, Nouzilly
10. CHALIVOIX Stéphanie, doctorante, Nouzilly
11. ANDERS Doreen, doctorante, Bâle
12. GIRARDET Clémence, doctorante, Marseille
13. FAIVRE Thierry, Psychiatre spécialiste sommeil, Meyzieu
14. CHELLAPPA Sarah, doctorante, Bâle

Leur adhésion est approuvée à l'unanimité par l'assemblée.

Pour l'année 2009-2010, la cotisation annuelle reste inchangée à 25 € par adhérent, à 12,50 € pour les retraités. La cotisation est toujours gratuite pour les étudiants sous réserve qu'ils publient un article dans RYTHMES. A noter cependant le changement de supplément qui s'élèvera désormais à 10 € pour l'envoi papier du bulletin RYTHMES.

4. Informations sur les prochains congrès SFC

L'an prochain, Franck DELAUNAY et Howard COOPER organisent le congrès annuel de la SFC près d'Antibes, du 15 au 18 septembre 2010. Un tarif préférentiel sera proposé aux étudiants. Par ailleurs, 5 bourses d'inscription/hébergement seront allouées par le CA de la SFC à des doctorant(e)s.

Contrairement aux années passées, Franck et Howard souhaitent que la gestion financière de leur congrès se fasse via une ligne budgétaire sur le compte de la SFC.

Pour 2011, l'organisation du congrès annuel pourrait être assurée par Benoît MALPAUX et ses collègues de Nouzilly.

5. Bulletin RYTHMES

Le bulletin RYTHMES a maintenu sa fréquence de parution trimestrielle. Le chargement électronique des bulletins par les membres est possible par le site

web de la SFC (http://www.sf-chronobiologie.org/revue.php).

Fabienne AUJARD, rédactrice en chef, rappelle une fois encore le problème récurrent de l'approvisionnement en articles pour la revue, qui doit pourtant être un support d'échanges scientifiques entre les membres de la société. Il est rappelé, par exemple, que chaque résumé de thèse peut paraître dans RYTHMES. Enfin, Fabienne rappelle que les doctorants, membres à titre gracieux de la SFC, ainsi que les récipiendaires du prix SFC se sont engagés à soumettre un article dans RYTHMES, ce que peu d'entre eux ont fait jusqu'à présent.

Il est par ailleurs décidé que la version papier de RYTHMES ne sera plus reliée à partir de 2010 pour éviter le problème du nombre de pages (multiple de 4) inhérent à cette présentation.

6. Site internet de la SFC (http://www.sf-chronobiologie.org/)

C'est toujours Sophie LUMINEAU, secrétaire adjointe, s'occupe de la maintenance du site de la SFC. Le site visible aux membres de la SFC et au grand public assure bien notre visibilité nationale et internationale. Il est rappelé à chacun qu'il peut participer à sa dynamique en y postant des annonces d'évènements, d'offres…, qui sont encore essentiellement remplies par Etienne CHALLET. De plus, le forum de discussion reste toujours exploité.

Afin de compléter le site, une page recensant les enseignements de la chronobiologie en France sera créée.

Par ailleurs, notre président propose aussi de rendre accessibles en ligne les articles parus dans RYTHMES. Ils seraient alors classés sur le site par ordre d'arrivée.

Bruno suggère également d'alimenter le site avec des articles de vulgarisation des grands thèmes de la chronobiologie qui pourraient être intéressants pour un large public (hibernation, chronothérapie). Cher(e)s collègues membres de la SFC, à vos plumes (claviers) !

Autre proposition complémentaire de Bruno : il s'agirait d'inclure un glossaire des termes de chronobiologie sur le site. François ROUYER suggère de créer un lien avec la base de données Wikipedia.

7. Renouvellement d'une partie du Conseil d'Administration

En ce qui concerne le renouvellement du Conseil d'Administration, voici quelles sont les échéances cette année :

- Trois membres sont en fin de 2e mandat (non renouvelable): Bernard BRUGUEROLLE (ex-président), Isabelle MENEY-ESSABER et Berthe VIVIEN-ROELS (trésorière-adjointe)
- Deux membres sont en fin de 1er mandat (renouvelable) : Howard COOPER et Benoît MALPAUX

L'assemblée générale remercie chaleureusement les trois membres (Bernard, Isabelle et Berthe) qui ne peuvent se représenter au CA.

Six personnes font acte de candidature au CA pour 5 postes à pourvoir : Howard et Benoît pour un second mandat ainsi que :

- Xavier BONNEFOND (chargé de recherches au CNRS, chronobiologie des systèmes neuroendocrines, Montpellier)
- Olivier COSTE (médecin en chef, responsable de l'équipe « vigilance, performance et chronobiologie » à l'IMASSA, Toulon)
- Bertrand KAEFFER (chargé de recherches à l'INRA, chronobiologie du nouveau-né, Nantes)
- André KLARSFELD (chargé de recherches au CNRS, chronobiologie de la drosophile, Gif-sur-Yvette)

Les résultats du vote à bulletin secret (35 votants et 1 bulletin blanc) sont les suivants :

- X. BONNEFOND: 31 (Elu),
- H. COOPER: 30 (Elu),
- O. COSTES: 23 (Elu),
- B. KAEFFER: 20 *(Non élu),*
- A. KLARSFELD: 30 (Elu),
- B. MALPAUX: 31 (Elu).

8. Renouvellement du secrétaire, de la secrétaire-adjointe, de la trésorière et de la trésorière-adjointe

Suite au départ de Berthe VIVIEN, le poste de trésorier adjoint était vacant. Franck accepte de reprendre cette fonction.

Les postes de secrétaire, de secrétaire-adjointe et trésorière étaient également vacants, mais aucun des membres du CA n'a présenté sa candidature. Pour assurer la transition et en attendant que des volontaires se manifestent, les trois personnes concernées acceptent de continuer à assurer leur fonction pour la ou les deux années à venir (date de fin de 2e mandat au CA pour les 3). Le CA accepte cette solution transitoire.

9. Bourse de voyage 2009

Un jury composé de tous les membres du CA a attribué deux bourses de voyage couvrant les frais de voyage pour venir assister au congrès de l'EBRS, l'une à Céline LEGROS, chercheuse post-doctorante à Londres (montant attribué de 145 €), l'autre à Christine MERLIN, chercheuse post-doctorante à

Worcester (Etats-Unis, montant attribué de 1000 €).

10. Prix Jeune chercheur/jeune chercheuse 2009

Pour rappel, le prix Jeune chercheur/jeune chercheuse, d'un montant de 1500 €, récompense un chercheur chronobiologiste de moins de 35 ans.

Un jury composé de 4 personnes du CA, n'ayant pas co-publié avec les candidats (Olivier BOSLER, René CLARISSE, Franck DELAUNAY, Sophie LUMINEAU) sous la direction de Bruno CLAUSTRAT a été attribué cette année à Jorge MENDOZA, chargé de recherche à Strasbourg.

Comme convenu l'an dernier, le/la lauréat(e) du prix Jeune chercheur/jeune chercheuse a été choisi(e) avant le congrès EBRS (juin 2009) afin de prévoir une présentation orale lors du congrès.

11. Informations et points divers

Le CA a le regret d'annoncer le décès du Professeur Jean DE PRINS le 15 mai 2009. le Pr. J. DE PRINS, physicien belge et spécialiste du traitement du signal, est l'un des fondateurs du « Groupe d'Etudes Des Rythmes Biologiques » (GERB) en 1969, GERB qui a été renommé « Société Francophone de Chronobiologie » en 1998. Madame Thérèse VANDEN DRIESSCHE a accepté d'écrire un article dans RYTHMES en hommage au Pr. DE PRINS.

La société suisse MEDIWATCH qui commercialise des montres dites « chronobiologiques » a émis le souhait de s'associer à la SFC. Dans la mesure où notre société est à but non lucratif, le CA décline cette proposition.

Le reliquat de trésorerie (moins de 1000€) de l'Association de Chronobiologie Médicale (ACM) sera versé à la SFC sur une ligne budgétaire identifiée. Howard Cooper propose que les modalités de gestion de cette ligne soient étudiées (par exemple délégation de signature temporaire le temps de l'organisation d'un séminaire thématique).

Etienne Challet,

Secrétaire.

Neuroscience 2009

Neuroscience 2009 (Oct. 17-21, in Chicago, Ill)

will offer unequalled scientific content in a world class city. Chicago, a hub of science and nightlife, is a welcoming host for the 39th annual meeting of the Society for Neuroscience.

Browse the Web site http://www.sfn.org/am2009/ to learn more and check back frequently for updates.

Contribute to the Neuroscience 2009 scientific program.

Abstract submission opens April 23 and closes May 14. Last year, SfN received nearly 16,000 abstract submissions.

Each year satellite events occur during SfN's annual meeting. These events are for annual meeting attendees, but are not planned or sponsored by SfN. Learn how to reserve space for your event in Chicago.

L'entraînement maternel prénatal chez les mammifères

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Introduction

Chez les mammifères, la principale horloge circadienne qui régule la physiologie et le comportement est localisée dans les noyaux suprachiasmatiques (NSC) de l'hypothalamus antérieur (Ralph *et al*., 1990 ; Klein *et al*., 1991). Chez les adultes, le premier signal environnemental entraînant cette horloge centrale est le cycle photopériodique (Aschoff, 1981) qui agit par voie neurale via les tractus rétinohypothalamiques (TRH) monosynaptiques, de la rétine vers les NSC (Moore & Lenn, 1972 ; Moore & Card, 1985). L'horloge centrale localisée dans les NSC entraîne, via une voie neurale, la glande pinéale, principal site de production de mélatonine. La glande pinéale des mammifères n'est ni photo-réceptive ni une horloge indépendante. L'horloge des NSC coordonne également des horloges périphériques situées dans des organes tels que le cœur, les poumons, le foie ou les reins, l'utérus… (Hastings *et al*., 2007). Ces horloges périphériques peuvent aussi être synchronisées par des zeitgebers non-photiques tels que la nourriture (Revues : Stephan, 2002 ; Pardini & Kaeffer, 2006) et pourront alors éventuellement être découplées de l'horloge centrale selon la nature et la force du nouveau zeitgeber.

Des études, chez le rat, ont montré que les NSC des fœtus oscillaient déjà durant la fin de la phase prénatale (Reppert & Schwartz, 1983, 1984) : les NSC fœtaux manifestent un rythme journalier de l'utilisation du glucose avec une activité importante la journée et faible la nuit. De la même manière, des études menées chez le mouton (Constandil *et al*., 1995 ; Breen *et al*., 1996) ont mis en évidence un rythme nycthéméral de l'activité des neurones des NSC fœtaux (expression de Fos) à partir du 90ème jour de gestation (sur 146 jours). Ainsi, l'horloge circadienne localisée dans les NSC serait fonctionnelle dès la fin du développement fœtal et synchronisée sur le cycle jour/nuit.

Les noyaux fœtaux sont-ils, comme chez les animaux adultes, entraînés par le cycle photopériodique de l'environnement ? La lumière peut pénétrer dans l'abdomen et l'utérus de la mère. Néanmoins, chez le rat, espèce nidicole, les yeux des fœtus ne sont pas encore reliés aux NSC via le TRH (Speh & Moore, 1993) et donc il semble peu probable que chez cette espèce la lumière synchronise directement les NSC du fœtus. La synchronisation aux conditions lumineuses environnantes pourrait alors se faire via le système circadien de la mère. En revanche, chez les moutons, espèce nidifuge, les NSC fœtaux sont innervés par le TRH dès le milieu de la gestation (Torrealba *et al*., 1993). Chez cette espèce, il est donc plus difficile de trancher pour un entraînement direct de la lumière ou pour un entraînement indirect via la mère. Breen *et al*. (1996) ont remarqué qu'un fœtus optiquement énucléé au 100ème jour de gestation présente un rythme nycthéméral de FOS dans les NSC tout à fait similaire au rythme trouvé chez son jumeau non opéré. Cette étude suggère que, chez le mouton, l'effet direct de la lumière sur la rétine des fœtus est négligeable. Breen *et al*. (1996) proposent donc que la mère serait à l'origine de la synchronisation du fœtus aux conditions lumineuses de l'environnement.

Nous nous proposons de réaliser une revue des connaissances actuelles sur les influences que la mère peut avoir sur la rythmicité du fœtus pour tenter de tirer des conclusions sur les processus d'entraînement fœtal. Dans un deuxième temps, nous nous intéresserons à l'impact que peut avoir cet entraînement prénatal sur la rythmicité de l'individu après sa naissance.

Entraînement du fœtus par la mère

Rôle des noyaux suprachiasmatiques maternels

Reppert & Schwartz (1986a) ont examiné l'effet de lésions des NSC (opération connue pour éliminer la rythmicité circadienne chez les mammifères) de rattes gestantes (au jour 7 de gestation), maintenues en LD, sur le fonctionnement de l'horloge des fœtus. Ils ont, pour cela, mesuré par autoradiographie (Schwartz & Gainer, 1977), l'utilisation du glucose (reflet de l'activité métabolique) dans les NSC fœtaux (aux jours 20-21 de la gestation). Chez les fœtus de mères non opérées, les chercheurs trouvent un rythme nycthéméral très clair de l'utilisation

(Suite page 72)

(Suite de la page 71)

du glucose dans les NSC. C'est-à-dire que les NSC fœtaux sont métaboliquement actifs durant la photophase et à l'inverse, métaboliquement inactifs durant la scotophase. A l'opposé, aucun rythme journalier de l'activité métabolique des NSC n'est observé chez les fœtus de mères ayant subit des lésions des noyaux. Dans ce cas, les valeurs moyennes de l'activité métabolique des NCS fœtaux sont intermédiaires entre les valeurs attendues pour la photophase et la scotophase.

Les rythmes journaliers mesurés dans cette étude correspondent à ceux de portées (tous les fœtus d'une même mère) et donc la disparition de ces rythmes chez les fœtus de mères opérées pourrait refléter une perte de la rythmicité d'une part ou bien une désynchronisation des rythmes de chacun des fœtus d'une même mère d'autre part. Pour Reppert & Schwartz (1986a), la deuxième interprétation serait la plus probable. En effet, l'inspection visuelle des autoradiographies montre que l'activité métabolique individuelle des fœtus d'une même mère est très variable avec des phases actives et des phases inactives comme si l'horloge centrale des fœtus oscillait et générait un rythme circadien en libre cours.

Shibata & Moore (1988) ont eux placé des rattes gestantes (dès le jour 10 de gestation) dans des conditions constantes d'illumination (conditions connues pour éliminer la rythmicité circadienne chez cette espèce). Les fœtus des mères ayant subit ce traitement lumineux ne présentent pas de rythme d'activité métabolique dans leurs NSC au jour 22 de la gestation, contrairement aux fœtus de mères contrôles (placées en conditions photopériodiques LD durant toute la durée de la gestation) qui présentent un rythme nycthéméral de l'utilisation du glucose dans les noyaux.

De la même manière que dans l'expérience de Reppert & Schwartz (1986a), lorsque que les fœtus des rattes gestantes placées en LL sont examinés individuellement, un rythme d'utilisation du glucose dans les NSC semble se dessiner mais les rythmes de chaque fœtus ne paraissent plus synchronisés les uns avec les autres (Shibata & Moore, 1988). Ceci suggère donc à nouveau que les NSC fœtaux oscilleraient en libre cours indépendamment les uns des autres quand ils ne sont plus entraînés par la rythmicité maternelle. Cela semble également suggérer que les fœtus d'une même mère ne se synchronisent pas mutuellement.

A la vue de ces expériences, l'horloge circadienne centrale du fœtus apparaît être entraînée par la mère mais pas directement par le cycle photopériodique. En effet, dans la première expérience (Reppert & Schwartz, 1986a) les fœtus de mères opérées restent sous conditions photopériodiques mais ne sont pourtant pas synchronisés sur ce cycle. Et dans la deuxième expérience (Shibata & Moore, 1988), les conditions constantes de lumière ne semblent pas, comme pour les mères, supprimer la rythmicité circadienne en libre cours des fœtus. Comment alors l'information lumineuse est-elle transmise de la mère au fœtus étant donné que le fœtus est anatomiquement séparé de la mère par le placenta et qu'il n'existe aucune communication neurale entre eux ?

Rôle de la mélatonine maternelle

D'après Breen *et al*. (1996) ou Goldman (2003), le signal chimique susceptible de transférer l'information lumineuse de la mère au fœtus est la mélatonine. La mélatonine est l'une des rares hormones maternelles à pouvoir traverser le placenta sans être altérée (Klein, 1972). Des expériences d'injection de mélatonine radioactive chez la brebis gestante montrent que l'hormone peut passer dans le système circulatoire fœtal (Zemdegs *et al*., 1988).

Chez la brebis gestante maintenue sous conditions photopériodiques (LD), Zemdegs *et al*. (1988) ont trouvé un rythme nycthéméral robuste de mélatonine dans le système circulatoire de la mère ainsi que dans le système circulatoire du fœtus avec des concentrations élevées la nuit et faibles le jour. Yel-

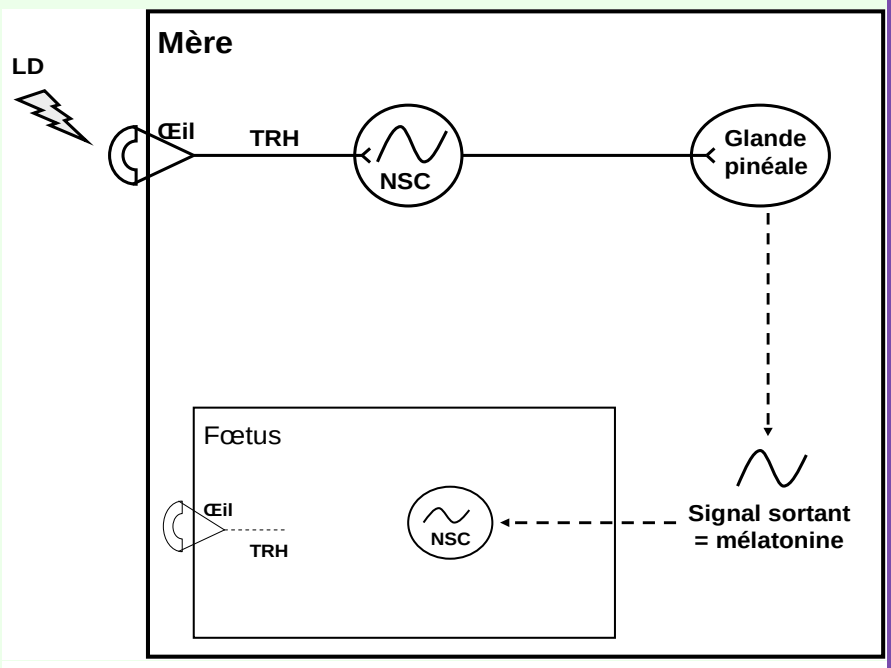

Figure 1. *Modèle conceptuel de l'entraînement maternel prénatal chez les mammifères, sous conditions photopériodiques (LD). TRH : tractus rétinohypothalamique. NSC : noyaux suprachiasmatiques.*

(Suite page 73)

(Suite de la page 72)

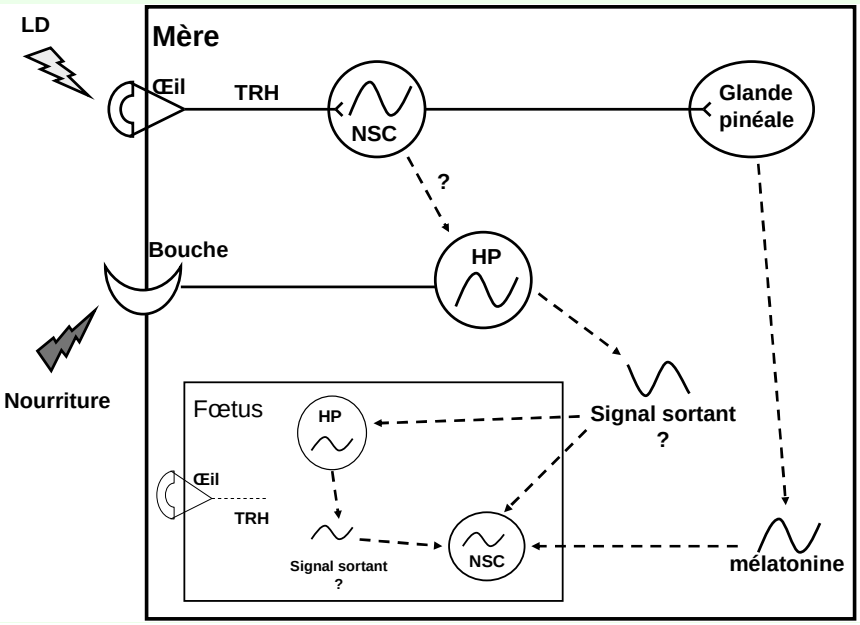

***Figure 2**. Modèle conceptuel de l'entraînement maternel prénatal chez les mammifères, sous conditions photopériodiques (LD). TRH : tractus rétinohypothalamique. NSC : noyaux suprachiasmatiques. HP : horloges périphériques.*

lon & Longo (1988), d'une part, puis McMillen & Nowak (1989), d'autre part, ont montré que lorsque des brebis gestantes subissaient une pinéalectomie entre le 104ème et le 118ème jour de gestation, le rythme journalier de mélatonine plasmatique (mesuré entre le 125ème et le 140ème jour de gestation), précédemment mis en évidence, disparaissait chez les mères ainsi que chez les fœtus. Ceci semble indiquer que le fœtus ne produit pas de mélatonine de façon rythmique à ce stade du développement et que la variation journalière de mélatonine observable chez les fœtus de mères non opérées est entièrement imputable à la mélatonine sécrétée par la glande pinéale maternelle. En effet, plusieurs équipes ont montré que la synthèse de mélatonine pinéale ne démarrait qu'après la naissance chez le rat (Deguchi, 1975), le mouton (Nowak *et al*., 1990) et l'homme (Kennaway *et al*., 1992).

Le rôle de zeitgeber de la mélatonine maternelle pour les NSC du fœtus de capucin a été très récemment mis en évidence par Torres-Farfan *et al*. (2006). Les chercheurs ont placés des femelles gestantes en condition constante d'illumination durant le dernier tiers de la gestation ce qui supprime la rythmicité circadienne maternelle. Cela a entraîné un déphasage de l'expression des gènes de l'horloge Bmal1 et Per2 ainsi que du récepteur de mélatonine MT1 dans les NSC fœtaux (indiquant que les NSC fœtaux sont en libre cours). Ces gènes sont réentraînés par l'injection cyclique de mélatonine. Ainsi, il semble que le signal chimique entraînant l'horloge centrale des fœtus est la mélatonine maternelle.

Grâce aux diverses études décrites jusque là un premier modèle de l'entraînement maternel prénatal peut être proposé (Figure 1).

En revanche, Reppert & Schwartz (1986b) ont montré qu'une pinéalectomie chez la ratte gestante, placée sous conditions photopériodiques, ne supprimait pas le rythme d'activité métabolique journalier des NSC fœtaux. Ici, la première hypothèse venant à l'esprit pourrait être que les NSC de la mère (toujours entraînés par le cycle photopériodique) suffisent, à eux seuls, à entraîner les NSC fœtaux. Cela semble toutefois peu probable étant donné qu'il n'existe aucune liaison neurale entre la mère et le fœtus et que les NSC maternelles ne produisent pas de mélatonine.

Rôle des autres horloges périphériques maternelles

McMillen *et al*. (1990) a montré que, sous conditions photopériodiques (LD), le rythme respiratoire des fœtus de brebis gestantes pinéalectomisées ne disparaît pas. Par contre, le profil journalier des mouvements respiratoires fœtaux est significativement différent de celui des fœtus de mère non pinéalectomisée : le pic de fréquence respiratoire des fœtus de mère non pinéalectomisée a lieu au début de la scotophase alors que celui des fœtus de mère pinéalectomisée coïncide avec l'heure de la prise alimentaire des brebis (milieu de la photophase). Ainsi, en absence de rythme de mélatonine maternelle, le rythme de prise alimentaire de la brebis pourrait devenir le signal dominant entraînant le rythme journalier de respiration fœtal.

Ohta *et al*. (2008) ont examiné de plus près quel pouvait être le processus mis en jeu ici. Les chercheurs ont imposé un cycle de nourrissage à des rattes gestantes. La nourriture n'était disponible que 4h de suite durant la photophase d'un cycle LD 12:12h. Ce type de restriction alimentaire est connu pour entraîner les horloges périphériques des animaux adultes, sans influencer toutefois l'expression des gènes de l'horloge dans les NSC (Damiola *et al*., 2000 ; Stokkan *et al*., 2001). Après 21 jours d'entraînement par la nourriture, Ohta *et al*. (2008) ont examiné l'expression du gène Per1 dans les NSC et le foie de la mère ainsi que des fœtus. Comme prévu, la phase du rythme d'expression de Per1 dans les NSC de la mère n'a pas été affectée. En revanche, l'horloge des NSC fœtaux est clairement affectée par le cycle de nourrissage imposé à la mè-

(Suite page 74)

(Suite de la page 73)

re et est déphasée de 4,7h en comparaison des animaux contrôles (ayant accès *ad libitum* à la nourriture). Cela suggère que les NSC fœtaux, contrairement aux NSC des animaux adultes, pourraient avoir la capacité de s'adapter temporellement aux cycles de prise alimentaire maternelle. D'autre part, le rythme d'expression de Per1 dans le foie maternel est déphasé de 9,1h en comparaison des animaux contrôles. Et l'horloge circadienne située dans le foie du fœtus a également été entraînée et est déphasée de 7,4h. Cette forte avance de phase comparée à celle trouvée dans les NSC fœtaux pourrait s'expliquer par une compétition des signaux affectant les NSC fœtaux. En effet, les femelles gestantes, toujours sous conditions photopériodiques, continuent de sécréter rythmiquement de la mélatonine. Les cycles maternels de mélatonine et de nourrissage pourraient alors être des signaux contradictoires pour les NSC fœtaux.

Ainsi, le signal maternel semble redondant : plusieurs rythmes maternels pourraient agir de concert pour entraîner le système circadien fœtal. A partir de ces nouvelles données, nous proposons un modèle théorique de l'entraînement maternel prénatal (Figure 2).

L'étude d'Ohta *et al*. (2008) semble donner une explication aux résultats trouvés pas Reppert & Schwartz (1986b). En effet, sous conditions photopériodiques (LD), la ratte gestante, malgré la pinéalectomie, exprime toujours une rythmicité circadienne grâce à son horloge principale, située dans les NSC, qui coordonne les horloges périphériques. Dans cette expérience, la femelle gestante doit donc continuer à ingérer de la nourriture de façon journalière. Ce nourrissage cyclique entraîne certainement les horloges du fœtus et le synchronise ainsi sur le cycle jour/nuit.

Les signaux chimiques intervenant dans ce processus restent à découvrir et donc des études complémentaires devront être menées pour en comprendre les mécanismes.

Impact de l'entraînement maternel prénatal sur la rythmicité du jeune animal après la naissance

Rôle des noyaux suprachiasmatiques maternels

Davis & Gorski (1983) (dans Reppert & Schwartz, 1986) ont été les premiers à montrer, chez le hamster syrien, que l'ablation des NSC de la mère (au jour 7 de la gestation) pouvait modifier l'ontogénèse du système circadien de ses jeunes. Les scientifiques ont mesuré l'activité locomotrice de jeunes hamsters élevés avec leur mère en conditions constantes dès la naissance. Le jour du sevrage, les petits nés de mères ayant subi une ablation des NSC expriment des phases actives non synchronisées alors que les petits de mères témoins expriment des phases synchronisées. Des résultats similaires ont été trouvés chez le rat pour le rythme d'activité alimentaire (Reppert & Schwartz, 1984) ou encore pour le rythme d'activité du N-acétyltransférase (NAT) pinéal (Reppert & Schwartz, 1986) (l'activité du NAT régule le rythme de synthèse de la mélatonine dans la glande pinéale ; Deguchi, 1978). Ces résultats tendent à confirmer l'hypothèse posée dans la première partie de cette revue : les NSC fœtaux oscilleraient en libre cours quand ils ne sont plus entraînés par la rythmicité maternelle. Et les fœtus ne se synchronisent pas mutuellement. Après la naissance, les jeunes d'une même portée continuent à exprimer un rythme circadien en libre cours mais ne sont toujours pas synchronisés les uns avec les autres.

Davis & Gorski (1988) ont renouvelé leur expérience sur les hamsters (1983), mais cette fois-ci les lésions des NSC ont été réalisées 2 jours avant la mise bas (soit à 14 jours de gestation). Les effets des lésions au jour 14 n'ont pas été aussi perturbants que les effets de celles réalisées au jour 7, suggérant que les noyaux maternels sont importants entre le jour 7 et 14 de la gestation, et que la synchronisation observée au sevrage chez les jeunes de mères n'ayant pas reçu de lésions, est déjà au moins en partie établie avant le jour 14 de gestation.

Davis & Gorski (1986) avaient préalablement mis en évidence ces effets prénataux grâce à une expérience d'adoptions croisées. Deux groupes de jeunes hamsters sont nés de femelles qui ont été entraînées, avant la mise-bas, à des cycles photopériodiques de 12h aux phases opposées (LD et DL). A la naissance, les deux groupes de jeunes sont élevés dans des conditions constantes de lumière de faible intensité (conditions ne supprimant pas la rythmicité) par des mères adoptives qui ont été entraînées uniquement par le cycle prénatal LD. Malgré ce traitement postnatal identique, les deux groupes de jeunes hamsters montrent des rythmes d'activité, au moment du sevrage, dont les phases sont très différentes. Les petits LD sont plutôt synchronisés entre eux et avec leur mère adoptive alors que les petits DL ne sont pas synchronisés avec leur mère adoptive. La moitié de ces petits montrent des phases en prédiction avec leur traitement prénatal. Ces résultats confirment que l'horloge circadienne contrôlant le rythme d'activité est déjà fonctionnelle et entraînée avant la naissance mais montrent également que la mère peut encore modifier en partie le rythme de ses jeunes après la naissance.

Rôle de la mélatonine maternelle

Bellavia *et al*. (2006) ont montré que la pinéalectomie d'une ratte gestante (au jour 7 de la gestation) modifiait significativement le comportement de prise de boisson de ses jeunes : après le sevrage, les phases de rythme des jeunes (élevés en DD avec

(Suite page 75)

(Suite de la page 74)

leur mère après la naissance) de mère pinéalectomisée sont désynchronisées contrairement à celles des jeunes de mères contrôles. En revanche, lorsque les chercheurs ont injecté cycliquement de la mélatonine en remplacement de l'activité sécrétrice de la glande pinéale maternelle durant la phase finale de la gestation, le traitement a annulé les effets de la pinéalectomie. Dans cette expérience les rattes étaient élevées en LD pendant la période de gestation. Ainsi leur NSC devait être entraîné par le cycle photopériodique. Pourtant cela n'a visiblement pas suffit à synchroniser les phases de prise de boisson des ratons.

Davis & Mannion (1988) ont trouvé des résultats similaires chez le hamster doré. Ils ont injecté cycliquement de la mélatonine à des femelles gestantes ayant subit des lésions des NSC (lorsque les NSC ne sont plus fonctionnels, la glande pinéale n'est plus entraînée et ne sécrète plus cycliquement de mélatonine). Cette injection prénatale restaure la synchronisation des phases d'activité locomotrice des petits d'une même mère au moment du sevrage.

La glande pinéale et la mélatonine maternelle affectent donc, directement ou indirectement, la rythmicité des fœtus et sont nécessaires à l'établissement d'une synchronisation du jeune animal avec son environnement social et/ou physique après la naissance.

Rôle des autres horloges périphériques maternelles

Weaver & Reppert (1989) ont confirmé que l'ablation des NSC de rattes gestantes supprimait la communication de l'information photopériodique de la mère au fœtus : les jeunes issus de ces mères et élevés en DD dès la naissance présentent, au moment du sevrage, des phases de prise de boisson totalement désynchronisées. En revanche, les chercheurs montrent que lorsqu'ils nourrissent cycliquement les rates gestantes sans NSC, les jeunes, cette fois, présentent des phases de prise de boisson synchronisées suggérant que la prise de nourriture cyclique a entraîné le fœtus. L'horloge centrale de la mère n'est plus fonctionnelle dans cette expérience. Ses horloges périphériques doivent donc très probablement entrer en jeux dans ce processus d'entraînement.

Conclusion

Cette revue démontre que l'horloge circadienne du jeune animal est entraînée par celle de la mère avant la naissance. La mère transmet l'information lumineuse au fœtus et ainsi ce dernier est synchronisé sur le cycle photopériodique externe. La deuxième partie de cette revue montre l'impact à long terme de l'entraînement maternel prénatal : en effet, des preuves de cette influence maternelle ont pu être mises en évidence après le sevrage des jeunes.

Il existe donc deux voies possibles pour l'étude de l'entraînement maternel prénatal. La première est une méthode directe qui consiste à mettre en évidence les rythmes fœtaux. La deuxième est une méthode indirecte qui consiste à mesurer les rythmes des animaux après la naissance en interprétant le résultat ontogénétique de l'influence maternelle.

L'entraînement maternel apparaît comme un processus particulièrement complexe puisque faisant visiblement intervenir l'ensemble des horloges (centrale et périphériques) de la mère, et probablement différents signaux chimiques transférés au fœtus (dont la mélatonine). Pour l'instant le nombre d'études assez restreint ne permet pas une compréhension globale du phénomène.

Toutefois, Seron-Ferre *et al*. (2007) proposent une idée tout à fait innovante pour la compréhension du phénomène d'entraînement maternel prénatal : ils émettent l'hypothèse que durant la vie fœtal, les NSC fœtaux pourraient être une « horloge périphérique » pour la mère comme le seraient les autres organes du fœtus. Les NSC et les organes périphériques du fœtus seraient ainsi entraînés par différents signaux maternels d'une manière relativement semblable à l'entraînement des horloges périphériques propres de la mère par les NSC. Cette hypothèse est effectivement plausible sur le versant hormonal de la synchronisation interne. En revanche, elle ne peut pas s'appliquer à la synchronisation interne par voie neurale étant donné qu'il n'existe aucune communication de ce type entre la mère et le fœtus.

Une horloge entraînée au cours de la vie fœtale rend le jeune mammifère en développement mieux préparé à la vie dans le monde extérieur. Ceci semble particulièrement important chez les animaux nidifuges car les soins parentaux postnataux chez ces animaux sont moins importants que chez les animaux nidicoles chez lesquels on pourrait penser que les jeunes ont plus de temps pour s'ajuster au monde extérieur via leurs parents. De plus il semble exister, chez les mammifères, une différence de maturité du système circadien entre les espèces nidifuges et nidicoles. Chez les espèce nidifuges, de nombreux rythmes journaliers sont présents chez le fœtus: rythmes cardiaque et respiratoire, rythmes de cortisol ou de prolactine plasmatiques, mouvements fœtaux… De plus, certains gènes de l'horloge sont exprimés rythmiquement dans les NSC fœtaux avant la fin de la gestation. Enfin, la neurogénèse des NSC et leur innervation par les TRH sont terminées en milieu de gestation.

A l'opposé, les espèces nidicoles ne développent qu'après la naissance des rythmes de température, de corticostérone plasmatique, d'activité locomotri-

(Suite page 76)

(Suite de la page 75)

ce… De plus, les gènes de l'horloge ne commencent à être exprimés rythmiquement que quelques jours après la naissance (Pour revue : Seron-Ferre *et al*., 2007). Enfin, la neurogénèse des NSC se termine près du terme et l'innervation des NSC par les TRH a lieu après la naissance

Dans cette revue nous avons justement des représentants de ces deux groupes d'animaux. Les études menées jusqu'à présent semblent montrer que les processus mis en jeux dans l'entraînement maternel prénatal sont sensiblement les mêmes dans les deux groupes. Toutefois les questions posées dans chacune des études étant différentes, elles ne permettent pas de tirer des conclusions générales. Ils pourraient être intéressant de réaliser une étude comparative entre espèces nidifuges et nidicoles afin d'estimer l'importance relative des effets parentaux prénataux *versus* postnataux dans chaque espèce. Nous postulons que les effets prénataux chez les espèces nidifuges devraient être plus importants que chez les espèces nidicoles et inversement pour les effets postnataux. Ou encore que l'entraînement prénatal devrait apparaître à un stade plus tardif du développement fœtal chez les animaux nidicoles que chez les animaux nidifuges.

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

Résumés des communications

Première partie

The J. Ariens-Kappers lecture

The autonomic and endocrine system as carrier of rhythmic information

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The hypothalamus integrates information from brain and body. As a result, countless functions are regulated by neuroendocrine and autonomic hypothalamic processes in concert with the appropriate behaviour that is mediated by neuronal influences on other brain areas. Within the hypothalamus de suprachiasmatic nucleus (SCN) imposes its rhythm onto the body via three different routes of communication: Via the secretion of hormones; it uses separate connections via either the sympathetic or the parasympathetic system not only to prepare the body for the coming change in activity cycle but also to prepare the body and its organs for the hormones that are associated with such change. Hereby the SCN determines the set point of various physiological variables. In the past years we have collected evidence for the mechanisms and the brain structures involved in mediating the output of the SCN. The hypothesis for our present work is that in view of the role of the SCN in determining the physiological day-night levels, the SCN also needs to be informed about the accurate values of these variables. Apart from light, activity and melatonin little is known about the information that is provided to the SCN in order to execute its functions especially at the physiological level. Consequently in the present presentation attention will be paid not only to the mechanisms the SCN is using to organize the set point in hormones, glucose, heart rate and temperature but also how the SCN is informed about these physiological variables. Hereby the role of the circumventricular organs in passing circulating information to the SCN and the role of the nucleus Tractus Solitarius in providing visceral sensory information to the SCN is discussed. Finally the role of the SCN in controlling cardiovascular functions is discussed in relation with the post-mortem finding that the activity of the SCN is diminished in people who had a long history of hypertension.

Supported by grants CONACyT 79797, DGAPA PAPIIT IN215308-3.

The Axelrod lecture

The role of Aurora kinase and salt inducible kinase in the regulation of Aa-nat transcription.

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The circadian rhythm of circulating melatonin in rat is driven by the expression of Aa-nat in the pineal gland. Although the initiation of Aa-nat gene expression requires the phosphorylation of CREB by PKA, recent studies have indicated that the temporal profile of this transcription is modulated by additional PKA-regulated events such as activation of histone kinases. In whole animal studies, we found a rapid nocturnal activation of the histone kinase, Aurora C, which parallels the increase in the level of phospho-Ser10 histone H3. Studies with cultured pinealocytes indicate that Aurora C activation is induced by norepinephrine (NE). Treatment of pinealocytes with Aurora C inhibitors, while having no effect on NE-stimulated phosphorylated CREB level, suppresses the NE-stimulated histone phosphorylation and more importantly, the induction of a subset of the CRE-targeted genes, including Aa-nat. These results suggest that histone modification is important for the transcription of specific CRE-targeted genes. Studies on another reported histone kinase, salt inducible kinase 1 (SIK1), showed a rapid nocturnal induction of this kinase in the rat pineal gland and in pinealocytes after NE stimulation. However, over-expression of SIK1 has no effect on histone phosphorylation and suppresses the NE-stimulated Aa-nat transcription. Moreover, knockdown of SIK1 amplifies the NE-stimulated Aa-nat expression, indicating that SIK1, while not functioning as a histone kinase, may serve as an endogenous repressor for the transcription of Aa-nat. Downstream from SIK1, over-expression of transducer of regulated CREB activity (TORC), a reported target of SIK, reduces the inhibitory effect of SIK1 on Aa-nat transcription. Together, these results suggest that, beside CREB phosphorylation, the temporal profile of Aa-nat transcription, and hence the pattern of circulating melatonin, is subjected to the regulation of multiple PKA-mediated signaling events.

The Gwinner lecture

(Suite page 78)

(Suite de la page 77)

Does a circannual pacemaker exist?

Lincoln GA

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Endogenous circannual clocks regulate internal time domains over months and years. They drive long-term rhythms in emergence, reproduction, metabolism, hibernation, migration and/or moult as a pre-emptive strategy – allowing physiological change to be complete in advance of environmental change. The endogenous nature of circannual timing is well established in organisms from different taxa based on the free-running and entrainment properties of seasonal rhythms. The molecular basis remains a mystery. We have begun to characterise a mammalian circannual pacemaker in our Soay sheep model where the neuroendocrinology is simplified by hypothalamo-pituitary disconnection (HPD). Remarkably, HPD sheep express a circannual rhythm in prolactin secretion with an endogenous period of 10 months under constant long days, that can be modulated in amplitude and timing by changes in photoperiod - transduced via melatonin signalling. Recent studies have shown that manipulating the adrenal axis in HPD sheep profoundly alters the expression of the circannual clock. We believe that this is the result of effects of glucocorticoids on cell division and differentiation in target tissues (e.g. pituitary, hypothalamus and hippocampus). These tissues may store seasonal temporal information as a type of vegetative memory. The proposed hypothesis is that circannual rhythm generating mechanisms are localized in various organs and govern different physiological systems. They are tissue-based depending on cell proliferation, differentiation and delayed feedback signals, with coupling between the organ timers co-ordinating circannual rhythmicity. The answer to the question 'does a circannual pacemaker exist' – is likely to be 'yes'. The new challenge is to establish whether there is a family of circannual clock genes expressed in core timer cells (calendar cells) and circannual timing depends on a cell autonomous oscillator.

The JCS lecture

Genetic dissection of the circadian clock in Mammals

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The molecular mechanism of circadian clocks has been uncovered by the use of phenotype-driven (forward) genetic analysis in a number of model systems. In mammals, circadian oscillations are generated by a set of genes forming a transcriptional autoregulatory feedback loop: these include: Clock, Bmal1, Per1, Per2, Cry1, Cry2 and Casein kinase 1 epsilon. Another dozen candidate genes have been identified and play additional roles in the circadian gene network such as the feedback loop involving Rev-erba. Despite this remarkable progress, it is clear that a significant number of genes that regulate circadian rhythms in mammals remain to be discovered and identified. As part of a large-scale N-ethyl-N-nitrosourea (ENU) mutagenesis screen using a wide range of nervous system and behavioral phenotypes, we have identified new circadian mutants in mice. These include new alleles of known circadian genes as well as novel circadian loci such, Fbxl3, an orphan member of the F-box protein family, which we have found to interact selectively with the CRY proteins to target them for degradation through the proteasome pathway. The discovery of 'clock genes' also led to the realization that the capacity for circadian gene expression is widespread throughout the body. Using circadian gene reporter methods, one can demonstrate that most peripheral organs and tissues can express circadian oscillations in isolation, yet still receive and may require input from the dominant circadian pacemaker in the suprachiasmatic nucleus (SCN) in vivo. We have used tissue-specific, conditional gene expression methods to analyze the relative contributions of central and peripheral circadian oscillators to circadian organization. The cellular autonomy of circadian clocks has raised a number of questions concerning synchronization and coherence of rhythms at the cellular level as well as circadian organization at the systems level.

Photoperiodic signalling through melatonin: molecular decoding

Hazlerigg DG

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In mammals, melatonin is essential for synchronisation of seasonal physiological rhythms to changes in photoperiod. Photoperiod is encoded in a nocturnal melatonin signal whose duration varies in proportion to the length of the night. Decoding of this signal takes place at multiple sites within the neuroendocrine system, which express high affinity G-protein coupled receptors. Of these melatonin targets, the pars tuberalis (PT) of the pituitary has come to prominence having been implicated in the seasonal regulation of both the reproductive and prolactin axes. Studies of melatonin signal decoding in the PT suggest that this entails distinctive morning and evening cascades of transcription factor expression and that these interact to control transcriptional output from PT cells. Recent studies highlight thyroid-stimulating hormone (TSH) as a functional output of the PT, which in turn acts within the mediobasal hypothalamus to control the reproductive axis. Exploration of the details of this mechanism suggests that positive autoregulation may be an important feature, allowing initial photoperiodic induction through durational decoding to be amplified into a sustained change in physiological output.

Neural circuitry, circadian rhythms and sleep in Drosophila

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Purpose: The neural circuits that regulate sleep and arousal as well as their integration with circadian circuits remain unclear. Previous work in Drosophila has defined two populations of circadian brain neurons, morning cells (M-cells) and evening cells (E-cells), connected to morning and evening locomotor activity, respectively. Functional organization of this cellular network generates the normal pattern of behavior in natural light-dark conditions, enabling the animal to anticipate and exploit environmental conditions associated with specific times of day. This circadian circuit intersects with those of photoreception and sleep/arousal, as light is both an arousal signal in

(Suite page 79)

(Suite de la page 78)

diurnal animals and an entraining signal for the circadian clock. In Drosophila, brain tissue involved in circadian photoreception intersects with photoreceptive neurons in the visual system. Methods: To identify neurons and circuits relevant to light-mediated arousal and circadian phase-shifting, we developed novel genetic techniques that link behavior to single cell-type resolution within the Drosophila central brain. This new intersectional mosaic technique can label, activate, inhibit or eliminate central brain neurons with single cell-type and even single cell spatial resolution. Combined with a temperature-sensitive dTRPA1 channel and mild temperature shifts, the methods also allow the acute activation of a variable number of defined neurons within each fly. We have also sought to identify primary light-responsive neurons within the brain. Results: These studies have led us to focus on the unknown function of the 10 PDF-containing large ventral lateral neurons (l-LNvs) of the Drosophila circadian brain network, which turn out to function in light-dependent arousal. They also are critical for phase shifting in the late-night (dawn). Considered together with other results including gene expression profiling of the l-LNvs, the data indicate that these cells respond to multiple arousal cues. As these cells are not the principal pacemaker cells, the circadian photoresponse is non cell-autonomous and therefore a network property. Importantly, the behavioral response reflects the number of activated or functional l-LNvs. Conclusions: To map the connectivity between l-LNvs and other brain regions, we are currently combining new genetic methods with UAS-RNAi transgenes, pharmacology and brain imaging.

Kai protein oscillator and cyanobacterial circadian clock

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We reconstituted the self-sustainable circadian oscillation of phosphorylation state of the cyanobacterial clock protein KaiC by incubating it with KaiA, KaiB, and ATP. This in vitro oscillation persisted robustly and the period was compensated against temperature changes. Period lengths observed in vivo in various kaiC mutants were consistent with those measured using in vitro mixtures containing the respective mutant KaiC proteins. These results indicate that the oscillation of KaiC phosphorylation is the primary pacemaker of the cyanobacterial circadian clock. We then found that the interactions between KaiA or KaiB with KaiC and mutual regulations of two neighboring phosphorylations of KaiC facilitated the phosphorylation cycle. Moreover, we showed that KaiC possesses extremely weak but temperature-compensated ATPase activity (15 ATPs per day) and that activities of wild-type KaiC and five period-mutant proteins are directly proportional to their in vivo circadian frequencies, indicating that the ATPase activity defines the circadian period. Based on these observations, we propose the KaiC ATPase activity as the most fundamental reaction underlying circadian periodicity of cyanobacteria. Moreover, KaiC also has novel mechanisms for synchronization. Firstly, the robustness of the KaiC phosphorylation rhythm arises from the rapid synchronization of the phosphorylation state of KaiC proteins. Secondly, we recently found that the KaiC phosphorylation cycle can be entrained by high/low temperature cycle in a parametric fashion. Thus, the Kai protein clock is designed as the master pacemaker of cyanobacterial circadian clock. In addition, we recently found that temperature-compensated circadian rhythm of gene expression could persist even when KaiC was arrested in the phosphorylated state. It is important to note that the characteristics of this oscillation are still under control of KaiC. Thus, even ifbothcyclescould apparently be considered to generate circadian rhythm, the ATPase activity of KaiC is the most fundamental pacemaker that regulates rhythms in gene expression and KaiC phosphorylation in vivo in a fashion so as to harmonize two rhythms together.

The migratory clock in monarch butterflies

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Purpose: To characterize the molecular clock in the eastern North American monarch butterfly (Danaus plexippus). Results: We have been developing this species as a model to examine the role of the circadian clock in time-compensated sun compass orientation and in the seasonal induction of the migratory state. Using clock protein expression patterns, we identified the location of circadian clock cells in the dorsolateral protocerebrum (pars lateralis) of the butterfly, which expresses PERIOD, TIMELESS and a Drosophila-like cryptochrome (designated CRY1; see below). We have also identified a CRY1-staining neural pathway that may connect the circadian (navigational) clock to polarized light input important for sun compass navigation and may connect the circadian clock to neurosecretory cells in the pars intercerebralis for the initiation of the migratory state. We also found that the butterflies, like all other non-drosophilid insects so far examined, expresses a second cry gene (designated insect CRY2) that encodes a vertebrate-like CRY. Functional studies in show that monarch CRY2 is light insensitive, but potently inhibits CLOCK:CYCLE-mediated transcription, while monarch CRY1 is light sensitive, but does not show transcriptional repressive activity. The expression of two functionally distinct crys in monarchs suggests that the butterfly clock use a novel clockwork mechanism not yet fully described in any organism. We have therefore used both in vitro and in vivo approaches, along with Drosophila carrying monarch cry1 or cry2 transgenes, to provide a more detailed molecular view of the clockwork mechanism and its photic entrainment in the butterfly. Conclusions: Our results strongly suggest that both monarch CRY1 and CRY2 have important roles, with CRY1 functioning as a blue-light photoreceptor for photic entrainment, while CRY2 functions within the clockwork as the major transcriptional repressor of an intracellular negative transcriptional feedback loop. Our results define the characteristics of an ancestral clock mechanism that may be common among lepidopteran species and other invertebrates expressing both CRY1 and CRY2.

Clocks, cancer and chronotherapeutics

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Purpose: To improve cancer treatments through their adjustment to the Circadian Timing System (CTS) of individ-

(Suite page 80)

(Suite de la page 79)
ual patients. CTS function can vary according to gender, age, genotype, lifestyle, diseases and treatments. This strategy requires shifting the paradigm of circadian biology from standardization to personalization. Methods: The relation between toxicity and efficacy of anticancer drugs, as well as their pharmacologic and cell cycle determinants, are investigated along the 24-h in ? B6D2F1 mice (LD12:12). This development results in standardized chronotherapeutics, where all the patients receive the same chronomodulated chemotherapy. Results . Circadian timing increases by >50% the tolerability of 42 of anticancer medications and the efficacy of 28 of them in rodents. The coincidence between chronotolerance and chronoefficacy is explained in vivo and in silico by 1) the shielding of the CTS when treatment is given at the least toxic time, and 2) the cell cycle variability and poor circadian entrainment of tumors. Standardized chronotherapeutics has been tested in 3 international randomized trials in 842 chemo-naïve patients with metastatic colorectal cancer receiving 5-Fluorouracil, Leucovorin and Oxaliplatin (chronoFLO) or conventional delivery. The meta-analysis of these trials show that chronoFLO deteriorates survival in women (Hazard Ratio, 1.23 [0.97-1.56]; p=0.09) and improves it in men (HR = 0.77 [0.62-0.92]; p = 0.005). Cancer-related circadian disruption also predicts for poor quality of life and poor survival in over 500 cancer patients. CTS disruption can further result from cancer treatments, and seems to correlate with adverse events and poor efficacy of chronotherapeutics in rodents and in patients. Conclusions Gender and genetic issues represent a major challenge for the personalization of cancer chronotherapeutics. The integration of in vivo, in vitro and in silico approaches drives dedicated technological developments through strong interactions with the clinic, including novel trial concepts and designs.

Support: EU (NoE BIOSIM, Contract No. LSHB-CT-2004-005137; STREP TEMPO, contract LSHG-CT-2006-037543) and ARTBC, Hospital P Brousse, Villejuif (France)

Molecular links between the circadian clock and diseases

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Rhythmic change of the internal milieu is a fundamental principle in all living organisms. Indeed, evidences are accumulating that mitosis of most cells of the body, migration stem cells from bone marrow to systemic circulation, and calcification of osteoblasts occur at specific times of the day. These phenomena and a variety of physiological and pathological symptoms as well show daily rhythms. This timing is determined by the endogenous oscillatory system called circadian system. Circadian system is consisted with a central clock in the suprachiasmatic nucleus (SCN), peripheral clocks, and their connecting neuronal and hormonal routes. In the last ten years, the molecular dissection of circadian clock has dramatically progressed, and now, it is known that circadian time is generated by an autoregulatory transcription-(post)translational feedback loop of clock genes. This oscillation at the levels of genes reflects at cells, tissues, and system levels through a variety of conducting system. For example, at the SCN level, the intercellular system of amplification and transmission of each cellular rhythm is necessary to generate effective output of the time information to the non-SCN brain regions and peripheral organs. Increasing evidence suggests that disruption of one or multiple sites of this circadian clock system is associated with various pathogenic conditions and may be highly relevant to various diseases including sleep-wake abnormality, metabolic syndrome, osteoporosis, abnormal estrous cycle and hypertension. To reveal the link between the circadian clock and the diseases, we begin the pathophysiological analyses of genetically deficient mice for clock genes or for genes that are revealed by the SCN-gene project.

Banquet lecture

Pineal, melatonin and biological rhythms: a European perspective

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The European Pineal Study Group, founded by Paul Pevet and Johannes Ariëns Kappers in 1977 has metamorphosed into the European Biological Rhythms Society. This underlines the broad appeal of this enigmatic organ, much derided by grant reviewers in the past. The pineal itself has almost ceased to be a specialist area of study since it is implicated in so many aspects of the 'rhythms of life'. The clear function of the pineal as a seasonal time giver, via melatonin production, provided the first recognition of its real importance in physiology. The use of melatonin as a light sensitive hormone and as the best marker rhythm of the circadian system, together with its own time-giving properties, has raised its profile to the extent that spin off merchandising (melatonin ties, scarves, appearances at art exhibitions, subject of questions in quiz shows etc) is becoming embarrassing. However as universal artificial lighting and the 24 h society intrude on urban lives we have the means both to understand the physiological problems and to apply therapeutic solutions thanks to melatonin, light and biological rhythms. Much pioneering work in this field took place in Europe. In the same decade for example, were observations of the human endogenous circadian clock in a cave and in a bunker, the evolution of the pineal as a sensory organ and its innervation, and the birth of a field called chronobiology, both here and across the world. Our host Paul Pévet has been instrumental in Europe in bringing coherence and collaboration to our subject. His enormous enthusiasm, knowledge, research success, political acumen, recognition of historical roots and support of young scientists has inspired great efforts. We honour Paul for his outstanding contributions to our field - he has founded a scientific dynasty.

Abstracts of conferences and selected communications

Symposium 1. Photodetection by the circadian system

VA opsin-based photoreceptors in the hypothalamus of birds

***Chairman*: Foster RG**

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Background: In the Japanese quail, daylength (photoperiod) alters the activity of thyrotrophs within the pars tuberalis (PT) to release thyrotrophin (TSH) which in-

(Suite page 81)

(Suite de la page 80)

turn triggers a cascade of events within the hypothalamus leading to a reproductive response. Despite these notable advances in understanding the avian photoperiodic response, the cellular and molecular identity of the photoreceptors which detect daylength and initiate these seasonal changes in physiology have remained a mystery. Birds possess photoreceptors located deep within the brain which regulate both seasonal and circadian responses to photoperiod. The existence of these "deep brain photoreceptors" was first shown by Benoit in the 1930's. Fine glass rods were placed within the hypothalamus of ducks and used to illuminate this region of the brain with artificial daylengths. Spring-like daylengths stimulated testicular growth whilst short winter photoperiods had no effect upon reproduction. Refinements of this approach during the 1970's confirmed that birds utilize photoreceptors somewhere within the hypothalamus to regulate their seasonal physiology, and in the 1980's an action spectrum for photoperiodic induction provided strong evidence that these receptors utilize an opsin/vitamin A based photopigment system. Multiple attempts to localize these hypothalamic photoreceptors using immunocytochemical approaches and employing antibodies raised against rod- or cone-opsin have failed, suggesting that these photoreceptors utilize another form of opsin. Results: A decade ago a new photopigment family was isolated and termed VA opsin. This photopigment class was thought to have a restricted taxonomic distribution, confined to the agnatha and teleost fish. We report the isolation of a full-length orthologue of VA opsin from the chicken (cVA) and demonstrate using a cellular expression system that cVA forms a functional photopigment. Further, this photopigment is expressed within a population of hypothalamic neurones with extensive projections to the median eminence of both the chicken and Japanese quail. Conclusions: These results provide the most complete cellular and molecular description of a deep brain photoreceptor in any vertebrate and strongly implicate these photoreceptors in daylength detection for the regulation of the avian photoperiodic response.

Melanopsin expresses invertebrate-like bistable properties in the human retina

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Purpose: Invertebrate rhabdomeric photopigments (IRP) use light to drive both sensory transduction and chromophore regeneration, a photo-reversible mechanism known as bistability. Rods and cones lack this mechanism. However, vertebrate melanopsin expressed in vitro has been shown to possess the fundamental properties of bistable photopigment systems. Using the pupillary light reflex (PLR) as a tool we explored the in vivo spectral and temporal properties of melanopsin bistability in humans. Methods: The PLR was measured by exposing one eye to monochromatic lights and recording consensual pupil responses from the unilluminated eye using an infrared video pupil tracking system. The kinetics of the temporal components of PLR (phasic, steady state, post stimulus persistence) were analyzed following pre-exposures to adapting short and long wavelength lights. Results: Using long duration light exposures (5 min), we define photoequilibrium and difference spectra that are typical of IRPs. Prior exposure to long wavelength light increases, while short wavelength light decreases the amplitude of pupil constriction to a subsequent light exposure. By modelling these responses to invertebrate photopigment templates we obtain putative spectra for the two underlying melanopsin (11-cis retinal bound) and metamelanopsin (all-trans retinal bound) states. Together, these spectra describe the essential response functions of the melanopsin bistable photopigment system. Conclusions: The results suggest that the capacity of melanopsin to respond to light is modulated by prior spectral lighting conditions, emphasizing the importance of previous light history in chronobiology. The findings also raise the possibility that appropriate manipulation of spectral light composition in industrial, domestic and clinical phototherapy applications can be exploited to optimize the response capacity of melanopsin-dependent circadian and other non-visual responses.

Support: FP6- EUCLOCK, Rhône-Alpes Cible

Melatonin modulates visual function and cells viability in the mouse retina via the MT1 melatonin receptor

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The role played by specific melatonin receptors in the retina is not well defined. Melatonin receptors are expressed by several retinal cell types, including the photoreceptors, suggesting that melatonin receptors are involved in photoreceptor physiology. Indeed, melatonin influences the membrane conductance of dark adapted frog photoreceptors, acts directly on the rod photoreceptors to increase dark adaptation, and potentiates rod signals to ON type bipolar cells in fish retina. Melatonin is also involved in the regulation of the electroretinogram of reptiles, birds, and, possibly, humans. The vast majority of mouse strains do not produce melatonin and many that do so carry a mutation leading to degeneration of photoreceptors (e.g., C3H rd1). Mice with targeted deletion of the MT1 melatonin receptor gene (MT1-/- mice) were crossed onto the C3Hf+/+ background. These mice make melatonin but do not develop retinal degeneration. MT1 receptor transcripts were localized in photoreceptor cells, inner retinal neurons and ganglion cells. A diurnal rhythm of scotopic ERG responses was observed in WT mice, with higher a- and b-wave amplitudes at night, but this rhythm was absent in mice lacking MT1 receptors. Injection of melatonin during the day increased the scotopic threshold and the amplitude of the a- and b-waves in the WT mice, but not in the MT1-/- mice. These data demonstrate that melatonin and MT1 receptors regulate visual processing in the mouse retina, enhancing visual responses at night when melatonin is released. At 12 months MT1-/- mice have a significant reduction in the number of photoreceptor nuclei in the outer nuclear (10 %). At 18 months the loss of photoreceptor nuclei in the outer nuclear layer was further accentuated (29 %) and, surprisingly, we also observed a significant reduction in the number of ganglion cells (27 %). These data demonstrate the functional significance of melatonin and MT1 receptors in the mammalian retina and create the basis for future studies on the therapeutic use of melatonin in retinal diseases associated with decreased retina sensitivity to light and degeneration of retinal cells as occur in age related macular

(Suite page 82)

(Suite de la page 81)
degeneration and glaucoma.

The mammalian molecular clockwork controls rhythmic expression of the ryanodine receptor

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Purpose: To analyze the role of BMAL1 and CLOCK in light-induced mPer expression. Methods: Mice with an impaired molecular clockwork (BMAL1-/-) and corresponding WT mice were used. Light- induced mPer expression was analyzed by in situ hybridization. Levels of ryanodine receptor (Ryr) mRNA and protein in the SCN were examined by real time PCR and immunohistochemistry. The impact of clock gene proteins on Ryr promoter activity was demonstrated by luciferase transcription assays. Functionality of Ryr in acute SCN slice cultures from both BMAL1-/- and BMAL1+/+ mice was determined using calcium imaging and 2 photon microscopy. Results: In BMAL1-/- mice light–induced mPer expression was impaired during early but not during late night. This suggests a selective disturbance of the signal transduction pathway involving Ryrs. Ryr mRNA and RyR protein levels were dramatically reduced in the SCN of BMAL1-/- mice. Transcriptional activity of the Ryr promoter could be increased by cotransfection with CLOCK and BMAL1. The activation of Ryr promoter mediated by CLOCK:BMAL1 was inhibited by co-transfection with CRY1. This shows that Ryr promoter activity is under control of molecular clockwork components. A deletion of the first E-box element in the Ryr promoter suppressed the effects elicited by the CLOCK:BMAL1 heterodimer verifying an E-box mediated mechanism. Calcium imaging showed that the responsiveness of SCN cells towards the Ryr-agonist caffeine was drastically reduced in BMAL1-/- mice as compared to WT. This confirms impaired functionality of Ryr in the SCN of BMAL1-/- mice. Conclusion: Our findings provide the first direct evidence that the mammalian molecular clockwork controls Ryr expression and thus its own photic input pathway components.

Supported by the DFG (GA737/2-3)

Effects of constant monochromatic light on the locomotor rhythm of Drosophila melanogaster

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Purpose: The sensitivity of Drosophila's circadian clock toward coloured light was assessed to learn about the underlying photopigments. Methods: The locomotor activity of flies was recorded for 20 days under constant monochromatic light (395-630 nm) of different intensity. The activity was judged for the presence of rhythmic components, and if possible period length was determined. Results: We found that light between 395 and 540 nm lengthened the period in a dose-dependent manner up to 29 hours. At higher intensity these wavelengths caused arrhythmic behaviour. For both effects (period lengthening and arrhythmicity) 395 nm monochromatic light was most effective. At wavelengths above 540 nm we observed little period lengthening and rarely arrhythmicity. Instead, at intermediate intensities of 585-630 nm monochromatic light two rhythmic components occurred in the activity pattern, one free-running with short the other with long period. At higher intensities multiple components appeared, suggesting that different components of a multi-oscillator system desynchronized. Conclusion: CRY that has a spectral sensitivity in the blue (up to 540 nm) is may be responsible for strong period lengthening and arrhythmicity. Rhodopsins 1 and 6 that are most sensitive in the green (up to 640 nm) might be responsible for internal desynchronization into multiple rhythmic components.

Neuroglobin expression in the rat suprachiasmatic nucleus (SCN): Co-localisation, innervation and response to light

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Purpose: To characterise the expression pattern and neuronal phenotype of a newly discovered neurone specific globin, Neuroglobin (Ngb) in the suprachiasmatic nucleus (SCN) of rats. Methods: Using double and triple immuno florescence and confocal microscopy we determined innervations and co-localisation of Ngb with neurotransmitters of the three major input pathways to the SCN and key clock genes. Results: The majority of Ngb-expressing neurons in the SCN belong to a cell group not previously characterized by neurotransmitter content; only a small portion was found to co-store Gastrin releasing peptide (GRP) in the ventral SCN. Furthermore, Ngb-containing neurones are unresponsive to light stimulation as measured by c-fos induction and only a few cells were found to express the core clock gene Periode1 during the 24 h LD-cycle. The Ngb-containing cells received input from NPY containing nerve fibres of the geniticulo-hypothalamic tract (GHT), whereas no direct input from the eye or the midbrain raphe system was demonstrated. Conclusion: The results indicate that the Ngb could be involved in non-photic entrainment via input from the GHT.

Symposium 2. Entrainment by photic cues

Introduction

Chairwoman: Sumová A

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In most organisms, circadian clocks govern the temporal organization of biological functions and adjust timing of these functions to the light/dark cycle of the solar day. Photic stimuli are the most potent Zeitgebers entraining the molecular clockwork with the external world. In multicellular organisms, organization of circadian clocks is now seen as being decentralized. While in non-mammalian species many cells of the body are directly light-responsive, in mammals photoreception is restricted exclusively to the retina. Photic stimuli are then conveyed to the central clock located within the suprachiasmatic nucleus (SCN) of the hypothalamus. The photic signals impinge on the molecular machinery generating circadian rhythmicity in the clock cells so that the phase of the molecular

(Suite page 83)

(Suite de la page 82)
rhythms is reset. Molecular basis of this event is still only partially understood. In mammals, transcriptional induction of light sensitive components of the clockwork, Period genes, seems to be the crucial step, while in non-mammalian species, such as drosophila and zebrafish, Cryptochrome genes play key role in light entrainment of the clock. Multiple signaling cascades mediating these events have been recognized. Recently, post-transcriptional and post-translational regulation of the entraining pathways has begun to be uncovered. Moreover, the role of the morphological and functional complexity of the SCN clock has been emphasized and the photic entrainment appears to be conditional upon a crosstalk between subpopulations of the SCN cells. In non mammalian species, communication between the pacemaking cells, that are directly light-sensitive, is also likely. Altogether, future research is still needed to decipher the complex molecular scenario of events passing between the stage when light stimulus reaches the clock and the one when entrainment of the clock is accomplished.

PDF-modulated visual inputs and Cryptochrome define diurnal behavior in Drosophila

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Morning and evening circadian oscillators control the bimodal activity of Drosophila in light-dark (LD) cycles. The lateral neurons evening oscillator (LN-EO) plays a key role in promoting diurnal activity at dusk. We show that the LN-EO autonomously synchronizes to LD cycles through either the Cryptochrome (CRY) that it expresses or the visual system. In conditions where CRY is not activated, flies depleted for PDF or its receptor loose the evening activity and display reversed PER oscillations in the LN-EO. Rescue experiments indicate that normal PER cycling and the presence of evening activity rely on PDF secretion from the large LNvs and PDF receptor function in the LN-EO. The LN evening oscillator thus integrates light inputs and PDF signaling to control Drosophila diurnal behavior, revealing a new clock-independent function for PDF.

The clock control of cell cycle events and cell communication in zebrafish cells

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Circadian clocks are thought to be present in most cells and tissues of the body. Zebrafish have taken this decentralization of biological timing to the extreme in that not only do the majority of cells in the body possess a clock, but they are all also directly light responsive. When the sun rises in the morning, the cells within all tissues detect this illumination and use it to set the timing or phase of their cellular clocks. This light responsive, daily timing mechanism can be found from the earliest stages of embryo development. But what cellular events does this daily pacemaker control? A series of experiments, by ourselves and others, have shown that the timing of cell cycle events to particular periods of the day occurs at the single cell level. S-phase is timed by the circadian clock to occur in the late night/early morning, while mitosis is regulated to occur in the late day or early night. “Stopping” the clock within individual cells shows that this cell cycle regulation is not light driven, but the consequence of the cellular circadian pacemaker. We will discuss which key cell cycle regulators appear to be under daily, clock control, and how the circadian and cell cycle oscillators may be functionally linked. In addition, we will explore the role of cell communication through gap junctions on clock function, as well as the regulation of rhythmic clock output events.

The molecular mechanism underlying light regulation of the zebrafish Period2 gene

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Daily rhythms of the melatonin hormonal signal and of gene expression in the pineal gland are the earliest detected circadian rhythms in zebrafish, appearing as early as days 2-3 of development. Light exposure is mandatory for the functional development of the pineal circadian clock that drives these rhythms. Light induces the expression of period2 (per2) which is, in turn, important for the functional development of the pineal circadian clock. Interestingly, in zebrafish, light exposure induces per2 expression not only in the pineal gland but also throughout the body and in cell lines, by an unknown mechanism. In order to understand the mechanism underlying light-induced per2 expression, functional promoter analysis was performed using transient and transgenic expression of per2 promoter-reporter constructs in zebrafish embryos and by means of stable transfection of PAC-2 zebrafish cells that are known to contain directly light-entrainable circadian clocks. This analysis has revealed a light-responsive module (LRM) in the per2 promoter which is both necessary and sufficient for light induction. Interestingly, the LRM sequence is highly conserved throughout evolution and the human LRM can substitute for its zebrafish counterpart to confer direct light regulation of gene expression in zebrafish cells. Functional analyses revealed that within the LRM, a D box enhancer is critical for its light-induced expression, while an E-box element is responsible for its clock-regulated expression. By generating a transgenic zebrafish line, we were able to use in vivo approaches complemented by in vitro techniques, to identify, characterize and functionally test transcription factors that bind these elements. The findings of these studies suggest a hierarchic mechanism in which light enables clock regulation of per2. This study extends our understanding of the mechanisms underlying light-entrainment, functional development of the circadian clock and contributes to a general understanding of how clock gene regulation has evolved in vertebrates as well as of general photic cellular responses.

The role of light in the circadian phenotype of PER3 deficient mice

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Purpose: To characterise the phenotype of the Per3 functional knockout in C57BL/6 mice, which on a 129/sv background are perceived as mild circadian clock mutants. Methods: Running wheel activity was recorded from wild-

(Suite page 84)

(Suite de la page 83)

type and Per3 mutant C57BL/6 mice initially kept under 12:12 light-dark (LD) conditions and under constant light conditions ranging from complete dark (DD) to bright light (LL). A number of mice were tested for behavioural phase -shifting responses to a single light pulse in early or late dark period, and speed of re-entrainment to a 4-h phase delay and advance in the LD cycle. In a 3rd experiment, mice were kept under an ultradian 3.5:3.5 LD cycle of increasing light intensities to assess behavioural masking. We have used qPCR to assess clock gene expression and measured body temperature with telemetry. Results: In contrast to earlier findings (Shearman et al. 2000), we did not find a difference in behavioural free-running period in DD between the wild-type and Per3 mutant mice. When housed in LL, a difference in free running period emerged, extending up to 0.5 h in bright light (350 lux). This different response to light was not apparent in behavioural phase shifts after a light pulse. However, on the first day of re-entrainment to a four-hour phase delay, Per3 mutant mice showed a significantly reduced shift in the onset of behavioural activity than wild-type mice. In mice subjected to the ultradian protocol, light differently affected activity levels in wild-type and Per3 mutant mice. While both mice showed a consistently increasing level of activity suppression in increasing light intensities, Per3 mutant mice showed significantly lower levels of masking. Conclusions: The Per3 mutant mouse exhibits altered light sensitivity. The lower degree of ultradian masking indicates that this light sensitivity may not be directly linked to the circadian clock, but possibly upstream. Such upstream effects may also cause the differential responses to light in the experiments showing parametric effects of light exposure, such as LL and the shift in LD, while not being apparent after the 15-minute light pulse. These results are in line with other work showing an additional role for Per3 outside the circadian clock.

Parametric light effects on circadian activity rhythms in Djungarian hamsters

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Purpose: Djungarian hamsters (Phodopus sungorus) bred in our institute show activity patterns that seems incompatible with proper adjustment to a periodic environment. The activity onset in those animals is continuously delayed whereas the activity offset is stably coupled to “lights-on”, leading to a compression of the activity time. The differences between the activity patterns in those DAO-hamsters (DAO-delayed activity onset) compared to the Wild-type (WT) cannot be explained exclusively by differences in their free-running period and parametric light effects. Nonparametric light effects must be taken into account as potential causes. Methods: To construct a phase response curve (Aschoff type VI), animals were kept under standard lighting conditions (LD14:10). Light pulses (100 lx, 15 min) were applied at specific circadian times during the dark phase. The activity onset was taken as CT12 in hamsters of both phenotypes. Results: The phase response curve differed between DAO- and WT-hamsters. The phase shifts observed in WT-animals were larger compared to those obtained in DAO-hamsters, except of the activity offset in the early subjective night. In WT-hamsters, the light pulses induced phase delays of the activity onset in the early subjective night and phase advances in the late subjective night. In DAO hamsters, almost no response of the activity onset was observed, except at CT14. The activity offset was advanced at all circadian times in both phenotypes, with a maximum in the middle of the activity period. Conclusion: The delayed activity onset in the DAO-hamsters is obviously due to differences in the interaction with the light-dark cycle and the coupling strength between the morning and the evening oscillator, but to a lesser extent to differences in the circadian clockwork itself.

Symposium 3. Entrainment by non-photic cues

Non-photic time cues and animal life: Photic cue is not almighty

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Light is a potent synchronizer for the circadian clock in every organism from bacteria to humans. For many organisms, however, non-photic entrainment is also indispensable for the survival and better performance especially in the field. Photic and non-photic time cues in the environment cannot be separated. A light-dark cycle accompanies inevitably a warm-cool/ dry-humid cycle. At the same time, it is a feeding-non feeding and active-rest cycle for diurnal animals, and vice versa, for nocturnal ones. In spite of their significance in the field, the role of non-photic time cues have been overlooked or under-rated, probably because of a lack of knowledge on its molecular/ cellular mechanisms, input-output pathways and, above all, the site of oscillation. Responsive sites may be multiple, and there may be networks among them.

Recent advances in chronobiology, especially those in molecular clock mechanisms and technical advancements, enabled us to study these unsolved questions. In the symposium entitled “Entrainment to non-photic cues”, we will discuss about the roles of different non-photic time cues, such as food, temperature and physical exercise, in physiology and neural mechanisms for the entrainment from the sensors or receptors up to overt rhythm expression. Relation between the photic and non-photic time cues will also be an important theme. Through this symposium, we will update our knowledge of the mechanism how our circadian system utilize various different time cues.

Food as synchronizer

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Purpose: To assess the synchronizing effects of feeding and metabolic cues on the circadian system in Mammals. Methods: Rodents are challenged with meal timing without calorie restriction, as modulated by temporal restricted feeding, or with timed calorie restriction (i.e., when only a hypocaloric diet is given each day) to induce body mass loss and other metabolic changes. Others were fed chronically with a hypercaloric diet. Results: Meal timing is a potent synchronizer for secondary clocks in peripheral organs, but not for the master clock located in the suprachiasmatic nuclei of the hypothalamus (SCN) which is mainly reset by light. By contrast, timed calorie restriction (i.e., when only a hypocaloric diet is given each day) is a

(Suite page 85)

(Suite de la page 84)

synchronizer powerful enough to modify the SCN clockwork and increase the synchronizing effects of light. Conversely, a hypercaloric diet also affects the SCN clockwork, but decreases the synchronizing effects of light. Secondary extra-SCN clocks in the brain are differentially influenced by meal timing. Circadian oscillations can be either highly sensitive to feeding cues (i.e., their phase is shifted according to meal schedule) in some structures (e.g., cerebellum, hypothalamic ventromedial and paraventricular nuclei) or hardly affected by meal timing in others (e.g., basolateral amygdala or hippocampus). Furthermore, food-anticipatory activity that animals manifest prior to meal time may rely on cerebellum integrity because chemical or genetic cerebellar damage without ataxia impairs or prevents anticipation of food availability. Conclusions: Taken together, these data indicate that feeding cues can markedly modulate the timing of the circadian system, not only at the periphery, but also within the brain. The cerebral clocks sensitive to meal time, such as those in the hypothalamus and cerebellum, likely define a network of coupled meal-entrainable oscillators within the brain. By contrast, the light-entrainable clock in the SCN is only sensitive to nutritional cues associated with metabolically challenging conditions.

Supported by CNRS, ANR JC

Phase regulation of the Drosophila circadian system by light and temperature

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The fruit fly, Drosophila melanogaster, shows a bimodal locomotor rhythm with a morning and an evening peak. Light and temperature are both powerful zeitgeber for the rhythm to synchronize to environmental cycles. Lines of evidence show that multiple oscillators with different entrainability to light and temperature are involved in the Drosophila circadian system. When a temperature cycle was given in constant darkness in such a manner that the thermophase corresponded to the previous night phase, the morning peak split into two components, which resynchronized to the temperature cycle with different time-courses. When subjected to a light cycle (LD) combined with a temperature cycle advanced by 6 h relative to the LD, the onset of evening peak advanced accordingly but the offset stayed at the previous phase. These results suggest that both the morning and the evening peaks are driven by two separate oscillators with different entrainability to light and temperature. Immunohistochemical analysis using anti-TIM revealed that clock neurons located lateral protocerebrum (LNs) are light entrainable and other clock neurons prefer to synchronize to temperature cycles. In the natural conditions, the LNs probably synchronize to LD to determine the frame of activity rhythm while the other clock neurons may set active phase within the frame in response to ambient temperature. This hypothesis is supported by the finding that in pdf01 mutant flies, both the onset and the offset of evening peak occurred earlier than in wild-type flies when a temperature cycle was advanced by 6h relative to LD. Molecular analysis revealed that temperature steps affected mRNA levels of the clock genes, per, tim, Clk, vri and Pdp1. The changes in the mRNA levels differed dependent on the direction of the temperature steps. The effect of temperature steps could not be observed in ClkJrk mutant flies, suggesting that temperature resets the clock through the Clk gene.

Light and temperature produce distinct entrainment patterns in the circadian clock network of the Drosophila larval brain

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Purpose: In the adult Drosophila brain, the circadian clock which drives behavioral rhythms comprises ~ 75 neurons (per hemisphere), 9 of which are already present in larvae. Light and temperature impart a similar phase to all adult clock neurons, but some may entrain preferentially to one or the other input. Rhodopsins and CRYPTOCHROME (CRY) mediate light responses. Little is known about temperature inputs. We investigated how the simpler larval network is entrained by light and temperature. Methods: larvae were entrained by either light or temperature cycles. The levels of clock proteins were assessed by immunocytofluorescence on dissected brains. Adult activity rhythms were assessed after larval-only entrainment. Results: Two CRY-negative dorsal neurons (the DN2s) appeared intrinsically blind, since their light entrainment, to a phase opposite that of all other clock neurons, required PIGMENT-DISPERSING FACTOR (PDF) neuropeptide signaling from four lateral neurons (LNs). In contrast, temperature entrainment of the DN2s was PDF-independent. The phase of their molecular oscillations did not change in thermocycles, whereas the PDF-positive LNs switched their phase, and were now in synchrony with the DN2s. This phase-switch was reflected in the phase of adult activity after larval-only entrainment by light or temperature. Rescue experiments suggest that temperature entrainment of the LNs requires signals from a functional clock in the DN2s. Larval clock neurons thus respond very differently to light and temperature, with the CRY-negative DN2s playing a major role in temperature entrainment. We are currently investigating the signaling pathways involved. They may include thermosensitive TRP channels and recently described effectors of temperature entrainment in the adult.

Glaser FT & Stanewsky R Curr Biol (2005); Klarsfeld A et al. J Neurosci (2004); Picot M et al. J Neurosci (2009).

Nonphotic time cues phase-dependently accelerate phase-shifts of mouse peripheral clocks

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Purpose: After an abrupt phase-shift of a light-dark (LD) cycle, Per1 expression rhythm in the SCN are reported to re-entrain instantaneously to a new LD cycle, whereas that in peripheral organs re-entrains slowly. Using a transgenic mouse carrying a Per1 luciferase reporter gene (Per1-luc), we previously reported that a scheduled exposure to novel environments with a running-wheel accelerated the re-entrainment of circadian behavioral rhythms and clock gene expression rhythms of peripheral clocks differentially, but it did not affect the SCN rhythms. However, it remains unknown whether or not the acceleration of re-entrainment by the scheduled exposures is dependent on timing of the exposures. Methods: To study this issue, we compared the effect of timing of the scheduled

(Suite page 86)

(Suite de la page 85)

exposures on re-entrainment of circadian rhythms in the SCN and peripheral clocks after an 8 h phase-advance or -delay shift of LD cycles. We used adult male wild type (WT) and Per1-luc mice. On the day of LD shift, the scheduled exposures for 3 h was started either from dark onset (ZT12, dark onset of new LD cycles) or 3-h prior to dark offset (ZT21). After 4 exposures, WT mice were released into constant darkness to evaluate phase-shifts of behavioral rhythms, while Per1-luc mice were killed for culture preparation. The Per1-luc rhythms in the cultured SCN and peripheral tissues were measured for 5 days. Results: In the phase-advance experiment, the circadian behavioral rhythm in the ZT12 mice completely re-entrained to the new LD, whereas that in the ZT21 mice did not. The Per1-luc rhythms in the cultured SCN immediately re-entrained regardless of the exposure timing. In contrast, the effect on the lung and skeletal muscle was phase-dependent; exposure at ZT12 completely re-entrained the rhythms whereas that ZT21 did not. In the phase-delay experiment, the timing of exposure affected the re-entrainment of Per1-luc rhythms in the lung and skeletal muscle, while it didn't in the behavioral rhythms. Conclusions: Non-photic time cues phase-shift the mouse peripheral clocks phase-dependently and tissue-dependently.

Food time or scheduled melatonin induces rapid re-entrainment after a 6 hours phase advance

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Purpose: Evaluating the effect and influences of food restriction or melatonin administration in the prevention of Jet-lag in rats. Methods: Rats were maintained under 12-12 light-dark conditions with water and food ad libitum for 2 weeks, and were then exposed to a 6h phase advance. During the 5 days prior to the phase shift a group of rats (N=16) was submitted to restricted food or a subcutaneous melatonin (10mg/kg b. w.) injection scheduled at the new ZT12 phase. A second group (N=16) received the treatment simultaneously and for the first 5 days after the phase advance at the new ZT12. Locomotor and temperature rhythmicity were monitored and ZT12 was defined as lights off. Results: The transitory cycles necessary for re-entrainment, in the control group took 7- 9 days, the food group took 3 - 5 days, and the melatonin group took 5 - 8 days. In the second food restriction reduced transitory cycles to 2 - 3days, and melatonin reduced them to 2 - 5 days. Conclusion: Food scheduled previously to the expected new phase, significantly reduced the transitory cycles for re-entrainment of locomotor and temperature rhythms to a new light/dark cycle. When food or melatonin administration was schedules simultaneous with phase shift the transient days were minimized. We may presume that food entrained peripheral oscillators facilitating the task of the SCN to couple peripheral functions to the new LD cycle.

Supported by DGAPA-PAPIT IN205809, CONACyT 82462

Symposium 4. Clocks in model species

Entrainment in Homo sapiens and other model species

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Purpose: Understanding entrainment and dysentrainment of the human clock in real life. Methods: We use an interactive systems approach involving databases, field studies, constant routines, biochemical, physiological and genetic analyses as well as modelling. Results: The reliable assessment of chronotype by a few simple questions (MCTQ) has been validated against sleep-logs, actimetry, and biochemical markers. Human entrainment is tightly coupled to the light:dark cycle. In urban societies weak and/or irregular light cycles elicit weak or dys-entrainment. Social jetlag describes the discrepancy between internal (circadian) and external (social) time. Although shift-work elicits probably the most severe form of social jetlag, it is not the most common form: over 40% of the non-shift-working population suffers from a social jetlag of =2 hours/day. Social jetlag results in chronic sleep debt, which in turn is probably the most important variable underlying the detrimental health effects of dys-entrainment. Our modelling efforts in different organisms show that entrainment can be explained by relatively simple formalisms. Our genetic studies in Neurospora and Homo sapiens have identified novel map locations, indicating the involvement of as yet unknown genes in controlling entrainment in the two species and sleep-wake behaviour in humans. Conclusions: Nothing in entrainment makes sense except in the light of internal time.

A molecular connection between hormone signalling and the Arabidopsis circadian clock

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Purpose: To explore the molecular nodes where plant hormone signalling and the circadian clock converge to translate the environmental information into a physiological response, Methods: we made use of physiological, biochemical and molecular approaches using Arabidopsis thaliana as a model system. Results: we found evidence of a feedback mechanism linking the circadian clock with abscisic acid (ABA) mediated plant responses to drought. A key clock component (TOC1, Timing of CAB expression 1) binds to the promoter of the ABA-related gene (ABAR/CHLH/GUN5) and controls its circadian expression. TOC1 is in turn acutely induced by ABA and this induction changes de timing of TOC1 binding and modulates ABAR circadian expression. Moreover, the gated induction of TOC1 by ABA is abolished in ABAR RNAi plants suggesting that the reciprocal regulation between ABAR and TOC1 expression is important for sensitized ABA activity. In addition, genetic studies with TOC1 and ABAR over-expressing and RNAi plants showed defective responses to drought. Conclusions: our studies support the notion that clock-dependent gating of ABA function is important for cellular homeostasis under dry environments.

Targeting CK1d as the principal regulator of PER-mediated circadian timing in mammals

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(Suite page 87)

(Suite de la page 86)

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Purpose: To define the contribution of CK1 isoforms to circadian timing. Methods: We used novel pharmacological inhibitors targeting either CK1e or CK1d combined with gene knock-out, and knock-down, in vivo models, as well as tissue and cell culture. Results: Use of a CK1e-selective drug had no significant action on circadian rhythmicity in WT or CK1e null animals, tissues or cells, but counteracted accelerated periods of the behavioural activity and clock gene oscillation in CK1etau mice. In contrast, a CK1d selective inhibitor dose-dependently lengthened circadian wheel-running periods of WT and CK1e null mice as well as clock gene oscillations in the SCN, peripheral tissues and fibroblasts. CK1e knockdown had little impact, but CK1d knock-down reduced amplitude of oscillation and lengthened period. At high doses, d inhibition caused reversible cellular arrhythmia, and prolonged nuclear retention of PER2, suggesting CK1 enzymes are crucial for nuclear clearance of target PER2 proteins. Nuclear retention may extend actions of PER2 within the E-box mediated negative feedback loop. This was confirmed in cells, where d inhibition lengthened periods of transcriptional oscillations (Bmal1, Per2 and Rev-erba), but asymmetrically, significantly extending intervals between Per2 and Bmal1 and Rev-erba cycles, but not between Bmal1 and Rev-erba. Conclusions: CK1d but not CK1e is a critical endogenous circadian regulator and, despite remarkable structural similarities, these enzymes are not interchangeable. It is predicted that compounds which specifically target CK1d are likely to offer useful new pharmacologies for the therapeutic regulation of circadian timing.

Zeitgeber response of the circadian clock in cyanobacteria inferred by the phase oscillator model

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Purpose: The circadian clock in cyanobacteria has been previously shown to have a surprisingly high temporal precision. In this work we inquire this clock response to external perturbations, like light and/or temperature in the form of entrainment-like signals.

Methods: We expose a bioluminescent PkaiC reporter strain of cyanobacteria (AMC 462) to external periodic cycles of different phases and amplitudes and follow the dynamic response of the clock during several weeks. We use for that a 96-well plate reader where each perturbation condition is represented by 16 independent wells. The entrainment is realised in situ by illumination, temperature or both.

Results: We show that the in vivo cyanobacterial oscillator in interaction with external forces (temperature and/or light) can be described as a simple phase oscillator: the instantaneous average phase dynamic is fitted by the solution of the Adler equation. In this way, the result of each entrainment force on the circadian oscillator is described by a unique physical measure: the coupling constant. By extending this simple description we next infer into the origin of the clock noise.

Functional analysis of the TOC1 and CCA1 master plant-clock genes in the photosynthetic picoeucaryote Ostreococcus reveals similarities and divergences in the molecular regulations of "green" clocks

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Purpose: Characterisation of TOC1 and CCA1 function in a simple photosynthetic eukaryote. Methods: Implementation of genetic transformation and luciferase strategy to create reporter lines for functional analysis through gene repression (antisense) and overexpression. Results: In eukaryotes, circadian clocks rely mainly on autonomous transcriptional feedback loops. In higher plant many components of these loops have been identified and shown to interact in complex genes circuits. Only the two homologs of plant master clock genes Timing Of Cab expression 1 (TOC1) and Circadian Clock-Associated 1 (CCA1) were identified in the small genome of the green unicellular alga Ostreococcus tauri. TOC1 was peaking at dusk closely followed by CCA1 expression early in the night. Both exhibited similar phase responses to resetting and sustained oscillations under constant light. Deregulation of TOC1 and CCA1 abolished the rhythmicity of their own and reciprocal expressions as well as those of the output gene Chlorophyll A/B Binding (CAB) under free-running conditions. TOC1 or CCA1 misexpression prevented proper entrainment of the clock, as arrhythmic lines displayed increased sensitivity to resetting cues, suggesting that both genes play a central role in the clock. CCA1 was shown to bind the evening element consensus sequence of the TOC1 promoter, which is essential to its circadian regulation. Decreased TOC1 expression level in CCA1 overexpressing lines and increased CCA1 expression level in TOC1 overexpressing lines were consistent with TOC1 and CCA1 working in a feedback loop, which might correspond to a reduced version of the higher plant circadian oscillator. Adjustment of CCA1 and TOC1 expression and phenotypes of arrhythmic lines under short and long days further suggest that TOC1 is regulated at dusk and CCA1 at dawn. Conclusion: The emergence of functional genomics in a simple green cell with a minimal genome as Ostreococcus, will allow a better understanding of the regulation of complex cellular processes such as the circadian clock.

A circadian clock in Saccharomyces cerevisiae

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Purpose: Although circadian clocks are found widely in nature, they have been largely ignored in

Saccharomyces cerevisiae. We are looking for signatures of a circadian system in S. cerevisiae. Methods: We use chemostat cultures to establish conditions that reveal characteristic clock properties as described in so many other species, thereby showing circadian timing in budding yeast. We monitor the oscillation of respiration in yeast in a fermentor. To evoke daily oscillations, temperature cycles are imposed on the cultures. We have investi-

(Suite page 88)

(Suite de la page 87)
gated several readouts - pH change and dissolved oxygen in the media - to determine exactly what is regulated by a daily timer in yeast. Results: The temperature cycle protocols reveal distinct processes in S. cerevisiae, namely entrainment and a damped free-running rhythm, that are consistent with a circadian timing mechanism. Furthermore, we have shown clock-controlled molecular rhythms in gene expression of a key metabolic pathway that can be used to map out clock genes and behaviour in yeast. Conclusion: Features of the temperature protocols indicate a daily timing system in Saccharomyces cerevisiae. These observations are consistent with a circadian clock that is regulating numerous processes.

Symposium 5. Exploring spatiotemporal organization of SCN circuits

Individual neurons form nodes making up circuits that repeat a daily sequence of activity to produce the SCN master clock

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Purpose: We have explored the inputs and outputs of the SCN clock, together with its individual elements, with the goal of understanding its circuit organization. Methods: This has been achieved by repeated back and forth between top-down and bottom-up experimental, theoretical, and modeling studies. Results: We put forth two types of hypotheses. First, we show that under fixed conditions, individual SCN oscillators form identifiable, stable, phase-locked circuits that repeat a daily sequence of activity. This sequence of activated circuits functions as a master circadian clock that sets the phase of rhythms in the rest of the brain and body. This sequential circuit activation provides a more information rich set of timing cues than do component cellular oscillations. Furthermore, this is a plastic system; different patterns of stable oscillations of the underlying circuits can be achieved under a range of environments. Second, we review the many ways that circadian oscillation can be achieved. Circadian oscillation survives catastrophic abuses, including acute brain slice preparations, various mutations or knockouts of clock genes, dispersal of cells, and following many types of lesions. These insults do not destroy the ability to oscillate, but instead reveal resilience of the SCN timing system. Normally however, the circadian system achieves more than just oscillation: it entrains appropriately to environmental cues, coordinates its outputs, and does so under a range of environmental states indicating robustness. This plasticity and adaptability is a product of precise coordination of local, and ever more experimentally identifiable SCN circuits. Conclusions: We conclude that groups of neurons form local circuit “nodes” and that these are serially activated in an ordered manner to repeat a daily sequence of activity to produce a cell based brain clock.

Peptidergic signalling in the circadian oscillator

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The mammalian clockwork is viewed as a series of interlocked transcriptional/translational feedback loops synchronized and sustained by neuropeptidergic signaling. At the tissue level individual cells within the SCN are synchronized to form a coherent oscillator through this peptidergic intercellular coupling; these network interactions stabilize and enhance oscillatory behaviour resulting in a coherent behavioural output. In order to test the roles of peptides within the SCN the PER2:LUC knockin reporter line crossed with Vip (Vip-/-) and the Vip receptor (VPAC2r -/-) knockout mice. The persistence and dynamics of the molecular circadian rhythms were measured by real-time bioluminescence and CCD recording of SCN tissue explants. Over 10 days of recording, the Vip-/- and VPAC2r-/- explants gradually lost coherence in their rhythmicity as intercellular communication was lost. At this time, a 2nd SCN explant from a non-reporter mouse was then co-cultured with the existing SCN explant. Continuous monitoring of the bioluminescence for a further 10 days revealed an immediate rescue of PER2:LUC rhythmicity in the Vip-/- SCN explants which was robust, high amplitude and persisted for the 10th to 20th days of recording. Control grafts of cerebral cortex, however, did not sustain circadian network activity in the host SCN slices. The instantaneous resynchronisation by the grafted explant implies a paracrine rather than a neural mechanism of action. In contrast, the grafting of a non-reporter SCN explant to VPAC2r-/- SCN slices recovered rhythmicity gradually, over several cycles, until it reached a stable level of PER2:LUC bioluminescence expression. These results suggest that whereas VIP signalling is sufficient for intercellular synchronization across the SCN, it is not, however, the only factor- as rhythmicity was also restored to the VPAC2r-/- explants which are devoid of Vip signaling. Future studies will seek to identify these novel synchronizing factors.

Synchronization among SCN neurons determines the phase shifting capacity of the SCN and the amplitude of the rhythm

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Purpose: The phase shifting capacity of the circadian system depends strongly on photoperiod. An explanation for this phenomenon is currently lacking. 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 in darkness, they received a light pulse to construct a Phase Response Curve for long and short day length. We furthermore investigate the phase shifting capacity of the SCN in brain slices. Results: We observed large behavioral phase shifts in animals from short day length and small phase shifts in animals from long day length. In vitro we applied NMDA to mimic the effects of a light pulse. 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 neurons of the SCN are highly synchronized, and that the amplitude of the rhythm was larger than in long days. Conclusions: We observed large phase shifting responses in high amplitude rhythms in slices from short days, and small shifts in low amplitude rhythms in slices

(Suite page 89)

(Suite de la page 88)

from long days, and conclude that the photoperiodic dependent phase responses are intrinsic to the SCN. The relation between phase shifting magnitude and oscillator amplitude is in contrast to earlier predictions from limit cycle theory. This indicates that neuronal networks are governed by different rules than single cell oscillators. We propose that high synchronization among SCN neurons enhances the phase shifting capacity of the network ensemble, and provide experiments to support this hypothesis.

Daily electrical silencing in the mammalian circadian clock

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Purpose: To determine the bioelectrical properties of per1 and non-per1 expressing neurons in the mouse suprachiasmatic nucleus (SCN). Methods: Targeted whole-cell patch clamp electrophysiology was performed from 309 period1 (per1) and 120 non-per1 neurons from SCN of mice expressing a short half-life destabilized enhanced green fluorescent protein (d2EGFP) reporter of per1. Results: The electrophysiological properties of GFP-positive per1 neurons were completely different from those in which GFP could not be detected. We show, for the first time, that throughout most of the day, per1 neurons sustain an electrically excited state but unexpectedly do not spontaneously fire. During the night, all cells are less electrically excited, and for a period of time they do not fire. Between night and day phases, per1 neurons traverse numerous conditions of electrical excitability and show novel low threshold membrane oscillations not seen in other cells. Using a combined experimental and theoretical approach, we explain how circadian variation in ionic currents lead to these activities of per1 cells. Hence, neurons containing the molecular clock show unusual electrophysiological behaviors and, unlike other mammalian brain cells, can survive and function at depolarized states. Conclusion: For almost 30 years, SCN neurons have been thought to express time of day by changing their firing frequency, with high rates during the day and lower rates at night. Here, we show that this daily variation in firing rates is associated with the firing activity of non-per1 neurons.

Circadian firing rhythms in Cry1/Cry2 double-deficient mice in the cultured suprachiasmatic nucleus

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Purpose: The roles of Cryptochrome 1 and 2 (Cry1 and Cry2) are investigated in the circadian system of the mouse suprachiasmatic nucleus (SCN). Methods: Cry1/Cry2 deficient (Cry1-/-/Cry2-/-) and wild type mice of C57BL/6J background were used. Wheel-running activity was measured in LD for two weeks and successively in constant darkness (DD) for several weeks. Neural activity of the SCN slice culture was recorded by means of Multi-electrode array dish (MED). Coronal sections of the SCN were made by a tissue chopper and put on an MED, which was pre-coated with collagen gel. After culturing for 1-3weeks in 5%CO2/95%air at 37℃, spontaneous firing rhythms from the cultured SCN were recorded for 7-18 days. The number of spikes was counted every 1 min. Circadian rhythm and its period were evaluated by a chi-square periodogram. Results: Wild type mice exhibited free-running activity rhythms with periods shorter than 24h under DD. On the other hand, Cry1-/-/Cry2-/- mice became aperiodic under DD. In wild type mice, neural activity was detected from almost all electrodes in the SCN slice and exhibited significant circadian rhythms. In Cry1-/-/Cry2-/- mice, robust circadian rhythms were detected from several electrodes, but bimodal or tripmodal patterns were obtained from some electrodes. Conclusion: A lack of Cry1 and Cry2 leads to arrhythmicity in behavior under DD. However, spontaneous firing in cultured SCN exhibited robust circadian rhythms. The rhythms from some electrodes seemed to be modified by firing rhythms from other oscillating cells. These results suggest that the circadian oscillation exists in cultured SCN of Cry1-/-/Cry2-/- mice, but the mutual synchronization of oscillating neurons seems to be weak as compared to the wild type.

Daily remodelling of synapses in the suprachiasmatic nucleus

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Purpose: To determine the contribution of glutamatergic and GABAergic synapses to the neuronal-glial rearrangements that were found to occur in the suprachiasmatic nucleus (SCN) over the 24-h cycle. Methods: Immunoreacted SCN sections from adult rats maintained under a 12h:12h L/D cycle were taken at ZT02 (day) or ZT18 (night) time-points and processed for quantitative confocal imaging of glutamatergic and non-glutamatergic synapses made on neurons expressing vasoactive intestinal peptide (VIP) or vasopressin (AVP). Specific vesicular transporters were used as markers of glutamatergic (VGLUT1/2) or GABAergic (VGAT) terminals and Bassoon (Bas), a cytomatrix protein known to be associated with active zones, was used as a marker of synapses. The study was complemented by an electron-microscopic morphometric analysis. Results: The density of both glutamatergic synapses (VGLUT+/Bas+), including optic ones, and non-glutamatergic synapses (VGLUT-/Bas+), including GABAergic ones (VGAT+/Bas+), on VIP neurons in the retinorecipient region significantly increased at daytime (+36%). In contrast, synapses made on AVP neurons in the non-retinorecipient region did not change quantitatively with time of day. At the electron-microscopic level, available data showed that the ultrastructural features of glutamatergic terminals were not different at day and night in the retinorecipient region. To which extent GABAergic terminals may be submitted to synaptic remodelling at VIP vs non-VIP targets is under current investigation. Conclusions: The structural plastic events that are believed to subserve the light synchronization process in SCN involve a remodelling of synapses at sites of photic integration. Considering the 12-h time frame within which the synaptic rearrangements occur, they provide a further illustration of how the adult brain may rapidly and reversibly adapt its synaptic architecture to functional needs.

(Suite page 90)

(Suite de la page 89)

Symposium 6. Organization of the hierarchical multi-oscillator system in the suprachiasmatic nucleus

Introduction

Chairman: Honma KI

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It is well established that the suprachiasmatic nucleus (SCN) consists of a number of oscillating neurons which are not identical in the nature but coupled with each other to make a population oscillator. Recently, we found that the organization of SCN oscillating system in mice was changed by photoperiod, showing at least two sub-populations of oscillating cells. Under different photoperiods, the phases of these sub-population oscillators were correlated with the activity onset and end of activity of behavioral rhythms respectively. These findings suggest that the oscillatory system in the SCN circadian clock contains two sub-oscillators which correspond to the morning (M) and evening (E) oscillators proposed many years ago by C.Pittendrigh. The questions arise here about the mechanisms of differential couplings among the constituent oscillating cells; the coupling among the oscillating cells to make the sub-population oscillators and the coupling between the M and E oscillators.

In the present symposium, two appointed speakers talk about the possible mechanism of oscillatory coupling and the dynamics of population oscillators. Three speakers from the Abstracts will take related topics. The goal of this symposium is to find out the strategies to approach the hierarchical multi-oscillating system in the SCN.

Synchronization engineering: Role of neuropeptides

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The brain's biological clock is located in the hypothalamic suprachiasmatic nucleus (SCN) and contains approximately 10.000 neurons. The neurons house individual molecular clocks, which need to be synchronized to drive circadian rhythms of behaviour and physiology. Furthermore, the functional clock needs to be daily entrained by photic and non-photic cues to be in phase with the astronomic day length of 24 h. The neurons within the SCN can be characterized by the phenotype due to transmitter expression, clock gene expression and anatomical localization. Recent studies have demonstrated the several neuropeptides are involved in the entrainment process. Light information from the eye involves PACAP, non-photic input from the midbrain involves NPY and within the SCN, VIP is necessary for synchronization of the individual neurons to generate sustained rhythmicity whereas gastrin releasing peptide (GRP) is involved in light entrainment and argine-vasopressin (AVP) is in involved in output signalling form the SCN. The presentation will focus on two neuropeptide systems in the SCN: the VIP/VPAC2 receptor and the cholecystokinin (CCK), another neuropeptide system in the SCN which will be described in new details covering anatomical characterization, CCK neurons responsitivity to light and clock gene expression and its role in light entrainment as determined by studies in mice lacking the CCK gene.

Effects of intercellular communication on the entrainment to time cues

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Purpose: The circadian rhythm in mammal is orchestrated by the suprachiasmatic nucleus (SCN). The SCN consists of many clock cells. Each individual cell in the SCN has circadian rhythm and by synchronizing their rhythms the SCN can form a large rhythmic signal. Here, fundamental questions may be posed. Why is SCN a population of cells? Why do SCN cells form a complex network of communication pathway? In particular, a simple synchronization of clock cells would not require such a complex network structure. In this presentation, possible roles of the intercellular communications are discussed. We mainly focus on what kind of functions can be realized by what kind of network structure. Methods: A simple mathematical model, called a phase oscillator model, describing clock dynamics of a population of interacting cells is analyzed. Results: We find suitable network structures that enable the cell population to efficiently respond to external time-cues and to realize precise rhythmic behavior. Similarity of these found network structures and the anatomically known SCN structure is also discussed. Conclusions: Possible functions of the multi-oscillator structure in the SCN are proposed.

Two distinct oscillators in the suprachiasmatic nucleus.

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The hypothalamic suprachiasmatic nucleus (SCN) is the master regulator of the systemic circadian organization. Many neurons in the SCN can generate circadian outputs such as locomotor activity, sleep and wake, temperature, metabolic, and endocrine rhythms. The SCN neurons are synchronized in a constant dark condition. However, the SCN is composed of several subregions. The most prominent functionally distinct subregions are ventrolateral region (VLSCN) and dorsomedial region of the SCN (DMSCN). Only the VLSCN receives neural projections from the. This suggests that VLSCN directly accept the photic information while DMSCN does not. Previously, we demonstrated that the endogenous desynchrony in the SCN occurs after a rapid light:dark cycle shift in rats, which suggested that jet-lag syndrome is caused by endogenous desynchrony in the SCN. We have also investigated the functional and morphological difference between DMSCN and VLSCN. The VLSCN have direct projection from the retinal ganglion cells. Thus it receives photic information from the retina. Interestingly, the information seems to be gated during the most of daytime since the light exposure during the daytime hardly induces immediate early genes (IEG) such as c-fos and c-jun, and light-inducible clock genes such as Per1 and Per2. The light pulse during the night induces and increases the amplitude of Per1 and Per2 expression and the oscillation of Per1 and Per2 rhythm continues for more than three days in DD condition. In the DMSCN, light during the night does not induce IEGs so that we do not know when the phase

(Suite page 91)

(Suite de la page 90)

of the VLSCN is shifted after light exposure. Light exposure seems not to increase the amplitude of clock gene oscillations. The shift of the DMSCN is slow compared with the VLSCN. The findings suggest that VLSCN and DMSCN have distinct systems that regulate the resetting of overt circadian rhythms. We have assessed the characteristics of the two oscillators separately by using the slice cultures that contained one of VLSCN and DMSCN. Further, we constructed a mathematical model that fits to the complicated behavior of the two subregions.

Forced desynchronization revisited: modeling SCN neuronal population networks

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Purpose: understanding dynamics of two coupled oscillators under forced desynchronization protocols, using mathematical modeling and computer simulations. In particular, understanding experimentally observed temporal release of melatonin patterns when rats are maintained in LD22h and, consequently, the SCN neuronal subpopulations are under forced desynchronization. Methods: two coupled oscillators forced by an external zeitgeber are simulated for a wide set of period and coupling force configurations, using Pittendrigh-Pavlidis equations for oscillators and square waves as zeitgebers. Simulations are performed with Neurodynamix II software. Results: simulations show transition from synchronized to desynchronized, and then back to synchronized states of two oscillators as a result of gradual departure of zeitgeber periods from the intrinsic oscillator periods. We show that the long standing concept that the non-entrained oscillator is "free-running" under forced desynchronization is wrong, because it is always driven by the zeitegeber and is modulated through coupling to the entrained oscillator. In desynchronized states, the non-entrained oscillator is in "relative coordination". This explains melatonin patterns, which show periodic compression and decompression of release durations along with periodic phase jumps in rats under LD22. Relative coordination and masking by light account for its generation. These results indicate that the circadian control of pineal melatonin release is due to the dorsomedial SCN (the non-entrained, t>24h subpopulation of SCN neurons) while light inhibition of release is achieved through the ventrolateral SCN (the entrained t<24h subpopulation of SCN neurons). Conclusions: This model shows that SCN subdivision into dorsomedial and ventrolateral subpopulations account, respectively, to the two distinct mechanisms that restrict melatonin release to the dark phase of the day, namely entrainment and masking.

Mechanism of coupling between bilaterally paired suprachiasmatic nuclei

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Purpose: Investigate putative coupling pathways between left and right suprachiasmatic nuclei (SCN). Methods: Calcium imaging of mouse SCN neurons was performed in acute brain slices with electrical stimulation of the contralateral SCN. Patch clamp recording of evoked postsynaptic currents after contralateral stimulation were used to measure response delay and reversal potential. Blockers of several neurotransmitter receptors were bath-applied. Phase shift of electrical activity rhythm after brief (30 min) unilateral electrical stimulation was assessed by long-term multi-unit activity recording of both SCN. Results: Unilateral electrical stimulation of SCN slices at ZT 14 led to significant phase advances of the contralateral SCN rhythm (2.5h ±0.9, n=7). Calcium imaging experiments as well as patch clamp recordings provided evidence for synaptic signaling dependent on action potentials. Calcium imaging results revealed that the dorsocaudal part of the SCN contains most of the neurons responding to contralateral stimulation. Patch clamp recordings showed excitatory postsynaptic responses to contralateral stimulation. Delay times indicate monosynaptic (3-5 ms) as well as multisynaptic connections (7-15 ms). Our pharmacological data suggest a role of GABA in modulating inter-SCN coupling. Vasoactive intestinal peptide (VIP) antagonist and substance P attenuated contralateral calcium responses, but these were also still present in VIP-knockout mice. Application of AMPA receptor antagonist blocked calcium transients and blocked most of the postsynaptic responses in the patch clamp experiments. Conclusions: Communication between left and right SCN is based on chemical synaptic transmission driven by action potentials. Phase-shifts after contralateral stimulation indicate the functional significance of this pathway. A majority of recipient neurons of this pathway are in the dorsal part of the caudal SCN. It is possible that more than one neurotransmitter is involved in this signaling with GABA as a modulator. Glutamatergic, possibly indirect, pathways may contribute to communication between bilaterally paired SCN.

Symposium 7. Organization of the mammalian circadian timing system: centrifugal or centripetal

Introduction

***Chairman*: Schwartz WJ**

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The circadian pacemaker in the suprachiasmatic nucleus (SCN) governs a wide array of rhythms, from biosynthetic to behavioral. The synchronization, sequencing, and/or segregation of these rhythms, as also shaped by non-circadian factors, create a temporal program that adapts to the time of day, changing seasons, and local environment. Neural, hormonal, and behavioral substrates underlying this internal organization are being identified. These include direct axonal projections from the SCN to specific brain targets (e.g., for the activation of luteinizing hormone -releasing hormone neurons), SCN-secreted diffusible signals (e.g., for setting the temporal window for the cyclic alternation of rest and activity), and multi-step, multi-modal pathways for entraining the activities of extra-SCN oscillators (e.g., for regulating aspects of hepatic rhythmicity).

(Suite page 92)

(Suite de la page 91)

Further complicating these analyses are the existence of nested feedback loops, by which rhythmic outputs may act to gate zeitgeber sensitivity and alter oscillator properties. This symposium will highlight our emerging understanding of how such complex timing mechanisms are integrated for concerted action. Invited presentations by Andries Kalsbeek and Hugh Piggins will dissect the anatomy and physiology of central and peripheral timekeeping systems that regulate the internal milieu of brain and body.

SCN neuronal outputs as mediators of peripheral circadian structures

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The master biological clock, located in the hypothalamic suprachiasmatic nuclei (SCN), uses its projections to neuro-endocrine and pre-autonomic neurons in the hypothalamus to control daily hormone rhythms, e.g. adrenal corticosterone and pineal melatonin release. The hypothalamic paraventricular nucleus (PVN) is an important target area of biological clock output as it harbors the pre-autonomic neurons that control the sympathetic and parasympathetic branches of the autonomic nervous system (ANS). The SCN also plays an essential role in maintaining daily blood glucose concentrations between strict boundaries. To investigate further SCN control of ANS activity we focused our attention on the daily rhythm in plasma glucose concentrations. Using local intra-hypothalamic administration of GABA and glutamate receptor (ant)agonists we investigated how changes in ANS activity contribute to the daily control of plasma glucose and plasma insulin concentrations. We show that the major part of the SCN timing information is provided by a rhythmic GABAergic output to the pre-autonomic neurons, differentiated for the separate organs. With the help of selective hepatic denervations we were able to show that the ANS is an important gateway for the SCN to transmit the (phase-shifting) effects of light to the glucoregulatory and clock gene machinery of the liver. Finally, using ICV administration of neuropeptides and/or their (ant)agonists we were able to delineate how the SCN may also “use” the hypothalamic neuropeptide systems to control the daily rhythm in glucose production by the liver. For instance, GABAergic SCN inputs to the orexin-containing neurons in the lateral hypothalamus may form the molecular link between the plasma glucose and sleep/wake rhythms; whereas it’s input to the PACAP system in the ventromedial hypothalamus may form the molecular link between the metabolic and reproductive rhythms.

Extra-suprachiasmatic circadian oscillators in the mouse brain

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Purpose: To visualize dynamic clock gene expression in extra-suprachiasmatic (SCN) brain regions and to determine circadian properties in these extra-SCN oscillators. Methods: Coronal brain slices were made from adult male mPer2::Luc mice and cultured in luciferin-containing medium. Bioluminescence was assessed via luminometry or visualized on an EM-CCD camera system. Results: Using luminometry, circadian rhythms in PER2::LUC bioluminescence of varying robustness and longevity were detected in multiple brain regions. Low amplitude oscillations were sustained for 2-4 cycles in coronal sections containing the habenula, whereas in the amygdala, a single peak in bioluminescence was measured. In mediobasal hypothalamus (MBH) slices, low amplitude oscillations could be tracked for up to 8 days. To identify more precisely the anatomical origin of the PER2::LUC signals and to determine the cellular organization of these tissue oscillators, we next imaged PER2::LUC bioluminescence in these cultures. In the habenula, we visualized rhythmic PER2::LUC signal in the ependymal cell layer of the dorsal third ventricle and observed a small number of PER2::LUC cells in the medial component of the lateral habenula whose rhythmic activity damped over 2-4 days. In the amygdala, PER2::LUC signal was localized to the central nucleus, but single cells could not be discerned. In MBH, the ependymal cell layer rhythmically expressed PER2::LUC as did individual cells in the arcuate and dorsomedial nuclei. These single cells initially expressed synchronized rhythms, but they became desynchronized and the intensity of the signal declined over ~8 days. Conclusions: Collectively, these data indicate that low amplitude circadian oscillators are widespread in brain tissue and that a key factor in limiting their robustness and autonomy is the inability of individual cells in these areas to maintain synchronized clock activities.

Internal desynchronisation between the central circadian pacemaker and peripheral rhythms in CBA/CaJ mice using workload

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Purpose: To determine the internal desynchronisation between the central circadian pacemaker (SCN), and hormonal and peripheral rhythms in the mouse strain CBA/CaJ. Methods: Using workload, we forced mice to shift their activity into the light phase. To explore the physiological consequences we measured core body temperature using temperature loggers and 6-Sulphatoxymelatonin (aMT6s) production in the urine. Results: Under increased workload the onset of running wheel activity shifted into the light phase. Moreover, increased workload caused a significant decrease of the body temperature to below 30° C. The aMT6s profile in the urine was characterised by a peak at the beginning and at the end of the dark phase. The change of the photoperiod from LD 12:12 to DD in combination with a change from food restriction to food ad lib caused an immediate shift of the activity-onset back to the beginning of the dark phase. Currently we are measuring PER1 and PER2 protein expression in the SCN as well as in the peripheral organs in order to determine the internal rhythms. Analysis on aMT6s excretion and Per gene expression in liver and SCN should indicate whether the central and peripheral liver clock has shifted along with the activity rhythm. Conclusions: Workload seems to infer extreme energy demands and therefore causes a decrease of the core body temperature. Consequently torpor occurs to save energy. This phenomenon may be the reason underlying the activity-shift towards the light phase. The result from the change of the photoperiod from LD to DD, as well as no change in aMT6s production during

(Suite page 93)

(Suite de la page 92)
workload indicates desynchronisation between the central circadian pacemaker (SCN) and the peripheral clocks.

In the fetal rat, the adrenal circadian clock is functional before the SCN clock

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In the fetal rat, oscillatory expression of clock genes on the SCN occurs at 20 day of gestation. However, at 18 days of gestation, fetal corticosterone content shows a circadian oscillation, with a maximum at 0800-1200h. The rat fetal adrenal gland expresses clock genes and Mt1 melatonin receptor. The clock gene Bmal1 controls expression of steroidogenic acute regulatory protein (StAR) a key protein for corticosterone production in the adult adrenal. Purpose: To explore the intrinsic oscillation of Per2 and Bmal1 in the fetal adrenal gland and its relationship with StAR expression. Methods: Pregnant dams (n=12) were euthanized at 20-24 h at 18 days of gestational age by thiopental overdose. Fetal adrenal glands were dissected, pooled in 15 ml DMEM-F12 and preincubated by 6 hrs at 37ºC. Then the adrenal pool was aliquoted in culture dishes (about 8 adrenals per well) in triplicate and adrenals were collected every 4 hrs for 30 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 StAR were measured by real time-PCR. Results: We detected oscillatory expression in culture of the clock genes Bmal1 and Per2 as well as of Mt1 and StAR with acrophases at 2400, 1200h, 1600h and 1200h, respectively. The acrophase of StAR coincided with that of Per2 and with the timing of maximal plasma corticosterone concentration in the fetus. Conclusions: Our data show that, in vitro, the fetal rat adrenal gland sustains Bmal1 and Per2 oscillation, together with the oscillation of one of the key enzymes of corticosterone production, StAR and with MT1 receptor expression. Altogether, the present results support that rat fetal adrenal is a functional peripheral clock, active at 18 days of gestation. The presence of MT1 expression suggests that in vivo the rat fetal adrenal rhythms are synchronized by maternal melatonin.

Supported by Fondecyt-1080649

The SCN activates ?-MSH neurons in the ARC at the end of the activity period

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Purpose: The arcuate nucleus (ARC) and the suprachiasmatic nucleus (SCN) play important roles in the integration of metabolic signals and the temporal organization of physiology and activity of the organism, respectively. Recently, reciprocal neuronal connections between these nuclei have been demonstrated. In order to investigate the nature of the interaction between these two structures, and the significance of the metabolic state for such interaction, the pattern of activity in transmitter defined neurons in the ARC was determined under ad libitum and fasting conditions. Methods: Animals were perfused ad libitum or under 48 h fasting conditions at ZT2, 6, 10, 14, 18 and 22, tissue was stained using double immunocytochemistry for c-Fos and ?-MSH. Ad libitum and fasted SCN lesioned animals were sacrificed at ZT 22 and also stained. Results: Under ad libitum conditions the ARC showed a significant increase of c-Fos, ?-MSH co-localization at ZT 18 and 22. Because these time points coincide with the end of the feeding phase of the rat, it was investigated if the same pattern was maintained under fasting conditions. Without food the same daily pattern was observed, with the difference that more ?-MSH neurons were activated at those time points than in ad libitum conditions. SCN-lesioned animals did not show c-Fos in the ARC suggesting the involvement of the SCN in the activation of the ARC nucleus during the last part of the dark phase. Conclusions: The persistence of ?-MSH activation under fasting conditions and its disappearance after an SCN lesion indicates that without satiety, the activity of these neurons continues to follow a day-night pattern, showing the involvement of the SCN in the activation of ?-MSH neurons. The present results indicate that the SCN may affect food intake and energy balance, via a daily activation of the ?-MSH neurons in the ARC.

Supported by DGAPA project IN215308 and Conacyt 79797

Symposium 8. Interplay between circadian clocks and metabolism

Introduction

Chairman: Shibata S

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Disturbance of the circadian clock such as clock and/or clock-controlled gene mutation causes an impairment of metabolic process, and results in obesity, diabetes and/or hypercholesteremia. Actually food intake of same volume at night increases body weight gain than that of same volume in the morning. These results strongly suggest that circadian clock can control the metabolic process of food digestion and absorption. On the other hand, metabolic syndrome such as obesity and diabetes causes the impairment of circadian rhythms of clock gene expression in the peripheral clock. Restricted feeding can entrain not only behavioral and temperature rhythms but also and peripheral clock rhythm, suggesting that metabolic signal (s) may operate the entraining signals in peripheral clock. Thus, circadian clock and metabolism is now known to interplay each other. However, we still do not know which and how such signals operate as interplay signals. In the present symposium, we want to discuss the mechanism(s) of interplayer between circadian clocks and metabolic process.

Food and circadian organization: motivation versus metabolic cues

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In nature and for most species, access to food sources is restricted to a few hours daily. Therefore, animals have developed the capacity to estimate time, which allows them to anticipate the coming feeding opportunity, by approaching the right location and to prepare physiological

(Suite page 94)

(Suite de la page 93)
and digestive functions for the expected meal. In the laboratory restricted feeding schedules (RFS) induce anticipatory activity (FAA) and impose daily oscillations of c-Fos and clock proteins in brain structures and peripheral oscillators. We have proposed that food entrainment is initiated by alternation of a fasting / feeding cycle that will drive a network of brain nuclei in interaction with our organs. In addition, the metabolic state of scarcity leads to an increased motivational state associated with FAA and increased locomotion. Purpose: In order to dissect the contribution of the metabolic cycles and the motivational state to induce FAA, we compared the influence of daily access to food or chocolate on Fos and PER1 food entrained cycles in hypothalamic and corticolimbic structures in Wistar rats. Methods: Rats were exposed to daily restricted food access or 5 gr of chocolate at ZT6. Results: RFS produced FAA of higher intensity and durantion than daily chocolate delivery. Both induced a differential effect in Fos and PER1 rhythmicity, RFS entrained mainly hypothalamic structures while chocolate induced rhythmicity in corticolimbic structures with peak values at ZT12. This pattern persisted for up to 8 days after interruption of both entraining protocols. Conclusions: Present data evidence different oscillatory systems in the brain that can be driven differentially by metabolic stimuli or by motivation and reward.

Granted by CONACyT 43950-M and 82462

Nocturnin is a circadian regulator of lipid uptake

Green CB

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Purpose: Nocturnin is a deadenylase that controls mRNA expression in a circadian manner by degrading the poly-A tails of target mRNAs, leading to mRNA turnover or translational silencing. Previously we reported that a mouse lacking Nocturnin was resistant to diet-induced obesity and heptatic steatosis. The purpose of the present study is to discern the mechanism behind this lean phenotype. Methods: We exposed WT and Nocturnin KO mice to various dietary challenges and performed in vivo and in vitro analyses of lipid utiliziation and uptake in the liver and small intestine. Results: The lean phenotype was not due to increased activity, decreased food intake or a higher metabolic rate. When subjected to either a ketogenic diet or food restriction, the KO mice lost more weight than their WT counterparts. When on a standard mouse chow, the KO mouse exhibited lower circulating beta-hydroxybutyrate – a finding consistent with altered lipid availability in the KO. Moreover, this latter discrepancy in the KO is not due to hepatocyte malfunction as hepatocyte analysis showed normal rates of both lipid uptake and beta-oxidation. A gut motility assay indicated that the Nocturnin KO mice have a faster transit time for lipid, but not water. Following a lipid bolus , histological examination revealed that the lipid droplets formed along the jejunum of the small intestine were significantly larger in the Nocturnin KO mice than in the WT mice. Through a subsequent series of in vivo and in vitro studies, we demonstrated that the KO mice are deficient in their ability to take up lipids. These animals have significantly disrupted lipid trafficking in the enterocytes, resulting in decreased absorption via apoB-containing non-HDL lipoproteins. Conclusions: We propose that Nocturnin has a role in the absorption of dietary lipid in bowel, presumably by altering genes necessary for metabolism or digestion through circadian post-transcriptional modifications of targeted transcripts.

Possible involvement of the hypothalamic VIP and PACAP projection systems in the circadian control of hepatic glucose production via the autonomic nervous system

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Purpose: The biological clock, located in the hypothalamic suprachiasmatic nuclei (SCN), is involved in the control of carbohydrate metabolism. We studied whether the Vasoactive Intestinal Polypeptide (VIP) containing SCN-PVN projection has a glucoregulatory role. Methods: VIP shares its two receptors, VPAC1R and VPAC2R, with Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP). Endogenous glucose production (EGP) was determined during a 2-h intracerebroventricular (ICV) infusion of either VIP or PACAP-38. Receptor specificity was examined by ICV (co)infusions with specific receptor agonists or antagonists. The hypothalamo-hepatic neuronal pathway involved was studied by combining retrograde neuronal cholera toxin B tracing from the intermediolateral (IML) column of the thoracic spinal cord with Fos immunocytochemistry and by performing specific sympathetic or parasympathetic hepatic denervation. Results: ICV infusion of different doses of VIP and PACAP-38 significantly increased EGP via the VPAC2R, but not the VPAC1R. They also induced strong Fos immunoreactivity (ir) in the PVN. Fos-ir induced by PACAP-38 co-localized with the retrograde tracer in pre-autonomic PVN neurons projecting to the sympathetic preganglionic neurons in the IML. The PACAP-38 induced increase of EGP was significantly suppressed by a specific sympathetic but not parasympathetic hepatic denervation. Conclusion: This study presents a wired neuronal connection, involving VPAC2R and pre-autonomic PVN neurons, via which VIP (probably from the SCN) and PACAP-38 can regulate EGP.

Effect of total sleep deprivation on postprandial responses in shift workers and non-shift workers

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Purpose: To investigate the effects of one night total sleep deprivation (TSD) on postprandial metabolic and hormonal responses under controlled laboratory conditions. The responses of shift workers and non-shift workers were compared. Methods: Eleven shift workers (35.7±7.2 y (mean±SD), shift work for 8.7±5.25 y) were matched for age, body mass index, plasma cholesterol and high-density lipoprotein with 13 non-shift workers (32.8±6.4 y, shift work for 0.03±0.12 y). After an adaptation and baseline night (equal to habitual sleep), volunteers were kept awake for 30.5h, followed by a nap (4h) and recovery sleep. Blood samples were taken prior to and after a standard breakfast on the baseline day, following TSD and after recovery sleep (all in light <8 lux, interventions rela-

(Suite page 95)

(Suite de la page 94)

tive to wake up time, body posture and food controlled). Plasma triacylglycerol (TAG), non-esterified fatty acid (NEFA), glucose and insulin levels were measured. Results: No differences were observed between shift workers and non-shift workers in all baseline fasting levels. Analysis by three-factor ANOVA (repeated measures for 'time' and 'day' and 'group' as between subjects factor) showed no overall difference between shift workers and non-shift workers. In both groups, the TAG response was significantly increased after recovery sleep compared to TSD ($F_{2,22}=10.89$, $P<0.001$) and baseline sleep ($F_{2,22}=10.89$, $P<0.05$) whereas the NEFA response decreased after recovery sleep compared to baseline sleep ($F_{2,22}=5.20$, $P<0.01$). By contrast, there was no effect of day on glucose responses. In non-shift workers the insulin response was higher after recovery sleep than TSD and baseline sleep ($P<0.001$). Conclusions: These preliminary analyses show that there were no overall differences between shift workers and non-shift workers and that changes in postprandial responses were mainly observed after recovery sleep.

Supported by EU grant MCRTN-CT-2004-512362

Circadian rhythmicity in murine pre-adipocyte and adipocyte cells

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Purpose: Current evidence suggests that circadian rhythms play an important role in adipose physiology as the plasma concentration of multiple adipokines, and approximately 20% of the murine adipose transcriptome, undergo diurnal variation. However, due to the heterogeneous nature of adipose tissue and rhythmical input from both neuronal and humoral signals, the cellular basis of adipose rhythms is unclear. In this study, we tested the hypothesis that an endogenous adipocyte circadian clock drives the rhythmic synthesis and secretion of adipokines. Methods: Populations of murine 3T3-L1 pre-adipocytes and differentiated adipocytes were generated and treated with a 2-hour pulse of 50% horse serum. Cells were then sampled every 4-hours over a 48-hour period and mRNA expression of clock genes, nuclear receptors, the transcription factor SREBP1, and adipokines was analysed by quantitative real-time PCR. Secretion of the adipokines leptin and adiponectin were also measured in culture medium from differentiated adipocytes. Results: Following a serum pulse, circadian rhythms of the clock genes Per2, Rev-erba and Dbp, but not Per1, Cry1 and Bmal1 were observed in both pre- and post-differentiated adipocytes. The nuclear receptor genes PPARa, PPAR?, and transcription factor SREBP1 also failed to exhibit temporal changes in expression in pre- and post-differentiated adipocytes. Moreover, although there was no detectable rhythm of Leptin or Adiponectin mRNA in adipocytes, the rate of leptin accumulation in the culture medium increased with a circadian rhythm. Conclusion: These data suggest that 3T3-L1 pre- and post differentiated adipocytes possess an endogenous circadian clock. This clock may control leptin secretion in adipocytes, but is likely to be only partially responsible for rhythmic mRNA expression in adipose tissue.

Symposium 9. Regulation of pineal melatonin: New twists to an old story: introduction

Chairman: Stehle J

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In all vertebrate phyla, melatonin synthesis starts with the active uptake of tryptophan into pinealocytes, where it is converted to 5-hydroxy-tryptamine. The subsequent N-acetylation of the molecule by the arylalkylamine N-acetyltransferase (AANAT) is considered to be rate-limiting for melatonin synthesis. N-acetylserotonin is converted by the acetylserotonin O-methyltransferase (ASMT) to melatonin. It should be noted that ASMT may play a more important role than assumed so far, as a modulation of AANAT activity over a wide dynamic range is not followed by parallel changes in melatonin synthesis. Despite this common and highly preserved biochemical pathway for melatonin synthesis in all vertebrate species, there exist remarkable differences in the regulation of hormone synthesis. In non-mammalian vertebrates, pinealocytes are still directly photosensitive and photic input to the pineal gland adjusts ('phase-shifts') and gates melatonin synthesis directly. Moreover, pinealocytes of lower vertebrates take advantage of exactly anticipating daily changes in lighting conditions by inheriting in parallel an endogenous circadian clockwork that is tracked directly by prevailing lighting history. In rodents, recent studies have demonstrated that next to the pivotal transcriptional mechanisms, chromatin remodeling is a naturally occurring event to shape rhythmic melatonin synthesis. This cAMP-inducible phenomenon is restricted to nighttime, coinciding with the demand of an enhanced gene transcription. In the pineal gland of sheep and monkey, melatonin synthesis is based on a strongly attenuated control of transcription. It was shown that the destructive targeting of the constitutively present AANAT by the proteasome is switched during nighttime towards maintenance in activity via a cAMP-dependent and PKA-mediated formation of a complex of the AANAT protein and the 14-3-3 protein, thereby inhibiting AANAT dephosphorylation. However, in the human there exists evidence that this stabilizing complex is constitutively present, and that a dephosphorylation of the AANAT during daytime is not the reason for rhythmic AANAT activity.

Pineal function and development: Lessons from zebrafish

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The core circadian clock in zebrafish is similar to that described in mammals. Nevertheless, there are some notable differences and features that render the zebrafish an attractive model for chronobiologists from a comparative and evolutionary standpoint. 1) The zebrafish has more copies of each of the clock genes. 2) Circadian rhythms appear early in life; rhythms of melatonin production in the pineal gland begin two days after fertilization. 3) Zebrafish peripheral clock-containing structures and cell lines are directly light-entrainable. 4) The zebrafish model now offers a plethora of molecular-genetics techniques, such as gene knockdown and over expression, transgenesis, genome-wide transcriptome analysis (gene chip) and bioin-

(Suite page 96)

(Suite de la page 95)
formatics tools, including the entire genomic sequence.

Studies in our lab have indicated that circadian rhythms of pineal aanat2 expression appear on the third day of development and that light exposure is mandatory for the development of this rhythm. Additionally, light induces the expression of period2 (per2) in the pineal gland; an important event in the development of the pineal circadian clock. Utilization of the light-entrainable zebrafish cell lines enables to study the mechanisms underlying light-induced per2 expression and light-entrainment. These cell-based studies are being complimented by in vivo studies in wild type and per2:EGFP transgenic zebrafish line, where gene knockdown and over expression are used to determine the involvement of putative transcription factors in this process. Further, a genome-wide examination of pineal gene expression allows the detection of known and novel rhythmic genes, and their function in the pineal gland can be investigated in vivo by current molecular-genetic techniques. In conclusion, the use of zebrafish advances our understanding of the mechanisms underlying clock function, light entrainment and functional development of the pineal gland.

Fish pineal, a model to study melatonin biosynthetic pathways

Falcón J

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Abstract not provided

Novel aspects of melatonin synthesis regulation in the human pineal gland

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Purpose: To further investigate molecular details of melatonin synthesis in the human pineal gland on the basis of the earlier reported absence in daily variations in Asmt- and Aanat-transcripts. Methods: Using time-of-death-matched autoptic human and fresh sheep pineal organs, the presence and subcellular distribution of the AANAT and the ASMT proteins were investigated by immunoblot and immunohistochemical analyses. Results: ASMT- and AANAT-protein levels as well as the PKA-phosphorylated AANAT at threonin 31 did not show a diurnal variance in the human (and sheep) pineal tissue. Interestingly, AANAT dephosphorylation by alkaline phosphatase was readily observed in vitro in pineal gland homogenates from rats, but not from sheep or humans. A co-localisation of AANAT-, ASMT- and 14-3-3-proteins was demonstrated in tubular-like structures inside human and sheep pinealocytes, and co-localisation was confirmed by immunoprecipitation experiments. Tubular-like structures did not co-localise with sympathetic nerve fibres, or neurodegenerative structures. Conclusions: Findings in the human and sheep pineal gland render the earlier suggested adrenergic/cAMP/PKA-mediated protection from proteasomal degradation of AANAT protein during the night unlikely, but suggest phosphorylation of non-PKA-targeted sites as potentially relevant for regulation of AANAT activity, and thereby melatonin synthesis. While the exact nature and function of the tubular-like structures that embody the co-localisation of AANAT, ASMT, and 14-3-3 is yet unknown, these structures may be part of a compartmentalisation of the key components in melatonin biosynthesis.

Supported by Ebert-, Scheidel-, and Riese-Stiftung, and Stiftung Forensisches Forum

The rat pineal hosts an endogenous clock whose elements are differentially regulated by noradrenalin

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Purpose: To determine the occurrence of endogenous oscillation of various clock genes in cultured rat pineals and study their regulation by adrenergic agonist. Methods: To investigate for endogenous rhythmicity of Per1, Per2, Bmal1 and Rev-erba expression, pineals were sampled from adult Wistar rats, cut into 2-4 pieces and then cultured for 48h. Explants were harvested every 4h and the mRNA level of clock genes and Aa-nat was analyzed by qPCR to obtain an in vitro profile. In addition, pineals were collected from animals at different time of the day to examine in vivo expression patterns of all genes as controls. For stimulation experiment, 2µM of the isoproterenol (iso) or vehicle was added to the pineal at subjective mid-day and mid-night on the 2nd day of culture, and the explants were collected 1 and 3h after stimulation. Results: This study confirmed the daily rhythm of expression of all studied genes in the rat pineal in vivo. In vitro, the rhythm of Per1 and Aa-nat was severely damped with no more rhythm after 24h. However, exogenous stimulation with iso was able to increase their mRNA levels. By contrast, Per2, Bmal1 and Rev-erba displayed a significant and sustained rhythm of expression in vitro for 48h with a pattern similar to that observed in vivo. Strikingly, expression of Per2, Bmal1 and Rev-erba was unaltered by iso given at subjective day or night. Conclusions: The rat pineal hosts a circadian clock as represented by the sustained endogenous oscillations of Per2, Bmal1 and Rev-erba mRNA levels in culture. Expression of Per1 only was stimulated by iso suggesting that, in vivo, the adrenergic input could synchronise the pineal clock by acting selectively on Per1.

Arylalkylamine N-acetyltransferase 2 in goldfish (Carassius auratus): cDNA cloning and rhythmic expression in central and peripheral tissues

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Purpose: To clone the complete nucleotide sequence of the enzyme arylalkylamine N-acetyltranferase 2 (AANAT2) in goldfish and to investigate the tissue distribution and daily rhythmicity of aanat2 expression under different light conditions in pineal retina, liver and hindgut. Methods: Total mRNA from goldfish pineal glands was reverse transcripted and used as a template to clone AANAT2 with the RACE method. After sequence analysis, specific primers for qRT(Real Time)-PCR were designed. Goldfish were

(Suite page 97)

(Suite de la page 96)

exposed either to 12L:12D photoperiod or constant darkness (24D) conditions for 30 days, and tissues were sampled throughout 24-h to analyze the daily expression of goldfish Aanat2 (gAanat2) mRNA by RT-PCR. Results: Deduced goldfish AANAT2 protein sequence showed high similarity (around 90%) with other teleost sequences while homology with AANAT1 was lower (around 70%), confirming that the isolated sequence belongs to the gAanat2 gene. The gAanat2 is present in all the tissues studied and display daily expression rhythms in the light/dark cycle, either in both central and peripheral locations. The cosinor analysis indicates that the mesor and amplitude of such daily rhythms damped in pineal, retina and liver under constant darkness. In the liver, the acrophase occurs during the day or the subjective day (under 24D), in opposite to the expression profile in pineal, retina and hindgut where a nocturnal acrophase took place. Conclusions: The positive signal and daily rhythmic expression for AANAT2 transcript in several extrapineal sites support a possible peripheral synthesis of melatonin in goldfish. Besides, our results suggest that the gAanat2 expression rhythms in goldfish digestive tract may be entrained by non-photic cues.

Symposium 10. Melatonin: modes of action (part 1)

Melatonin receptors: new research directions

Chairwoman: Dubocovich ML

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Melatonin transmits photoperiodic information and modulates a number of physiological functions including sleep promotion, circadian phase, vascular function, retina physiology, metabolism, hormone secretion, and reproduction, through activation of MT1 and/or MT2 melatonin receptors. The discovery and development of novel and receptor type specific ligands, the elucidation of the molecular structures and signaling pathways for the receptors, and the development of models with genetic deletions of either the MT1 and/or MT2 melatonin receptors lead to significant advances in our understanding of melatonin receptor function. To mention a few: activation of MT1 melatonin receptors has been shown to phase shift locomotor activity rhythms (Dubocovich M.L., 2007), and regulate photoperiodic responses in mice (Yasuo S. et al, 2009). Furthermore, recent findings also demonstrated the involvement of both MT1 and MT2 melatonin receptors in glucose homeostasis and insulin release with the MT2 melatonin receptor being linked genetically to type 2 diabetes (for references see Mulder H. et al., 2009). Despite recent findings many questions remain unanswered. This symposium will address the molecular, cellular and functional consequences of MT1 and MT2 melatonin receptor activation in conjunction with genetic studies towards elucidating the physiological role of endogenous melatonin. Open questions and discussions leading towards understanding the role of endogenous melatonin in mammalian physiology and the mechanism of melatonin action include a) cellular colocalization of MT1 and/or MT2 melatonin receptors; b) modulation of drug efficacy by melatonin receptor heterodimerization and interaction with signaling molecules and protein complexes; c) modulatory role of endogenous melatonin and its receptors on circadian rhythms; and e) genetic linkage of melatonin receptors with diseases involving alterations of circadian rhythms disruptions. These questions will be addressed in the introduction to the symposium and/or in the presentations by the speakers.

Regulation of the melatonin receptors by associated protein complexes

Jockers R

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Melatonin receptors (MTR) belong to the G protein-coupled receptor (GPCR) super-family and constitute a small subfamily of 3 members in mammals. MT1 and MT2 receptors that bind melatonin with high subnanomolar affinity, and GPR50, an orphan receptor, which does not bind melatonin or any other endogenous ligand.

The function output of MTR is not only regulated at the transcriptional level but also at the gene level due to receptor polymorphisms. Several MT1 and MT2 polymophisms have been detected but their effects on receptor function are not known. MTR function is also regulated at the protein level by its dynamic interaction with receptor-associated protein complexes. Recent results on these two aspects of MTR regulation will be discussed.

Is MTNR1B (MT2) the link between circadian rhythms and glucose homeostasis?

Froguel P, Bonnefond A

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Type 2 diabetes (T2D) in humans has a strong genetic basis, as shown by twin and family studies. Although at the population levels dramatic changes in our environment are main triggers of the obesity/T2D epidemics, only individuals carrying a genetic susceptibility background usually develop T2D. Multiple genes are involved, each having individual modest effect, modulating different traits related to glucose control in interaction with the environment. Genome Wide Associations (GWA) studies using high density DNA polymorphism arrays have recently provided about 30 genes/loci increasing risk for T2D and/or modulating fasting plasma glucose (FPG) values. GWA data from 2,151 non-diabetic (ND) French subjects identified a common genetic variant rs1387153 near MTNR1B, encoding the melatonin receptor 2 (MT2), that modulates FPG. Analyses in several independent European populations confirmed the strong association of the rs1387153-T allele with increased FPG, HbA1c and with decreased insulin secretion. Furthermore the rs1387153-T allele increases the risk of T2D. Refinement of the association signal at the MTNR1B locus displayed that the G-allele of the MTNR1B intronic variant rs10830963 carries most of the haplotype effect on FPG. Furthermore the same allele strongly increases risk of T2D via a state of isolated impaired fasting glucose and not through elevated postprandial glucose levels. RT-PCR in a panel of human tissues confirmed that MTNR1B transcripts are present in the retina, the diencephalon including suprachiasmatic nucleus, and interestingly pancreatic islets and sorted ß cells from human donors, suggesting a possible direct (or indirect) link between the circadian rhythm regulation and insulin secretion. Ongoing studies are trying to identify

(Suite page 98)

(Suite de la page 97)
causative variation increasing T2D risk. Furthermore, direct sequencing of T2D patients found several non synonymous mutations that are currently under functional characterization.

The cerebrospinal fluid, source of melatoninergic physiological signal to the brain: demonstration by brain imaging

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Purpose: In previous experiments we demonstrated the presence of concentration gradients of melatonin (MLT) in the brain, which could result from MLT diffusion from the cerebrospinal fluid (CSF). To investigate this possibility, we studied in real time and in vivo the diffusion of MLT from the CSF into the brain by in vivo scintigraphy and autoradiography. Methods: Sheep received into the 3rd ventricle an injection of 2-[123I]-MLT (0.137±0.02mCi), free [123I] (1.296±1.2mCi) or [123I]-BSA (1.09±0.82mCi, n=3). The disappearance of these 3 molecules was followed with a ?-camera (15min acquisition, 1 image each 30sec). Their distribution within the cerebral tissue (2-[123I]-MLT, n=2 and [123I], n=2) after 10min scintigraphy, was studied by autoradiography (brain slices exposed to PhosphoImager screens). Results: Scintigraphy showed a very short half-life (time for the disappearance of 50% of the injected radioactivity) for MLT in comparison with [123I] and BSA (7.1±2.1, 16.8±2.4 and 129.6±15.2min respectively, $p<0.001$). Autoradiography showed a difference of distribution between MLT and [123I]. The MLT signal was more diffuse around the ventricles than [123I] and mainly in the anterior of the brain, and signal was seen in regions without ventricular structures. The [123I] signal was mainly at the posterior of the brain (aqueduct of Sylvius and 4th ventricle) and restricted to the vicinity of the ventricle wall. Conclusions: Scintigraphy and autoradiography showed that MLT penetrates quickly and efficiently from the CSF into the brain, whereas [123I] stays mainly in the CSF. In addition to our previous results on MLT gradients, these results are evidence that the CSF is the source of the melatoninergic physiological signal to the brain.

Melatonin and pigment dispersing hormone induce circadian time dependent effect in excitability of photoreceptor cells in crayfish: a comparative study

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Purpose: To demonstrate that melatonin and pigment dispersing hormone (PDH) affect, according to the circadian time, the excitability of photoreceptor cells in crayfish Procambarus clarkii. Methods: Intracellular electrical response to light of photoreceptor cells (receptor potential, RP) recorded at CT 8 and CT 20 was obtained from isolated eyestalks from previously adapted crayfish to cycles LD 12:12 and immersed in van Harreveld solution plus melatonin or PDH hormone. Results: At CT 8 both melatonin and PDH produce a diminution on the RP amplitude. Melatonin produces a minor effect with respect to CT 20 (91±2% and 45±5% of its original value, respectively), whereas PDH has a major effect at CT 8 with respect to CT 20 (84.7±0.6% and 94.3±0.6% of its original value, respectively). We found also that both hormones induce qualitative and opposite changes on RP duration: at CT 8, melatonin produces an increase to 121±1% whereas PDH induces a diminution to 85.1±1.1% of its original value; at CT 20 melatonin induces a diminution of 93±1% whereas PDH generates an increase of 116.9±1.0% of its original value. Conclusions: Our results show that melatonin and PDH affect in a complementary and circadian time dependent manner the dynamics of the ionic currents underlying the RP of the photoreceptors cells, which form the first step in the afferent via conducting light information to the pacemaker of the sensitivity to light circadian rhythm. These findings support the proposal that in the generation and synchronization of this rhythm the melatonin indicates the beginning of the photophase, and the PDH the beginning of scotophase.

Supported by DGPA IN200207 grant

Involvement of Gas and Gaq proteins in melatonin-induced prostate epithelial cell antiproliferation

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Purpose: Our laboratory has demonstrated a novel melatonin receptor-mediated antiproliferative signaling pathway, MT1/ PKA+PKC/ p27Kip1, in human prostate epithelial 22Rv1 and RWPE-1 cells. This study was conducted to identify the specific G proteins which mediate the antiproliferative action of melatonin on these cells. Methods: Expression of Gas, Gaq, or Gai proteins in human prostate epithelial 22Rv1 and RWPE-1 cells were knocked down by RNA interference, and the resultant effects on melatonin-induced p27 upregulation and antiproliferation in these cells were measured. The effects of melatonin or 2-iodomelatonin on intracellular cAMP levels in 22Rv1 and RWPE-1 cells were also quantified. Results: Knockdown of either Gas or Gaq, but not Gai expression by specific siRNAs abrogated melatonin-induced p27Kip1 upregulation and antiproliferation in 22Rv1 and RWPE-1 cells. Treatment of 22Rv1 cells with 10-8 M and 10-10 M melatonin in the presence of 10-5 M 3-isobutyl-1-methylxanthine (IBMX) resulted in 3.8- and 2.4-fold increases in intracellular cAMP levels, respectively. Similarly, treatment of 22Rv1 cells with 10-10 M 2-iodomelatonin in the presence of IBMX resulted in a 3.3-fold increase in intracellular cAMP. On the other hand, treatment of RWPE-1 cells with 10-8 M and 10-10 M melatonin in the presence of IBMX resulted in 2.9- and 2.2-fold increases in intracellular cAMP, respectively. Similarly, treatment of RWPE-1 cells with 10-10 M 2-iodomelatonin in the presence of IBMX resulted in a 3.1-fold increase in intracellular cAMP. Conclusions: The results suggested that activation of Gas and Gaq proteins are involved in the signal transduction of melatonin-induced antiproliferation in human prostate epithelial 22Rv1 and RWPE-1 cells. Our data also suggested functional coupling of MT1 receptor to Gas in these cells.

Symposium 11. Melatonin: modes of action (part 2)

Melatonin: multiplicity of actions in neutralizing free radicals and related reactants

(Suite page 99)

(Suite de la page 98)

Chairman: Reiter RJ

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Aim: To the review the radical scavenging actions of melatonin. Summary: Melatonin is a functionally diverse molecule. Membrane receptors for melatonin are uncommonly wide spread. Nuclear binding sites mediate some of the effects of melatonin. Finally, melatonin also has cytosolic actions after its interaction with molecules such as calmodulin. Besides melatonin's actions that involve receptors /binding sites, it also directly interacts with free radicals thereby detoxifying them. This discovery has greatly broadened melatonin's influence throughout the organism. Melatonin's distribution within organisms is not limited by conventional morphophysiological barriers, e.g., placenta, blood-brain barrier, and readily enters all cells. As a radical scavenger, melatonin has been shown to directly neutralize the superoxide anion radical (O2·-), hydrogen peroxide (H2O2), hydroxyl radical (•OH), singlet oxygen (1O2) and the peroxynitrite anion (ONOO-). Of these, the •OH and ONOO- are the most reactive and normally mediate a large percentage of the molecular damage meted out by radicals and radical products. The most reliable means used to confirm the radical scavenging activity of a free radical scavenger is electron resonance spectroscopy (ESR). Using ESR, melatonin has been shown to be equally effective as other antioxidants in scavenging highly toxic radical products; the rate constant for this process is 4.0-7.2 X 1010 M/s. Besides melatonin being a highly effective scavenger, its derivatives are likewise also effective in neutralizing toxic reactants. These by-products of melatonin include cyclic 3-hydroxymelatonin, N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK), N1-acetyl-5-methoxykynuramine (AMK) and perhaps others. The sequential scavenging of radicals by melatonin and its derivatives is referred to as the antioxidant cascade. Using fluorescence microscopy, we have confirmed that melatonin scavenges radicals at the level of mitochondria, a major site of free radical generation. Each of the metabolites formed when melatonin scavenges radicals has been confirmed using carbon and proton nuclear magnetic resonance and have been identified in the urine.

Melatonin binding sites: MT3 is QR2: facts and consequences

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In 2000, we purified an atypical melatonin binding site, described since 1986. This protein was quinone reductase 2. Our current effort on this enzyme is trying to understand the nature of the relationship between melatonin pharmacology and actions and what was known on QR2. Starting from the observations that (1) animals deleted from QR2 gene are not sensitive to menadione toxicity (a known substrate of QR2), and (2) cells are preserved from quinone toxicity either by siRNA deleting the QR2 mRNA or by treatments with two known QR2 inhibition (a potent one, resveratrol and a milder one, melatonin), it came that QR2 was producing ROS deleterious species causing cell toxicity. Indeed, using an electron paramagnetic resonance approach, we demonstrate that even when using natural substrates and cosubstrates, QR2 catalyses one electron reduction leading to the deleterious production of O2?-. This radical oxygen species is known for a long time to produce lethal damages to the cells. In the context of neurology, a little is known on the deleterious effects of an over-expression of this enzyme in brain. The possible explanation of these scattered observations is discussed.

Physiological concentrations of melatonin: what does it mean?

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Purpose: Melatonin, through the duration of its nocturnal secretion, is the primary transducer of photoperiodic information to the reproductive axis in seasonal breeders. Melatonin is released in the blood and it has been frequently assumed that melatonin concentrations throughout the body are the same as in the blood. The purpose of the reported studies was to test this hypothesis for the brain tissue. Results: In addition to the blood, melatonin is also released in the cerebrospinal fluid (CSF) through a specific site in the pineal recess where protruding pinealocytes contact directly the CSF. As a consequence, melatonin is present in much higher concentrations (100 times), in the cerebrospinal fluid (CSF) than in the blood. In addition, CSF melatonin can diffuse in the brain tissue quickly and efficiently and thus can reach periventricular structures in which 2-iodomelatonin binding sites are present, like the pre-mammillary hypothalamus involved in the control of seasonal reproduction. This diffusion of CSF melatonin is supported by the demonstration of a decreasing gradient of melatonin tissue contents from periventricular areas to more distal ones; tissue contents are orders of magnitude higher in some areas than in others and this is the consequence of the presence of melatonin at high concentrations in the CSF. Furthermore, in vivo scintigraphy, following injection of iodo-melatonin in the ventricles and combined with post-mortem autoradiography of brain sections, allowed to determine that melatonin diffuses efficiently from the CSF into the tissue before being eliminated by the CSF flow. Conclusion: These data raise the question of what is a physiological concentration of melatonin since tissue content in some brain areas is at least one-hundred-fold higher than in peripheral structures, and of what are the cellular mechanisms of action of melatonin in the brain.

Endothelial cells (EC) cultures are preconditioned by environmental lighting, melatonin (MEL) and lipopolyssacharide (LPS) treatment

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Purpose: EC are primary targets for circulating substances that regulate immune competent cells transmigration. We have shown that MEL inhibits the rolling and adherence of neutrophils to EC of microvasculature and reduces the constitutive and inducible nitric oxide synthase (NOS) activation. The mounting of an innate immune response is followed by suppression of pineal MEL synthesis. Here we investigate whether the conditioning of EC in vivo interferes on the cultured EC activation state; ie, expression of iNOS, adhesion molecules (ICAM and PECAM), and neutrophil adherence capability. Methods: Male rats (2 month-old, 12/12 h light/dark cycle), injected at ZT16 with LPS

(Suite page 100)

(Suite de la page 99)

(0.5 mg/Kg) or LPS+MEL (3 mg/Kg) and killed at ZT18. Control groups: animals killed at ZT6 (light) and ZT18 (naïve or saline injected at ZT16). EC were cultured according to Tamura et al. 2006; experiments were performed in confluence cells (14 days in vitro). Results: The expression of the adherence molecules (immunefluorescence) and the adherence of neutrophil (in vitro assay) were significantly lower during the dark when compared to the light phase (ICAM 69%, PECAM 68%, adherence 60%). The same profile was observed for iNOS (77%). LPS treatment increased ICAM, PECAM and iNOS expression, as well as, neutrophil adherence when compared to saline group. It was interesting to note that MEL co-administration blocked the priming of EC by LPS in an activated state. Conclusions: Our results are in accordance with the immune-pineal axis hypothesis, which predicts a lower ability of EC to adhere at the dark then at the light phase, and a reduction of LPS-induced effects by MEL. In addition, these results show that cultured EC keep a memory of the hour and the immune state of the donor. Taking into account that EC are being extensively tested for cell therapy, our results disclose the importance of a chronobiological approach in the protocols of cell banks.

Support: FAPESP, CAPES, CNPq

Melatonin stimulates endothelial release of tissue factor pathway inhibitor in vitro

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Purpose: Previous studies have reported decreased nighttime serum levels of melatonin and blunted diurnal variation of Tissue Factor Pathway Inhibitor (TFPI) in males with cervical spinal cord injuries. As TFPI is produced mainly by the endothelium, one may infer that melatonin exerts a regulatory effect on the endothelial production of TFPI. The objective of this in vitro study was to examine if melatonin would modify TFPI, Tissue Factor (TF), and von Willebrand (vW) protein production and TFPI and TF gene expression levels in endothelial cell cultures. Methods: We measured total TFPI, vW and TF protein and gene expressions in Human Umbilical Vein Endothelial Cells (HUVEC) and Aorta Endothelial Cells (AEC) incubated with melatonin at concentrations of 0, 10, 50, 100 and 300 pg/ml for 6 and 24 hours. The concentrations of proteins and gene expression levels were measured with standardized assays. Results: Incubation times of 6 and 24 hours showed similar responses in both HUVEC and AEC (data not shown), hence the data were pooled. Melatonin dose-dependently increased TFPI levels in HUVEC cells ($P<0.01$) from 24 ? 0.7 (median ? range) to 53.9? 5.0. AEC showed a similar pattern in terms of TFPI secretion. Neither TF and vW protein production or gene expression of TFPI and TF showed an apparent pattern of change upon melatonin incubation. Conclusion: Our data indicate that melatonin incubation of endothelial cells in vitro increases TFPI, but not TF or vW protein production. Furthermore we did not observe gene expression changes of either TFPI or TF after melatonin incubation.

Do time of day and genetics influence melatonin pharmacokinetics?

Claustrat B

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When administered orally, melatonin is rapidly metabolized by the liver, the half-life varying between 30-60min in young adults. Also, plasma profiles display dramatic heterogeneity. More than 80% of melatonin is excreted in the urine as 6-sulphatoxymelatonin (aMT6S); the proportion of N-acetylserotonin (NAS) represents 10-20% of administered melatonin. The metabolism of melatonin to aMT6S is catalyzed by cytochrome-P450 (CYP) isozyme CYP1A2 and to NAS by CYP2C19. CYP1A2 accounts for approximately 95% of caffeine metabolism and demonstrates wide variability in enzyme activity between individuals. An A?C substitution at position 734 (CYP1A2*1F) in the CYP1A2 gene decreases enzyme inducibility. Carriers of the variant CYP1A2*1F are “slow” caffeine metabolizers, whereas individuals who are homozygous for the CYP1A2*1A are “rapid” metabolizers. Pre-administration of caffeine results in increase in melatonin concentrations in few subjects. Recent data suggest that melatonin could be advantageous for CYP1A2 phenotyping, compared with the standard probe caffeine. The ratio between urine melatonin and aMT6S excretion determined in endogenous profiles shows significant diurnal variation. Also, AUCs determined after melatonin infusions at different times of day display heterogeneity, with the highest values in the morning.

Genetic and chronopharmacokinetic aspects should be taken in consideration when PRC or clinical trials involving oral melatonin preparations are performed.

Symposium 12. Seasonal timing

Seasonal timing: a little more spice!

Chairman: Morgan P

University of Aberdeen, Rowett Institute of Nutrition and Health, Aberdeen Scotland, UK

Many organisms change their phenotype according to the time of year. This well recognised phenomenon of physiological seasonality is an important adaptive mechanism that ensures synchrony between physiological state and environmental conditions. Of the seasonal responses, change in reproductive status is probably the best studied, but there are many others including energy balance, metabolism, growth and immune status, which represent major physiological axes robustly regulated by photoperiod. There are two central questions: firstly what are the mechanisms involved in decoding photoperiodic information for seasonal timing and secondly how does this information interface with the known pathways regulating physiology. In the light of recent advances in our understanding of photoperiod timing mechanisms in mammals and birds the talk will consider the current status of our understanding and examine the existing gaps in our knowledge. Photoperiodic regulation of energy balance and growth will be used as a primary exemplar, but the talk will also consider how this information may relate to the wider issues of the photoperiodic control of immune function in mammals, photoperiodic control of song in birds as well as other aspects covered in the symposium,

(Suite page 101)

(Suite de la page 100)
thereby serving as an introduction to the symposium overall.

Interactions among steroids, photoperiod and social stimuli in the regulation of the avian song control system

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In parallel with the acquisition of learned vocalizations, songbirds evolved a network of brain nuclei specifically devoted to song learning and production. Several of these nuclei, including HVC and its two main projection targets, exhibit marked seasonal changes in volume that involves, in the case of HVC, changes in neuron numbers. HVC expresses both androgen and estrogen receptors in many songbirds species and there is clear evidence that that this seasonal plasticity can be driven by a direct effect of testosterone (T) or its metabolite estradiol. However, the volume of these forebrain song nuclei can also vary independently of changes in steroid concentrations. For example, HVC volume increases in castrated tree sparrow shifted from short to long days and in castrated, T-treated, white-crowned sparrows or canaries after they are exposed to a female. In blue tits the vernal increase of song control nuclei volumes is also observed before any detectable increase in plasma T. These data indicate that the social environment or the photoperiod can modify the size of song control nuclei in the absence of changes in T concentration. Recent evidence indicates that the act of singing per se also increases song control nuclei volumes. We recently analyzed in canaries the brain expression of doublecortin (DCX), a microtubule-associated protein controlling the polymerization of the leading process and stabilization of the cytoskeleton during neuronal migration. BrdU injections confirmed that DCX labels recently born cells in the canary telencephalon. The number of DCX+ cells in HVC was increased in T-treated males compared to control castrates and in T-treated males paired with a female as compared with T-treated males paired with another male. Thus the endocrine state and social environment of canaries independently affects the expression of DCX, which may be one of the targets by which these factors induce seasonal changes in the volume of song nuclei.

Seasonal changes in vertebrate immune function

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Life represents energetic trade-offs between survival and investment in offspring. At higher latitudes the combined challenge of food shortage and low temperatures makes winter a particularly difficult time to reproduce and survive, and the trade-off is shifted towards investment in survival. Physiological and behavioral adaptations have evolved among nontropical animals to cope with this winter energetic bottleneck. Individuals use short days to determine time of year to shift investment to immune function, a proxy for survival. Field studies indicate that immune function is compromised and prevalence of many diseases is elevated during winter. Individuals should enjoy a survival advantage if seasonally-recurring stressors could be anticipated and countered by shunting energy reserves to bolster immune function. The primary environmental cue that permits physiological anticipation of season is daily photoperiod, a cue that is mediated by melatonin. This talk will review laboratory studies that consistently report enhanced immune function and reduced sickness behaviors, in short day lengths. Prolonged melatonin treatment mimics short days, and also enhances immune function in rodents both in vitro and in vivo. Melatonin appears to be part of an integrative system that coordinates reproductive, immunological, and other physiological processes to cope successfully with energetic stressors during winter. In addition to adult photoperiod, early photoperiodic conditions can organize (i.e., program) important physiological and behavioral survival responses later in adulthood. Our studies use day length, a simple and precise environmental factor, to probe gene expression to understand development of phenotypes. Differences in early day length exposure program adult neuroimmune responses; both peripheral and central inflammation are affected by photoperiod. The influence of photoperiodic influences on inflammatory responses and their effects on disease processes will be reviewed.

Photoperiodism in mammals: what are the long day signals?

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It is well known that the pars tuberalis (PT) of the pituitary in mammals is involved in the seasonal regulation of hormone secretion, including prolactin. The external day length reflected by the nocturnal melatonin signal is translated in the PT into neuroendocrine signals which are yet to be fully characterised. We used a bovine cDNA array to define the molecular events in the PT associated with photoactivation of the endocrine system in sheep. Animals were placed either in short photoperiod (SP, LD 8:16) or 1, 7 or 28 days in long photoperiod (LP, LD 16:8) and culled 3h after the light onset and offset. In response to LP exposure, we observed significant expression of Eya3 in the PT, a gene recently identified as LP-activated in Japanese quail. In situ hybridizations interestingly revealed a biphasic expression of Eya3 in LP in the PT, with a peak at dawn and dusk of the photophase. We also detected an up-regulation in TAC1 expression in LP conditions in the PT. TAC1 codes for tachykinins, including the low molecular weight peptides Substance P and Neurokinin A. Immunohistochemistry in the sheep brain confirmed that both bioactive peptides are expressed in the PT with their receptors (NK1R, NK2R and NK3R) expressed in hormone secreting cells of the pituitary pars distalis. Our results reveal that core mechanisms driving photoperiodism may be conserved across Vertebrates. Eya3 is a key transcription factors involved in photoreceptor differentiation, suggesting this developmental pathway may have been co-opted for photoperiodic time-measurement in the PT. Substance P is known to have prolactin releasing properties in the pituitary, we therefore propose that substance P and/or other tachykinins encoded by TAC1 in the PT could serve as the elusive long day "tuberalin" signal to drive the seasonal activity of the pituitary gland in mammals.

Photoperiod induces morphological reorganization within several hypothalamic regions controlling the reproductive function in the ewe

(Suite page 102)

(Suite de la page 101)

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Cellular events occurring following a change in photoperiod and resulting in the synchronization of seasonal reproduction remain mostly unknown. Purpose: Our study was performed i) to evaluate the scale and functional significance of the morphological reorganizations induced by a switch of photoperiodic treatment from long days (LD) to short days (SD) or the reverse and, ii) to identify neuronal populations undergoing synaptic plasticity. Methods: Variations in PSA-NCAM levels, a plasticity marker, were estimated by Western blot in brain areas, known to be involved in melatonin effects on reproduction and/or for GnRH secretion, dissected at different time-points following a passage from LD (16L: 8D) to SD (8L: 16D) or from SD to LD. Functional significance for seasonal reproduction of the variations in PSA-NCAM levels in the premamillary hypothalamus (PMH) or in the preoptic area (POA) was determined by blocking PSA-NCAM synthesis and measuring LH levels. Variations of association between GnRH neurons and PSA-NCAM or synaptophysin were studied by immunocytochemistry between D0 and D112 after a transition to SD. Results: PSA-NCAM content decreased in most areas 15 days after the transition to SD. In contrast, following a passage to LD, PSA-NCAM levels increased in all areas except in the PMH. Blockade of PSA -NCAM synthesis in the PMH failed to produce a significant effect on LH secretion suggesting compensatory plastic mechanisms. Function of PSA-NCAM changes in the POA is currently studied. Finally, association between GnRH neurons and PSA-NCAM was twice as large on D112 ewes compared to D0 and D60 ewes. Experiments are currently performed to determine whether this variation is associated with a modulation of synapse density. Conclusions: Variations in the duration of melatonin secretion induced by a photoperiodic transition seem able to induce a neuronal plasticity that may be necessary for the reproductive status.

Circannual phase response curves to short and long photoperiod in the European hamster

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Purpose: This study investigated in male European hamsters (Cricetus cricetus) whether entrainment of circannual rhythms follows the principles of the non-parametric entrainment model. Methods: (a) 5 groups of 10 European hamsters were subjected to constant long photoperiod (LP) interrupted by a one-month pulse of short photoperiod (SP) in regular non-365-day intervals which were different for each group. In two further experiments the times of the year when LP or SP are able to synchronize the reproductive cycle were determined, by recording phase response curves (PRCs). 28 groups of 10 hamsters were synchronized by SP, before being subjected to two converse experiments: (b) 14 groups were transferred to constant LP, only interrupted by SP for one month (SP pulse), the pulse being increasingly delayed between groups by two weeks or one month steps; (c) the remaining 14 groups stayed in constant SP interrupted by LP for one month (LP pulse) at different phases of the cycle. The reproductive state was checked every 2-4 weeks. Results: All 50 animals entrained for 2 or more consecutive cycles to the period given by the interval of the SP-pulses (a). The PRCs revealed that a SP pulse had a very strong phase resetting capability of up to 180° and +81° in subjective summer (b). During subjective winter when the animals hibernate, a SP pulse had only weak effectiveness (b) while a LP pulse advanced the circannual clock by up to +41°(c). In the latter conditions a furth er advance of up to +156° was achieved by the decrease in phot operiod at the return to SP conditions, which terminated the reproductive phase already after 4-5 weeks. Conclusions: i) the decrease in photoperiod that occurs naturally after the summer solstice is most important to keep European hamsters in phase with the environment and ii) the resetting of a circannual clock follows the same principles as the resetting of circadian clocks.

Symposium 13. Molecular mechanisms controlling vertebrate seasonal functions

Introduction

Chairman: Ebihara S

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Photoperiodism is an important mechanism for animals to adapt to various seasonal changes. The retina receives seasonal changes in day length and the photic information is processed in the suprachiasmatic nucleus (SCN) and converted to pineal melatonin secretion pattern in mammals, whereas the information is directly received by the deep brain photoreceptors in birds. In mammals, organization of the SCN is altered by long day photoperiod with phase changes of clock gene expression rhythms in multiple SCN regions. However, the photoreceptors mediating day length in avian brain are not yet determined. Recently, the downstream pathway after receiving the photic information began to be disclosed. Day length information of both birds (via the brain photoreceptors) and mammals (via melatonin) reaches the pars tuberalis (PT) of the pituitary gland. Exposure to long days induces thyroid-stimulating hormone (TSH) in PT, which triggers the expression of type 2 iodothyronine deiodinase (DIO2) in the mediobasal hypothalamus (MBH). DIO2 is a thyroid hormone-activating enzyme that converts the prohormone thyroxine (T4) to bioactive triiodothyronine (T3). Induction of DIO2 causes local increases in T3 concentration in the MBH under long-day conditions. These events lead to GnRH secretion in the median eminence and gonadotropins in the pituitary. Recent evidence suggests the kisspeptins neurons are implicated in the regulation of GnRH release. In this sympoium, we will discuss in view of the latest findings concerning molecular mechanisms of photoperiodism and hope to provide new insight into vertebrate seasonal functions.

Molecular mechanisms of seasonal reproduction in birds and mammals

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Purpose: Animals living outside the tropics use changes in photoperiod to adapt to seasonal changes in environment. Local thyroid hormone catabolism within the mediobasal hypothalamus (MBH) by thyroid hormone-activating en-

(Suite page 103)

(Suite de la page 102)
zyme (DIO2) regulates the seasonal reproduction. To address the identity of the photoperiodic signal transduction pathway, functional genomics analysis was performed in Japanese quail. Methods: We have dissected the molecular dynamics of gene expression regulating photoinduced thyroid hormone metabolism using a chicken high-density oligonucleotide microarray. Results: We identified two waves of gene expression. The first was initiated ~14 h after dawn of the first long day and included increased thyrotropin (TSH) ß subunit expression in the pars tuberalis of the pituitary gland; the second occurred ~4 h later and included increased DIO2 expression. TSH receptor was found in the ependymal cells of the MBH and intracerebroventricular administration of TSH to short day quail stimulated gonadal growth, and expression of DIO2. This TSH induced expression of DIO2 was shown to be mediated through a thyrotropin receptor-cAMP signaling pathway by the promoter analysis. We also provide evidence in mice that TSH participates in this photoperiodic signal transduction. Robust photoperiodic response of TSH and DIO2 was observed in melatonin-proficient CBA/N mice. Although these responses were not observed in melatonin-proficient C57BL mice, melatonin administration mimicked the effect of short days. Finally, melatonin administration did not affect the TSH and DIO2 in TSHR-null mice. Conclusions: Increased pars tuberalis TSH therefore appears to trigger long day photoinduced seasonal breeding in birds and mammals.

Kiss1 and rfrp: new hypothalamic genes for the seasonal control of reproduction

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Seasonal control of reproduction is crucial in order for birth and weaning of the offspring to occur at the most favourable time of year. In mammals, synchronisation of reproduction with seasons is controlled by melatonin, a pineal hormone which's secretion depends on photoperiod. However the anatomical substrate and the cellular mechanism through which melatonin modulates sexual activity are far from understood. In the male Syrian hamster, we have demonstrated that expression of two hypothalamic genes, Kiss1 in the arcuate nucleus and rfrp (Arg-Phe-related peptide) in the dorso/ventro medial hypothalamus, are strongly inhibited in short photoperiod by melatonin. Chronic central or peripheral administration of kisspeptin fully reactivates testicular activity in hamsters kept in an inhibitory short photoperiod, indicating that the peptide is crucial for the seasonal regulation of reproduction. However the lack of melatonin receptors in the hamster arcuate nucleus suggests that melatonin regulates Kiss1 expression indirectly. Melatonin receptors have been reported in the mediobasal hypothalamus where rfrp is expressed. Intracerebroventricular injection of RFRP-3 leads to neural activation in the arcuate nucleus and the anterior part of paraventricular nucleus of the thalamus, together with a marked increase in LH/FSH release. Furthermore, chronic central administration of RFRP-3 in short-day hamsters induces an increase in arcuate Kiss1 levels of expression and a moderate reactivation of testicular activity. Taken together, these findings suggest that RFRP neurons in the dorso/ventro medial hypothalamus may be one of the missing links between the seasonal message of melatonin and the kisspeptinergic control of reproduction in the Syrian hamster.

DmpARC neurons are activated by short day photoperiod in the brain of Siberian hamsters

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Purpose: To identify activated neurons in response to short day photoperiod in the seasonal Siberian hamster. Methods: Siberian hamsters were housed in long (LD, 16h light:8h dark) or short (SD, 8h light:16h dark) photoperiod for 12 weeks. In situ hybridization was performed on 14µm sections. Immunohistochemistry was performed on paraformaldehyde perfused brains cut at 40 µM. Electrophysiological recordings were made by the patch clamp technique on 300µm brain slice preparations. The retrograde tracer, pseudorabies virus (PRV) was injected unilaterally into inguinal fat pads. Hamsters were culled 6 days later and processed for immunochemical detection of PRV.

Results: In situ hybridization and immunohistochemistry show c-fos expression (a marker for neuronal activation) is activated in dmpARC neurons in SD. There was no circadian variation in c-fos over 24h and c-fos was not expressed in LD in the dmpARC. Patch clamp recordings demonstrated an increased spontaneous firing rate in dmpARC neurons in SD, accompanied by an increase in membrane resistance. Application of a histamine H3 receptor agonist (imetit) or antagonist (clobenpropit) on brain slices further suppressed or increased respectively spontaneous firing rate. GABA receptor antagonism in LD brain slices mimicked the effects of clobenpropit with an increase in membrane depolarization and increase in resistance and occluded the additional effect of clobenpropit. Immunochemical staining for PRV revealed bilateral dmpARC infected cells 6 days post-injection. Conclusion: DmpARC neurons are activated in SD by a mechanism that involves down-regulation of the H3 receptor and reduced tonic inhibition by GABA. These dmpARC neurons have a CNS pathway to adipose tissue. Together these data provide evidence for a mechanism contributing to the decrease in adiposity in the SD Siberian hamster.

Supported by Scottish Government for Rural and Environment Research and Analysis Directorate

Torpor in mice lacking the melatonin-related receptor, GPR50

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Purpose: Here we detail hypothalamic pathways involved in torpor in a unique model of hypometabolism, the GPR50 knockout mouse. Methods: Metabolic rate and body temperature were monitored in male mice during ad libitum feeding and during 24-48 hr fasting using indirect calorimetry and remote telemetry. Genotype and feeding-state dependent changes in gene expression were assayed using in situ hybridisation and immunohistochemistry. In vivo modulation of the torpor response was achieved using both peripheral injection (e.g. 2-

(Suite page 104)

(Suite de la page 103)
deoxyglucose, 2-DG) and intracereboventricular (icv) injection (e.g. leptin). Results: Mice lacking GPR50 were found to be highly prone to torpor in response to fasting or 2-DG administration. The torpor response in Gpr50-/- mice was not attenuated by central administration of leptin (4µg), but could be blocked by the thyrotrophin releasing hormone (TRH) analogue RX7883 (1µg), suggesting a role for altered thyroid hormone signalling in these mice. GPR50 expression was localised to tanycytes of the hypothalamic 3rd ventricle, cells which are central to thyroid hormone regulation in the brain. Importantly, direct measurement of T3 within the hypothalamus, combined with mRNA expression profiling of deiodinase type 2, MCT8 and TRH, suggest that T3 availability is correlated to the depth of torpor and arousal status of the mice. Conclusions: Using the Gpr50-/- mouse, our studies strongly implicate thyroid hormone availability in the hypothalamus as a key regulator in the expression of torpor.

Gene expression in the suprachiasmatic nuclei and the photoperiodic time measurement

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Purpose: To investigate the role of molecular elements of the circadian timing system in integrating photoperiodic information. Methods: Male Syrian hamsters were exposed to a change from long photoperiod (LP) to short photoperiod (SP). They were killed throughout 24 hours cycles, over the induction period of a winter physiological state (at days 4, 21 and week 8) and following the development of photorefractoriness (week 26). The daily clock and clock-controlled genes expression were measured by in situ hybridization in the suprachiasmatic nuclei (SCN). Results: The modifications in the 24h mRNA profiles induced by the photoperiod reduction occurred rapidly for selected clock genes (Per2, Per3) and more slowly for selected clock-controlled genes (Avp, Vip) – as might be predicted if the SCN drives physiological change. Moreover, all genes analyzed presented a similar daily expression in SP-refractory and in SP groups with the notable exception of Clock. Its expression was high and non-rhythmic in LP, rhythmic in SP, then low and non-rhythmic when animals restored their sexual activity during the SP-refractory physiological state. Conclusions: the SCN integrate new photoperiod quickly in term of clock genes and slowly in term of clock-controlled genes. This difference of delay of integration seems to be the basis of the construction of a new seasonal daily message by the circadian clock. Furthermore, Clock expression is dependent on the photoperiod and on the time duration under short photoperiod. It is thus possible that Clock plays an essential role in the seasonal physiology.

Symposium 14. Links between circadian clocks, clock genes and diseases

Role of clock genes in the regulation of human sleep

Chairwoman: Skene DJ

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The quality and timing of sleep are determined by the interaction of two processes, the circadian timing system (process C) and the sleep homeostat (process S). Clock gene mutations in mouse models have been shown to affect circadian timing (phase and period (?)). As sleep structure and timing are strongly controlled by Process C, individuals with clock gene mutations may have alterations in sleep timing and diurnal preference (morningness or eveningness). A number of clock gene polymorphisms have been shown to be associated with extreme diurnal preference (extreme morningness - PER1, PER2; extreme eveningness – CLOCK, PER3). The circadian rhythm sleep disorders, advanced (ASPS) and delayed (DSPS) sleep phase insomnia, have also been shown to be associated with clock gene polymorphisms (ASPS - PER2 and CSNK1D; DSPS - CLOCK and PER3). Recent research suggests that clock genes may also play a role in sleep homeostasis (Process S). Individuals with the longer allele of PER3 (PER35/5) coped less well with sleep deprivation (reduced cognitive performance) than individuals with the shorter allele (PER34/4) (Viola et al., 2007). Slow wave sleep and EEG slow wave activity in non-REM sleep, theta/alpha activity during wakefulness and REM sleep, were all increased in PER35/5 compared to PER34/4 individuals. This PER3 VNTR polymorphism may thus contribute to the inter-individual differences in performance during sleep loss. The study of clock genotype-phenotype relationships will continue to elucidate multiple roles of circadian clocks in physiological and pathological processes.

Viola AU, Archer SN, James LM, Groeger JA, Lo JCY, Skene DJ, von Schantz M, Dijk DJ (2007). PER3 polymorphism predicts sleep structure and waking performance. Curr. Biol. 17:613-618.

Abnormal synaptic homeostasis in autism spectrum disorders

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Autism spectrum disorders (ASD) affect at least 1/200 individuals and are characterized by impairments in communication skills and social interaction, as well as restricted, repetitive and stereotyped patterns of behavior. Our previous studies pointed at one synaptic pathway, including synaptic cell adhesion molecules (neuroligins and neurexins) and scaffolding proteins (SHANK3) associated with the disorder. These proteins are crucial for synapse formation/maintenance as well as correct balance between GABA and glutamate synaptic currents. In parallel, we could show that mutations within the ASMT gene, encoding the last enzyme of melatonin synthesis, lead to melatonin deficiency in a subset of patients with ASD. Melatonin is known to play a key role in the regulation of circadian rhythms such as sleep-wake cycles and was shown to modulate GABAergic currents, as well as neurite and memory formation in different animal models such as fish, birds, and mammals.

Based on these results, we propose that, in some cases, ASD could be the consequence of an alteration in the homeostasis of the synaptic currents in specific regions of the brain. Indeed, abnormal synaptic proteins could lead to an imbalance of excitatory/inhibitory currents. This imbalance could be revealed or amplified by an alteration of the circadian rhythms. Consistent with this hypothesis, a better characterization of the interplay between synaptic and clock genes may shed light on several features that are atypical in ASD such as sleep and memory formation.

Meta-analysis of genome-wide association data on

(Suite page 105)

(Suite de la page 104)

the circadian and sleep phenotypes of 7 European populations

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Purpose: To identify genes associated with circadian and sleep phenotypes using human population genetics studies. Methods: We have conducted genome-wide association studies with circadian and sleep phenotypes in 7 European populations. Assessments of sleep duration, mid-sleep-phase (chronotype), sleep inertia, and sleep latency were conducted using the Munich ChronoType Questionnaire (MCTQ). Genome-wide genotyping was based on the Illumina and Perlegen platforms for circa 317,000 and 600,000 single nucleotide polymorphisms (SNPs), respectively. Independent association analyses were conducted for every population per trait, and meta-analyses aggregated the phene-gene association results from more than 4,000 subjects. Results: We found significant association signals at the genome-wide level ($P < 0.0000001$) for independent populations, and in the joint analysis for individual traits. Conclusions: Our results indicate that the investigated traits have a strong genetic component, and that genes (beyond the known clock and sleep candidate genes) play a role in the molecular mechanisms modulating these phenotypes.

A role for REV-ERBa in pulmonary inflammation

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Purpose: To investigate the role of the clock protein REV-ERBa in the pulmonary inflammatory response. Methods: A rev-erb a gene knockout mouse was utilised in an in vivo model to test the innate pulmonary inflammatory response. Mice were exposed to increasing doses of aerosolised lipopolysaccharide (LPS; 0-2mg/ml in saline) for 20 min. 5h later the lungs were lavaged and lung tissue was harvested. Cytokine and chemokine release into bronchoalveolar lavage (BAL) fluid was determined (bead arrays), inflammatory cell recruitment to the lungs quantified (flow cytometry using BAL), and histological analysis undertaken. In further experiments macrophages were cultured from bone marrow, and challenged with LPS in the presence or absence of a REV-ERBa ligand. Results: In mice, loss of rev-erb a resulted in a significantly enhanced inflammatory response to LPS challenge: KC, MIP -2 and TNF alpha showed highly elevated levels in the lungs which correlated with a highly significant increase in levels of neutrophil recruitment (key cell types involved in the pathology of chronic obstructive pulmonary disease). Application of a novel small molecule ligand REV-ERBa (which enhances activity of REV-ERBa) significantly reduced LPS induced cytokine production in macrophages. Conclusions: REV-ERBa plays an important role in the pulmonary innate immune response. Whether this altered immunological response is a consequence of a disrupted circadian clockwork, or a more general pleiotropic effects of the gene, is currently under investigation.

Differential involvement of the mPer1 gene in the development of morphine dependence

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Purpose: Increasing evidence for a link between clock genes and addiction arises in the literature. We conducted the present study in order to better investigate the implication of the clock gene mPer1 in different morphine-induced behaviours reflecting the development of dependence towards that drug of abuse. Methods: Therefore, we assessed morphine-induced tolerance and withdrawal, as well as behaviour sensitization and conditioned place-preference in Per1Brdm1 mutant mice and their respective wild-type littermates. Tolerance towards the analgesic effect of morphine was measured using the tail-immersion and the hot-plate tests. Physical signs of withdrawal were evaluated following naloxone challenge (1 mg/kg, i.p.) after a chronic treatment of increasing doses of morphine (20, 40, 60, 80, 100 mg/kg; i.p.; twice a day, for 6 days). The development and expression of the behaviour sensitization to morphine was tested measuring the open-field morphine-induced (20 mg/kg, i.p.) locomotion, for 1h, once every two days for 6 sessions, and one session 7 days later (for the expression). The reinforcement properties of morphine were assessed successively using the conditioned-place preference test. Results: Although the Per1Brdm1 mutant mice did not show any significant difference in tolerance to the analgesic effects of morphine nor in the physical withdrawal signs compared to their controls, they showed a clear impairment in the development and expression of morphine-induced behaviour sensitization, as well as in conditioned-place preference. Conclusions: The present results reveal the selective implication of the mPer1 gene in the development of behaviours reflecting different neurobiological adaptations leading to morphine dependence

Symposium 15. Circadian rhythm disorders: from sleep problems to psychiatric troubles

Introduction

Chairwoman: Wirz-Justice A

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

Abstract not provided

(Suite page 106)

(Suite de la page 105)

Psychiatric co-morbidities in delayed sleep phase disorder and evening types

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Purpose: Psychiatric co-morbidities have been reported to be common among patients with Delayed Sleep Phase Disorder (DSPD). In this study, structured clinical interviews were used to define the types of psychiatric disorders and the prevalence of psychiatric co-morbidities in DSPD, evening-types and intermediate-types. Methods: Forty-eight DSPD (35 ± 11.4 yrs, 27 female), 25 evening-types only (34.2 ± 11.8 yrs, 12 female) and 23 intermediate-type control participants (34.1 ± 15.9 yrs, 10 female) as determined by the Horne-Ostberg questionnaire wore wrist activity monitors for several weeks and completed a daily sleep log and questionnaires including the Pittsburgh Sleep Quality Index (PSQI), Epworth Sleepiness Scale (ESS) and the Functional Outcomes of Sleep Questionnaire (FOSQ). All participants completed a Structured Clinical Interview (SCID) for DSM-IV Disorders. Groups DSPD, evening-type only, evening-types (DSPD + evening-type only), and intermediate-types were compared using χ^2 and Fisher exact tests. Results: Evening-types (DSPD and evening types only) were significantly more likely to have a history of an Axis-I diagnosis than intermediate-types (p=0.029). There was no significant difference between the DPSD and evening-type only group. Both DSPD and evening-type only groups were significantly more likely to be have a history of an anxiety disorder (p=0.026) by history than intermediate-types. Both DSPD and evening-types were significantly more likely to be diagnosed with major depressive disorder and a specific phobia by history than intermediate-types. Conclusion: These results indicate that having an evening-type circadian preference, and not necessarily having DSPD, increases the likelihood of having anxiety or depressive disorders compared to those with a neutral circadian preference. These results highlight the link between the circadian system and mental health. Recognition of the increased risk of psychiatric disorders in individuals with specific chronotypes is important for the effective management of not only circadian rhythm sleep disorders, but also psychiatric disorders.

Molecular interaction between circadian rhythm and mood disorders

Takumi T

Hiroshima University

Our biological clock counts daily rhythms with approximately 24 hours called circadian rhythm in our body. The mammalian circadian system consists of three components: input, pacemaker, and output. Almost all physiological phenomena including mental states, in addition to sleep-wake cycles, can be considered as circadian outputs. The recent molecular advances revealed that molecular clocks were located not only in the central oscillator, suprachiasmatic nuclei (SCN), but also in peripheral tissues, even in cultured cells. We established both in vivo and in vitro rhythm monitoring system. To understand molecular interaction between circadian rhythm and depression or mood disorders, we investigated circadian rhythm of the learned helplessness (LH) rat, an animal model of depression, at the behavioral and cellular level. The locomotor activity rhythm in vivo and circadian transcriptional rhythm in vitro seemed to be correlated with each other. The phosphorylated glycogen synthase kinase–3? (pGSK-3?) was likely to be the key molecule that connects behavioral rhythm with cellular ones. Clock genes were included in the downstream targets of GSK3??? The phenotypes including circadian rhythm in fibroblasts correlate to those in vivo, suggesting that the fibroblasts from the patients can be used as a diagnostic material and a therapeutic tool.

Impact of bright light therapy as a possible countermeasure against disruption of the rest-activity rhythm induced by general propofol anesthesia : preliminary results

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We previously demonstrated that propofol anesthesia was associated with a desynchronization of the daily rest-activity rhythm in both animal models and ambulatory patients during at least the 48h following anesthesia. Purpose: To test bright light as a therapy measure to diminish the disruption of the rest-activity rhythm induced by general anesthesia in ambulatory patients. Methods: Nineteen healthy patients scheduled for ambulatory colonoscopy were included in this randomized prospective study. Rest-activity rhythm was assessed using wrist actigraphy during the weeks before and after anesthesia. At recovery from anesthesia, patients were exposed either to normal light (n= 10, placebo group) or to bright light (n=9, treatment group) during the first 90 minutes. Results: The phase shift of the acrophase of the rest-activity rhythm observed in patients the days following anesthesia was significantly reduced in patients exposed to bright light (22 min) as compared to patients exposed to normal light conditions (57 min). The decrease of the Interdaily Stability Index (represents the strength of the coupling rate to external synchronizers supposed stable) the days following anesthesia was no more observed in patients exposed to bright light as compared to normal light conditions. Conclusions: Our first results showed that the rest activity disorders usually observed during the 72 h following anesthesia were largely amended in patients exposed to bright light conditions as compared to the group of patients exposed to normal light.

A new integrated index, based on thermometry, actimetry and body position (TAP) to evaluate circadian system status in human

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Purpose: Disruption of human circadian system has been associated with the development of chronic illnesses and the impairment of pre-existing pathologies. Therefore, the assessment of human circadian system functioning under free-style living conditions using non-invasive techniques

(Suite page 107)

(Suite de la page 106)

becomes a current issue that needs improvement. Traditionally, overt rhythms such as activity and temperature have been separately monitored; however, a comprehensive index could reduce individual recording artefacts. Thus, a new integrated index (TAP) for the circadian system status based in three simultaneous recordings: skin wrist temperature (T), locomotor activity (A) and body position (P), has been developed. Methods: An actimeter (Hobo® Pendant G) and a temperature sensor (ThermoChron®, I-button) were placed in the arm and wrist of the non dominant hand, respectively, during a week in 37 healthy volunteers. Each activity or temperature value per subject was scored between 0 and 1, where 0 corresponds to very high temperature, very low activity and horizontal position and 1 corresponds to very low temperature, very high activity and vertical position. Results: Each variable, wrist temperature, activity, position or TAP index was independently correlated with the sleep diary recordings. The highest correlation, together with higher specificity and sensitivity, was obtained when sleep recordings were compared with TAP. Conclusion: Our results demonstrate that TAP index improves the accuracy to differentiate activity from rest when compared to isolated temperature or activity recordings.

Acknowledgements: Seneca Foundation (PI/05700/07), the Instituto de Salud Carlos III (RETICEF, RD06/0013/0019), the Ministry of Education and Science (BFU2007-60658/BFI) to JAM and to the University of Murcia for the research fellowship to AMN.

Beyond blue light: from circadian photoreception to light therapies

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Purpose: Non-visual effects of light involve rods, cones and melanopsin-expressing ganglion cells in animals but the relative contribution of classical and non-classical photoreceptors is unknown. The goal of the study is to clarify the mechanisms involved in visual and non-visual functions in humans. Methods: Sensitivity of light-induced melatonin suppression was assessed in healthy young males and females. In a within-subject design, each subject was exposed to monochromatic lights of equal photon density (3.16x1012 photons/cm2/sec) at 9 different wavelengths spread over the visual spectrum (420–620 nm) as well as to combinations of monochromatic lights. Blood samples were collected every 15-60 min before, during, and after a 60-min nocturnal light exposure session in subjects with fully dilated pupils. Results: Our results confirm a peak sensitivity of melatonin suppression to wavelengths between 460-480 nm, whereas wavelengths below 460 and above 500 nm are much less effective. Combinations of monochromatic lights suggest that mechanisms involved in human circadian photoreception involve melanopsin ganglion cells. Conclusions: Our results confirm the peak sensitivity of the circadian timing system to mid-wavelength lights (460-480 nm). They also show that the effects of light on non-visual functions can be modulated by manipulation of prior light history. Our findings are relevant to for the optimization of current clinical photic strategies used for the treatment of chronobiological and affective disorders.

Supported by Cible Rhône-Alpes, FP6-EUCLOCK

Symposium 16. Ageing and the circadian system

Validation of animal-model circadian-system changes in aging for human brain disorders

Chairman: Swaab DF

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Animal models show circadian alterations in aging. A major question is how to validate the relevance of animal experimental data for human disorders. We study for this purpose postmortem samples from the Netherlands Brain Bank. The function of the human circadian system appeared indeed to be affected in aging, and in age-related disorders, such as Alzheimer's disease (AD) and depression. From the earliest AD stage onwards a functional disconnection was found between the suprachiasmatic nucleus (SCN) and the pineal, which accounts for pineal clock gene changes and circadian rhythm disturbances in AD (Wu et al., FASEB J. 20, E1171, 2006). Compared to young controls, the number and density of AVP/VIP-expressing neurons in the SCN did not change in the aged, but the number and density of malatonine receptor (MT)1-expressing neurons in the SCN significantly decreased. Moreover, both MT1-expressing neurons and AVP/VIP expressing neurons were strongly diminished in the last stages of AD (Braak V–VI), but not in the earliest stages (Braak I–II), compared to aged controls (Braak stage 0). MT1-mediated effects of melatonin on the SCN may thus be disturbed during aging and even more so in late stage AD (Wu et al., Neurob. of Aging 28, 1239, 2007. In depression, circadian rhythm disturbances are generally present. In addition, polymorphisms in clock genes are risk factors for depression. Administration of melatonin has been proposed as a potential therapy. In depressed patients the density and numbers of MT1- and AVP/VIP-expressing neurons was increased in the SCN. Moreover, the number and density of MT1-expressing neurons was negatively correlated with the age of onset of depression, while they showed a positive correlation with the duration of depression, indicating a relationship with the disease process. Recently we found that the MT2 receptors did not change in the same patients. Our results suggest the presence of an increasing number of MT1 receptors in the SCN of depressed patients during the course of disease.

Effects of the aging biological clock and treatment in demented eldery

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Brain areas involved in sleep and biological rhythm regulation are sensitive to light, the evolutionary oldest cyclically varying physical aspect the environment. Hypofunction of these areas is involved in nocturnal restlessness in demented elderly – a primary risk factor for transfer to a nursing home. In an attempt to reactivate the biological clock 189 demented elderly were treated and tracked for up to 3 ½ years in the first ever long-term multicenter randomized clinical trial on the effect of light. Published recently (JAMA 2008; 299:2642-55) the results are striking,

(Suite page 108)

(Suite de la page 107)

with great societal consequences. Increasing the amount of light reduced nocturnal restlessness by 9% per year; cognitive impairments by 5%, depressive symptoms by 19% and the deterioration in functional abilities (activities of daily living) by 53%. Without a doubt these effects can compete with the effects of individual symptomatic pharmacological treatments for each of these disorders, but without the side effects. Indeed, everyday health problems were less in the active light condition. Ongoing follow-up brain imaging studies on the mechanisms involved in the effect of better sleep on memory function suggest a specific sensitivity of the medial temporal lobe, including the hippocampus, to even mild disruption of sleep and sleep-wake patterns (Nat Neurosci 2009; 12:122-3; J Sleep Res 2009; 18:129-35). The results have great societal relevance. Implementation of high intensity environmental light in care centers is a relatively inexpensive measure that will lead to an improvement in the quality of life of many elderly people and a decrease of the burden of care for many careers.

The circadian system: a biomarker of aging and a potential target for anti-aging actions

Aujard F

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Purpose: Calorie restriction (CR) is the only anti-aging protocol which increases life span while delaying the onset of age-related diseases in short lived animal species. Its action on longevity involves the stimulation of sirtuin pathway. Since recent data showed that sirtuins are also involved in the functioning of the circadian system, we tested whether sirtuin activating anti-aging protocols improved the expression of circadian rhythms parameters validated as biomarkers of aging. Methods: We studied the circadian rhythms of locomotor activity (LA) and body temperature (Tb) in adult and aged mouse lemurs (nocturnal non human primates) fed with either a moderate CR (-30% of control diet) or a control diet supplemented with a sirtuin activator, potential mimetic of CR, the resveratrol (RES). Data were recorded by telemetry under LD 14/10 and under constant dim red light. Rhythms parameters were first tested for their evolution with age and molecular markers of the aging process. Results: Compared to adults, aged mouse lemurs showed a high percentage of diurnal activity, a phase advance of the activity onset, a short free-running period and a delayed occurrence of minimal Tb. The magnitude of these disturbances was correlated to the age-associated increase in interferon-?, a cytokine marker of chronic low-grade inflammatory state associated to aging. CR animals exhibited immediate but transient phase advance of the LA onset and increased amplitude in Tb daily variation. By contrast, RES diet improved synchronisation of LA onset and increased nocturnal activity, particularly in aged individuals. Conclusions: The observed transient effect of CR on circadian rhythms expression suggests a primary modulation of Tb and LA variations in relation to energetic regulation. RES diet, devoid of energetic constraint, improved rhythms expression. Sirtuins, as a link between metabolism and circadian rhythms, may be a good target for specific anti-aging actions on the circadian system.

Grants: FRM, ANR PNRA

Aging affects neural regulation of clock

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Aging is characterized by progressive decline in all physiological functions. The age-related sleep disturbances have been attributed to disturbances of circadian function. Neurotransmitter serotonin plays important role in the photic and non-photic regulation of circadian rhythms and is a precursor of melatonin, an internal zeitgeber. To understand the age induced changes in the functional integrity of circadian system, we studied daily serotonin rhythms in brain and SCN by measuring serotonin levels at variable time points in wide range of age groups such as 15 days, 1, 2, 3 (adult), 4, 6 and 9 months, 1, 1 ½ and 2 years old male Wistar rats. Animals were maintained under light-dark conditions (LD 12:12), two weeks prior to experiment. We report here that mean serotonin levels over 24 hour period in brain is highest at 3 months and daily serotonin rhythmicity reliably begins at 3 months and disintegrates at middle age and beyond. As the metabolome is the most predictive of phenotype, the changes in the metabolome are the ultimate answer of an organism to genetic alterations, disease, or environmental influences, we further extended to study age related changes in other serotonin related compounds such as tryptophan, 5-hydroxytryptophan, 5-hydroxytryptophol, 5-methoxytryptophol, N-acetyl serotonin, melatonin, 5-methoxy indole acetic acid and N-acetyl tryptamine. In addition effect of melatonin treatment on age induced changes in daily rhythms in serotonin and its related compounds, NAT and CaMKII activity rhythms and c-Fos levels in SCN were studied in SCN. The age induced changes obtained in present study will help in targeting novel treatments for age induced disorders of circadian function by identification of biomarkers of aging.

Human molecular circadian rhythms and ageing

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Purpose: Validation of an in vitro approach to study the human circadian rhythms and investigation of the effect of ageing on the circadian disturbances. Methods: In vitro circadian rhythms was obtained by infecting skin fibroblasts obtained from the different donors (10 sighted, 8 blind, 18 young and 18 older) with a lentivirus mBmal-1::luc. Measurements were conducted under standard experimental conditions (medium with bovine serum) or in human serum-containing medium. In vivo circadian period length was additionally measured in sighted and blind subjects. Results: A good correlation was observed between in vitro and in vivo period length of both sighted and blind people. Between young and older sex-matched subjects no change of the circadian period length of fibroblasts was found under standard experimental conditions. Of note, when human fibroblasts were measured in human serum

(Suite page 109)

(Suite de la page 108)
containing medium a reduction in period length was found only for cells treated with “older” serum. Heat-inactivation of sera from older donors almost undid the reduction in the circadian period length suggesting that protein/s is/are responsible for the circadian disturbances observed in the elderly. Conclusions: Fibroblasts are a good model to study circadian rhythms in vitro. The interplay between the molecular components of the skin fibroblasts oscillators does not change during ageing in general. However, the age-related changes in circadian rhythms in vivo and/or in vitro are possibly related to still unkown protein/s present in the serum.

Coordinated rhythmic retinal phagocytosis is lost during ageing in humans

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Purpose: To quantify retinal phagocytosis in humans. Methods: We counted phagosomes in electron micrographs prepared from post-mortem human retinas as a function of i) donor age, ii) time of day, iii) normal or age-related macular degeneration (AMD), and iv) macula or periphery. Phagosomes were identified as membrane-bound, disc-containing organelles within retinal pigment epithelium (RPE). Tissue was obtained from the Lions Eye Bank, from informed consenting donors according to ethical procedures established by the Univ. Iowa. 1000 donor eyes were used in the current study. Results: Phagosome presence showed a distinct clustering around late morning in donor retinas up to 49 years of age. By contrast, phagosome presence was random in retinas 50-99 years of age. As a function of age, the relative number of RPE containing phagosomes (+ve RPE) decreased, whereas the number of phagosomes per RPE was roughly constant (but highly variable). Phagocytic activity (+ve RPE x phagosome numbers) decreased steadily with increasing age. Macular phagocytic activity was higher than peripheral retinal activity at all ages. Phagocytic activity did not differ between normal and AMD retinas. Conclusions: Synchronised phagosome formation occurs in humans under 50 years, but is not seen in persons over 50 years. Phagocytic activity gradually declines with age, consistent with theories of decreasing RPE efficiency.

Symposium 17. Clocks and sleep

Joint workshop between the European Biological Rhythms Society and the European Sleep Research Society

Chairman: Bassetti C (Switzerland)

Circadian clock genes and sleep homeostasis

Franken P

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Purpose: Circadian and sleep-homeostatic processes both contribute to sleep timing and sleep structure. Elimination of circadian rhythms through lesions of the suprachiasmatic nuclei (SCN), the master circadian pacemaker, leads to fragmentation of wakefulness and sleep but does not eliminate the homeostatic response to sleep loss as indexed by the increase in EEG delta power. In humans, EEG delta power declines during sleep episodes nearly independently of circadian phase. Such observations have contributed to the prevailing notion that circadian and homeostatic processes are separate but recent data imply that this segregation may not extend to the molecular level. Results: Studies in mice with targeted disruption for core circadian clock genes have revealed alterations in circadian rhythmicity as well as changes in sleep duration, sleep structure and EEG delta power. Clock-gene expression in brain areas outside the SCN, in particular the cerebral cortex, depends to a large extent on prior sleep-wake history. Evidence for effects of clock genes on sleep homeostasis has also been obtained in Drosophila and humans, pointing to a phylogenetically preserved pathway. Conclusion: These findings suggest that, while within the SCN clock genes are utilized to set internal time-of-day, in the forebrain the same feedback circuitry may be utilized to track time spent awake and asleep.

The human circadian timing system and sleep-wake regulation

Cajochen C

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A remarkably tight association between the endogenous circadian rhythms of melatonin and sleep propensity has been described in humans. Besides circadian rhythmicity also sleep homeostasis contributes to sleep timing and sleep structure in humans. The circadian process and the sleep homeostat interact to consolidate the sleep-wake cycle. The circadian process generates a sleep-wake propensity rhythm that is timed to oppose homeostatic changes in sleep drive. In humans, circadian sleep propensity reaches its nadir just prior bedtime and its crest just prior wake time, in opposition to the wake-dependent increase and sleep-dependent dissipation of sleep drive. The fine-tuned interaction between circadian rhythmicity and sleep homeostasis implies that minor changes in either of these processes can contribute significantly to normal and pathological variation in sleep timing and duration. In fact, humans routinely cognitively “override” their internal circadian clock so that the timing of sleep and wake can be scheduled to meet the personal demands of their social and work schedules. In addition to this voluntary disruption of the normal temporal organization between the sleep and circadian clock systems, such disorganization can occur on an “involuntary” basis, such as in people with delayed and advanced sleep-wake syndrome or even more severe in patients suffering from depression and schizophrenia. However, any disorder of the human circadian system can result in circadian misalignment, which itself causes sleep disturbances, reduced attention, impaired daytime alertness, lack of energy, memory problems, negative mood and gastrointestinal disorders. Some of these syndromes and diseases mentioned above have been associated with alterations in genes, which are canonically implicated in the regulation of the circadian clock. Interestingly, current research in animals and humans indicate that some clock genes are also involved in the regulation of sleep homeostatic processes. On the other hand, SCN activity also directly depends on sleep structure; such that high EEG slow-wave activity decreases SCN multiunit activity- thus sleep per se impacts on the circadian clock. These data indicate that the sleep and circadian systems are interrelated even more closely than was previously recognized and that the interactions

(Suite page 110)

(Suite de la page 109)
between these systems are bidirectional.

Melatonin, sleep and sleep disturbance

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Melatonin is a time cue and soporific agent in humans. Brain correlates of melatonin sleep induction were studied in randomized, double-blind, placebo controlled functional magnetic resonance imaging studies. Reduced task related activations by melatonin but not placebo were found in discrete networks that correlated with subjective measurements of fatigue. Increased task related activity was found in the parahippocampus. These effects resembled actual sleep although subjects are fully awake. Similar changes were seen in the evening in subjects who by that time produced melatonin endogenously. The endogenous hormone obviated most effects of the exogenous hormone. Insomnia prevalence increases with age. Melatonin production declines with age, presumably due to pineal calcification. The degree of pineal calcification correlates with poor sleep in insomnia patients. Prolonged release melatonin (PRM; Circadin® 2 mg, Neurim Pharmaceuticals) has been shown effective for insomnia in patients aged >55. Because melatonin production declines with age it was pertinent to ask whether PRM efficacy is related to patient's endogenous melatonin or age and whether efficacy is maintained over long term (6 months) periods. Preliminary results from a large placebo controlled trial in insomnia patients aged 20-80 indicate that PRM is effective in >55 and older patients across multiple domains (e.g. sleep latency, quality of sleep, quality of life and in the long term period overall clinical status of patients, morning alertness and daytime functioning) and the effects are maintained and even improved during long-term treatment. Age is thus a better predictor of a relative melatonin deficiency (probably due to pineal calcification) and subsequently responsiveness to PRM than blood melatonin or urinary 6SMT levels. Our findings highlight the role of melatonin in priming sleep-associated brain activation patterns in anticipation of sleep. They further support a significant role of melatonin in sleep/wake regulation particularly in older patients.

Sleep, circadian timing and cardiovascular functioning

Scheer FAJL

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Purpose: The risk for adverse cardiovascular incidents peaks in the morning (between 6 and 12 h) and is increased in shift workers. We set out to determine the potential involvement of the endogenous circadian system and its interaction with the sleep/wake cycle (including the behavioral and fasting/feeding cycle) in these health issues. Methods: Hereto, we utilized Forced Desynchrony protocols (20-h or 28-h sleep/wake cycles), which enable the assessment of the independent influences of the sleep/wake cycle, the circadian cycle and their interaction. Results: First, in healthy subjects we demonstrated significant circadian rhythms in many hemodynamic, autonomic and hemostatic biomarkers, with a circadian peak or rise at a time equivalent to ~6-12 h for several autonomic and hemostatic markers. These effects were mainly additive to the effects of standardized mental, postural and exercise stressors to which the subjects were exposed at different circadian phases. Second, in healthy subjects we demonstrated that circadian misalignment—chronically experienced by shift workers—caused a systematic decrease of leptin, increase in plasma glucose, reversal of the sleep/wake cortisol and melatonin rhythms, increase in mean arterial pressure and reduction in sleep efficiency. Conclusions: First, these data suggest that the endogenous circadian system contributes to the morning peak in adverse cardiovascular events. Future studies are necessary to determine whether vulnerable populations show different circadian timing and/or amplitude of these biomarkers. Second, the findings on the effects of circadian misalignment on cardiometabolic risk markers, if borne out in longer term studies in an operational setting, could provide a physiological explanation for the increased risk for obesity, diabetes and cardiovascular disease in shift workers.

Symposium 18. The young researcher awards

Chairmen: Korf HF (Germany) & Foster RG (UK)

Young Investigator Award of the Japanase Society for Chronobiology.

Circadian output in the suprachiasmatic nucleus

Nakamura W

Laboratory of Oral Chronobiology, Osaka University Graduate School of Dentistry, Osaka, Japan

Purpose: In mammals, the neural output of suprachiasmatic nucleus (SCN) is essential for the circadian regulation of behavioral activity. We have studied the circadian property of SCN neurons from individual neurons to system level. Methods: We assessed circadian firing rhythms of SCN neurons on the recording electrodes of a multielectrode dish in both dispersed cell culture and slice culture. By using two culture methods, the significance of intercellular coupling among clock cells can be examined. For system level analysis, we have developed the technique of long-term recording of multiunit neural activity (MUA) in freely moving mice. That enabled us the direct monitoring of circadian clock comparing environmental input and behavioral output. Results: By using Clock mutant mice, we found that genetic perturbation affected autonomous circadian oscillation in the SCN neurons and inter-cellular coupling compensated for Clock deficiency. In system level analysis, two components of neural activity were observed transiently in the SCN when mice were forced desynchrony between environmental input and behavioral output by abrupt phase shift of light cycle. Clock mutant SCN did not show the dissociated two components. The kinetics of synchronization of locomotor activity was totally parallel to MUA in the SCN. Conclusions: Functional inter-cellular coupling within the SCN is important in the mechanism for sustaining robust circadian rhythms and entraining to environmental condition. Neural output of the SCN is totally parallel to behavioral circadian rhythm.

Young Investigator Award of the European Biological Rhythms Society

(Suite page 111)

(Suite de la page 110)

Tanycytes: the interface between photoperiod and the hypothalamus

Herwig A, Nilaweera K, Morgan PJ, Mercer JG, Barrett P

University of Aberdeen, UK

Purpose: To understand the role of hypothalamic tanycytes in photoperiodic regulation of physiology in the Siberian hamster (Phodopus sungorus). Methods: a) Adult Siberian hamsters were kept in long days (LD) or short days (SD) for 14 weeks. The hypothalamic tanycyte layer was dissected by laser capture microdissection. Differential gene expression was analyzed on micorarrays. b) Juvenile animals were transferred to LD or SD after weaning and culled after 1, 2, 8, 16 or 32 days. c) Adult hamsters were kept in LD or SD for 8 weeks before being starved for 48 hours. In situ hybridisations were performed on all experiments. Results: a) Microarray analysis identified differentially expressed genes involved in glucose metabolism. Expression of these genes increased in tanycytes of SD adult hamsters. These data lead to further identification of differential expression for genes involved in glycolysis and fatty acid synthesis. b) The importance of these genes and those involved in thyroid hormone metabolism, type 2 (D2, T3 synthesis) and type 3 (D3, T3 catabolism) deiodinases to the photoperiodic response was assessed in juvenile hamsters. In hamsters raised in LD or SD from weaning, body weight significantly differed by day 32. Expression of genes for glycolysis and fatty acid synthesis was augmented after 16 days in SD. D2 increased and D3 decreased by day 16 in SD. c) At 8 weeks in SD, adult hamsters show D2 to be decreased and D3 to be decreased. In addition the T4/T3 transporter, MCT8 was found to be increased in SD hamsters. Starvation reversed the direction of gene expression change for D2, D3 and MCT8 induced by SD. Conclusions: Tanycytes respond to altered photoperiod with multiple gene expression changes associated with thyroid hormone and glucose metabolism. Increase in glucose metabolic enzymes suggested increased activity in tanycytes. The early responses to photoperiod of these components in juvenile hamsters suggest tanycytes are important to the seasonal physiological response.

Young Investigator Award of the French speaking Society of Chronobiology

Food (reward) signaling in the circadian system

Mendoza J

Dept of Neurobiology of Rhythms, Institute of Cellular and Integrative Neuroscience, CNRS UPR3212, University of Strasbourg, France

Peripheral clocks can be entrained by various stimuli, food is the major synchronizer. The central suprachiasmatic (SCN) clock is principally entrained by an external cue: light. The SCN, however, also can receive and be affected by food signals under special feeding conditions. In rodents that are given a restricted food regimen where food is available with hypocaloric content during the day, the phase of behavioural, physiological and molecular rhythms is shifted. What signals arising from feeding affect the SCN clock? A hungry animal (during food restriction) is motivated to obtain food. Sensory inputs from the gut, changes in circulating hormones (e.g., insulin, leptin, ghrelin), or circulating nutrients (e.g., glucose, free fatty acids), as well as intracellular metabolites, signal "hunger" or "satiety," and thus could affect the SCN. However, the reward aspects of food by itself seem to be important in the SCN entrainment by food. Palatable diets exposure on a background of free feeding (ad libitum) of a regular diet can entrain circadian rhythms of the SCN. Moreover, light synchronization of the clock could be affected in animals under the regimen of palatable or reward diets. The classical dopaminergic and serotonergic signalling pathways that influence feeding and energy metabolism, can modulate the SCN entrainment to timed reward diets. In addition, the orexinergic system in the lateral hypothalamus can also act as an input to the SCN, involved in communicating non-photic (food) signals and in particular those with a higher arousal or reward background. Therefore, these central signalling systems (dopaminergic, serotonergic, orexinergic) may be involved in a feedback loop to link not only feeding and metabolic state, but also the rewarded status of the organism to the SCN clock.

Supported by ANR JC, CNRS, Institut Servier, Fondation pour la Recherche Médicale

Young Investigator Award given by the organising committee

Identification of an endocannabinoid system in the hypophysial pars tuberalis of the Syrian hamster: its possible role for seasonal prolactin secretion

Yasuo S[1,3], Koch M[1,3], Schmidt H[2,3], Ziebell S[2,3], Geisslinger G[2,3], Korf HW[1,3]

[1]Dr. Senckenbergische Anatomie, Institute of Anatomie II; [2]Institute of Clinical Pharmacology: [3]LOEWE Lipid Signaling Forschungszentrum Frankfurt, Goethe-University Frankfurt, Germany

Purpose: The pars tuberalis (PT) is essential for seasonal regulation of gonadotropic and lactotropic axes, although the factors through which the PT affects the hormonal secretion from the pars distalis (PD) are not yet identified. Here we examined the role of an endocannabinoid system, a lipidergic neuroendocrine regulatory system, in the PT and PD of Syrian hamsters. Methods: Male 8-9 week old hamsters were kept under long-day condition (16L:8D) for 3 weeks, and then separated into two groups: one group was transferred to and kept under short-day condition (8L:16D) for 4 weeks; the other group remained under long-day condition for additional 4 weeks. Tissues from animals sacrificed at ZT3, 9, 15 and 21 were analyzed by in situ hybridization, immunohistochemistry, and liquid chromatography tandem mass spectrometry. For organ cultures, PD was stimulated by each reagent for 48h, and prolactin levels in culture medium were measured by radioimmunoassay. Results: Most enzymes involved in endocannabinoid synthesis and degradation were expressed in the PT. Immunohistochemical investigation confirmed the localization of these enzymes in the PT. The expression of Daglb and protein levels of DAGLa, enzymes catalyzing 2-AG synthesis, and 2-AG content in the hypothalamus/PT tissue were upregulated in animals kept under long-day conditions. The cannabinoid receptor 1 (CB1) was expressed in the PD. 2-AG stimulated prolactin secretion from the PD in combination with forskolin; this effect was blocked by AM251, a CB1 antagonist. Conclusion: These results suggest that endocannabinoids are important signals from the PT which are involved in seasonal regulation of prolactin secretion.

Supported by LOEWE Lipid Signaling Forschungszentrum Frankfurt, Alfred und Gertrud Kassel-Stiftung

(Suite page 112)

(Suite de la page 111)

Symposium 19. Hot topics

Chairman: Hastings MH (UK)

Fatal effects of an immune challenge following repeated phase shifts

Davidson AJ[1], Castanon-Cervantes O[1], Ehlen C[1], Menaker M[2], Paul K[1]

[1] Morehouse School of Medicine, Atlanta, GA, USA; [2] University of Virginia, Charlottesville, VA, USA

Purpose: Shift work is a uniquely human lifestyle that results in chronic disruption of circadian timing, and also leads to a higher risk of a number of age-related pathologies. However the role played by biological timing in the prevention and ontogeny of disease is poorly understood. Work in animals has indicated that genetic and environmental disruption of rhythmicity can result in early aging, increased cancer, obesity and diabetes, early age-related and cardiac death, and an exacerbated response to a chemical irritant. Many of these conditions in both shift workers and experimental models are associated with a chronic inflammatory state, and this common feature may represent the cause of pathologies associated with circadian disruption. Methods: Adult mice were exposed to 4 weeks of 6h weekly phase advances of the light cycle, then exposed to 12.5mg/kg E. coli LPS, inducing sepsis. Measurements included basal immune status before challenge, body temperature, survival, serum cytokines, Per2:luciferase rhythmicity in spleen and SCN, and polysomnography. Results: White blood cells were reduced in shifting mice, and LPS-induced endotoxemic shock (sepsis) was greatly magnified leading to profound hypothermia and death (89% mortality compared with 21% in unshifted mice). Pro-inflammatory cytokines were significantly higher in shifted mice. Sepsis alone suppressed Per2:luc rhythms in spleen, but in shifted septic mice spleen and SCN rhythms were further suppressed or abolished. Sleep disruption is a potential mediator of the effect we describe. However sleep recording in shifting mice revealed no sleep loss or change in architecture, only a gradual shift in phase of the sleep-wake rhythm that mimicked the body temperature and activity transients during resynchronization to each phase shift. Conclusions: Circadian disruption, but not sleep loss, is associated with phase shift-related disregulation of the innate immune system.

From "on to off" DNA: a dynamic mechanism for PER repression in the Drosophila circadian clock

Menet JS, Abruzzi KC, Desrochers J, Rosbash M

Department of Biology, Howard Hughes Medical Institute, National Center for Behavioral Genomics, Brandeis University, Waltham, USA

Purpose: To determine the mechanisms by which PERIOD (PER) mediates the repression of CLOCK/CYCLE (CLK/CYC) transcriptional activation in Drosophila. Methods: We used a newly described strain containing a V5-tagged Clk transgene and performed a combination of immunoprecicipation, chromatin immunoprecipitation. We also characterized the molecular clock of some relevant genetic mutants. Results: We show that PER repression involves distinct sequential mechanisms. First, the beginning of the repression phase is associated with the binding of PER to circadian promoters, probably via a PER-CLK interaction. PER DNA-binding likely provides a decrease in CLK-mediated transcription despite CLK DNA-binding. This "on DNA" repression phase is followed by release of CLK from DNA and the concomitant formation of a strong 1:1 PER-CLK complex that is not bound to DNA. This "off DNA" phase is followed by PER degradation, CLK DNA-binding and the morning increase in circadian transcription. A CLK DNA-binding mutation that increases circadian period only extends the morning increase in circadian transcription, suggesting that CLK/CYC DNA binding is not rate-limiting for other phases of the transcriptional cycle. Surprisingly, the PER degradation phase in the morning is also extended by the CLK DNA binding mutation, indicating that CLK DNA binding can affect PER degradation rather than just the reverse. Conclusion: The results indicate a dynamic and integrated view of the PER repression mechanisms.

The antennae are necessary for the proper timing of sun compass orientation in migratory monarch butterflies

Merlin C, Gegear RJ, Reppert SM

Dept of Neurobiology, University of Massachusetts Medical School, Worcester, USA

Purpose: We examined the role of antennal clocks in time-compensated sun compass orientation of migratory monarch butterflies. Methods: Flight orientation of migratory monarchs was monitored in a flight simulator. Clock gene mRNA levels and protein abundance were measured by real-time PCR and western-blot analysis, respectively, in the brain and in antennae. Results: Compared to the normal southwestern flight orientation manifested by intact migratory butterflies, antennae-less migrants exhibited disrupted flight orientation, but unimpeded free flight. When subjected to a 6-hr delay in the lighting cycle, the orientation of intact butterflies was predictably shifted in the northwesterly direction, whereas the orientation of antennae-less butterflies was again completely disrupted. Molecular oscillations of the clock genes period and timeless in brain and their timing relative to the light-dark cycle were unaltered in antennae-less butterflies, suggesting a role of the antennae in the timing of sun compass orientation. Indeed, molecular and biochemical analyses showed that antennae possess circadian clocks, which are entrained by light and can function independently from brain. Painting the antennae with black enamel resulted in the loss of antennal light sensitivity, desynchronized antennal clocks, and severely altered sun compass orientation. In contrast, control butterflies with clear-painted antennae showed normal light entrainment of antennal clocks and manifested appropriate time-compensated sun compass orientation. Conclusions: The antennae are necessary for proper sun compass orientation in migratory monarch butterflies and may provide the primary timing component. This unexpected finding opens new avenues of investigation into clock-compass connections that may extend widely to other insects that use this orientation mechanism.

Reduced cognitive deficits and neurodegenerative indices after long-term melatonin treatment of Alzheimer transgenic mice

Olcese J[1], Cao C[2-4], Mori T[5], Mamcarz M[3], Maxwell A[1], Runfeldt M[3], Wang L[3], Zhang C[2], Lin X[2,3], Zhang G[3], Arendash GW[3,6]

[1]FSU College of Medicine, Tallahassee; [2]Byrd Alzheimer's Center

(Suite page 113)

(Suite de la page 112)

& Research Institute, Tampa; [3]Dept of Cell Biology, Microbiology & Molecular Biology USF, Tampa; [4]Dept of Molecular Pharmacology & Physiology, College of Medicine, USF, Tampa; [5]Saitama Medical Center/University, Saitama, Japan; [6]The Florida Alzheimer's Disease Research Center, Tampa, USA

Purpose: To comprehensively determine the potential for long-term melatonin (MEL) treatment to protect Alzheimer's transgenic mice against cognitive impairment and development of ß-amyloid (Aß) neuropathology. Methods: MEL (100 µg/mL) was provided to APP+PS1 transgenic (Tg) mice from age 2.5 months until euthanasia at age 7.5 months. Results: A comprehensive behavioral battery administered during the final 6 weeks of treatment revealed that Tg+MEL mice were protected from cognitive impairment in various tasks of working memory, spatial reference learning/memory, and basic mnemonic function — Tg control mice remained impaired in all of these cognitive tasks. Immunoreactive Aß deposition was significantly reduced in hippocampus (?43%) and entorhinal cortex (? 37%) of Tg+MEL mice. Although soluble and oligomeric forms of Aß1-40 and 1-42 were unchanged in hippocampus and cortex from the same Tg+MEL mice, their plasma Aß levels were elevated. These Aß results, together with data showing that MEL suppresses Aß aggregation in brain homogenates, are consistent with a MEL-facilitated removal of Aß from the brain. Inflammatory cytokines such as TNF-? were decreased in hippocampus of Tg+MEL mice. Cortical mRNA expression of three antioxidant enzymes (SOD-1, glutathione peroxidase, catalase) was significantly reduced to non-Tg levels in Tg+MEL mice. Conclusions: MEL's cognitive benefits appear to involve its anti-Aß aggregation, anti-inflammatory, and/or antioxidant properties. Our findings provide support for long-term MEL therapy as a strategy for abating the progression of Alzheimer disease.

Society for Research on Biological Rhythms

Meetings

SRBR 12th Biennial Meeting

May 22, 2010 - May 26, 2010

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

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

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

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

26th Conference of the I.S.C.

Vigo, Spain; July 5-9 2010

Conference

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

Abstract submission

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

Registration information

- December 1, 2009: on-line registrations opens

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

Formation

Neurobiologie des rythmes

Prise en compte de l'aspect rythmique en biologie

Personnes concernées

Toute personne possédant une formation de base en physiologie animale ou neurosciences et souhaitant acquérir des connaissances théoriques dans le domaine des rythmes biologiques et des compétences pratiques dans l'étude de ces derniers.

Objectifs

A l'issue du stage les participants auront acquis :

- **des connaissances fondamentales dans la rythmicité journalière et saisonnière des fonctions biologiques, en particulier chez les mammifères, depuis le niveau moléculaire jusqu'aux aspects les plus intégrés,**
- **les connaissances de bases pour l'élaboration de protocoles expérimentaux et la mise en oeuvre des techniques spécifiques à l'analyse des rythmes.**

Programme

Aspects théoriques :

- Définitions et concepts.
- Noyaux suprachiasmatiques : horloge circadienne des Mammifères ; gènes horloges.
- Synchronisation photique/non photique.
- Rétine et oscillateurs secondaires.
- Rythme veille/sommeil, description et mécanismes nerveux impliqués.
- Rythmes saisonniers (reproduction, hibernation) ; mélatonine, photopériodisme.

Aspects pratiques :

- Visite du Chronobiotron (Plateforme d'hébergement et d'exploration fonctionnelle dédiée à l'étude des rythmes chez les rongeurs), présentation du matériel et des modèles animaux.
- Éventail de méthodologies spécifiques : conditions environnementales constantes ou d'entraînement, échantillonnage longitudinal, télémétrie...
- Mise en oeuvre d'enregistrements d'activité locomotrice de roue d'un rongeur et d'activité générale des stagiaires volontaires.
- Prélèvements et observation histologique des structures clés impliquées dans les rythmes.
- Implantation chez l'animal d'un capteur de température corporelle.
- Traitements et exploitations des données.

A l'issue du stage : synthèse et discussion générale sur la valorisation des acquis dans les différents domaines des participants.

Méthodes pédagogiques

Les enseignements théoriques et pratiques sont assurés par des enseignants-chercheurs et des chercheurs des différentes équipes du Département de neurobiologie des rythmes de l'INCI.
Chaque participant recevra un support de cours polycopié ainsi qu'une clé USB contenant les documents de travail et les diaporamas des conférenciers.

Sanction de la formation

Cette formation donne lieu à la délivrance d'une attestation de participation.
Une évaluation en fin de formation permet de mesurer l'atteinte des objectifs et la satisfaction des stagiaires.

Responsables scientifiques

Sylvie RAISON et Patrick VUILLEZ, Maîtres de conférences, Faculté des sciences de la vie, Département de neurobiologie des rythmes de l'INCI.
Courriels : raison@neurochem.u-strasbg.fr / vuillez@neurochem.u-strasbg.fr

UNIVERSITÉ DE STRASBOURG

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Stage inter entreprises

Durée : 4 jours

En 2010 :
Stage 1 : Référence : 100283
du 8 juin 2010 à 9 h
au 11 juin 2010 à 16 h
Stage 2 : Référence : 110031
du 13 décembre 2010 à 9 h
au 16 décembre 2010 à 16 h

Lieu : Université de Strasbourg - Service Formation Continue
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Frais de participation :
1.605 € (pour toute inscription avant le 30/06/2010)
Repas de midi pris en charge par les organisateurs.
Nombre de stagiaires limité à 10.
Code : 0784

Ce stage ne peut pas être réalisé en intra

Renseignements et Inscriptions :
Stéphanie HOMMEL
Tél. : 03 68 85 49 30
Sauf les mercredi et vendredi
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s.hommel@unistra.fr

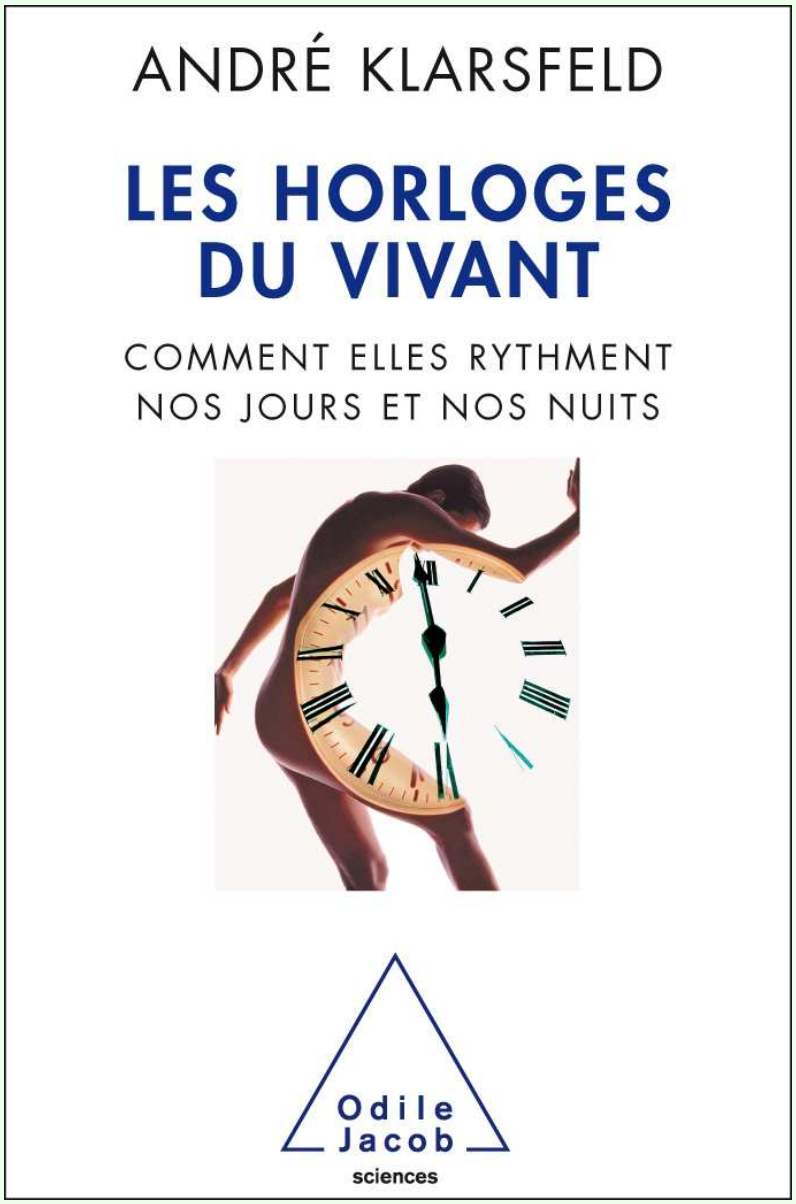
Les Horloges du Vivant

Comment elles rythment nos jours et nos nuits

(éditions Odile Jacob, octobre 2009)

André Klarsfeld

Qu'y a-t-il de commun au décalage horaire ressenti après un vol transatlantique, aux troubles de sommeil plus fréquents le dimanche soir, après deux grasses matinées consécutives, et aux enjeux de santé liés au travail de nuit, ou aux changements d'heure du printemps et de l'automne ? Ce sont les horloges biologiques, qui rythment nos vies sans que nous en ayons conscience la plupart du temps. Elles constituent une dimension essentielle du monde vivant, non seulement animal, mais aussi végétal, et même microbien, permettant aux organismes vivants de se préparer énergiquement à leur phase d'activité, diurne ou nocturne, alors qu'ils semblent se reposer – voire dormir du sommeil du juste.



Mais elles ont aussi bien d'autres fonctions fondamentales, parfois plus inattendues : déclencher la période des amours, guider des papillons des oiseaux ou tions saisonnières dans leur butinage dans leurs migrations, ou des abeilles…

Les biologistes disposaient des premiers indices d'une orchestration temporelle du vivant dès 1729. L'existence des horloges qui la dirigent ne sera pourtant admise que dans la seconde moitié du XXème siècle. Depuis lors, ils s'efforcent de comprendre à la fois comment le vivant mesure le temps avec une telle régularité, de l'intérieur, comment il met quotidiennement ses horloges internes à l'heure solaire, et comment il produit, en aval, tous les cycles physiologiques qui rythment ses journées. La quête des horloges du vivant a été une aventure intellectuelle semée d'embûches. Elle a aussi un impact qui nous concerne tous, notamment sur la compréhension et le traitement de nombreuses pathologies.

André Klarsfeld est chargé de recherche dans l'équipe *Génétique moléculaire des rythmes circadiens*, à l'Institut de neurobiologie Alfred-Fessard du CNRS (Gif-sur-Yvette). Il est membre de la Société francophone de chronobiologie. Avec Frédéric Revah, il a écrit *Biologie de la mort* (éd. O. Jacob, 2000).

Mélatonine; Rythme de la Température Corporelle et Organisation de l'Hypothalamus et des Noyaux Suprachiasmatiques chez le Dromadaire.

Khalid El Allali

Résumé

La plupart des mammifères doivent pour survivre s'adaptater à leurs biotopes. Dans les zones arides et désertiques, le Dromadaire (Camelus dromedarius) est exposé à des variations extrêmes de température, à un climat sec et chaud tout le long de l'année, à une rareté des parcours (??? alimenataire ?) et surtout à un manque des points d'abreuvement. Dans un tel biotope, à côté de la photopériode qui marque chaque saison, nous avons supposé que d'autres facteurs peuvent entraîner l'horloge biologique et permettent ainsi aux animaux d'adapter par anticipation leurs fonctions biologiques.

Dans un premier temps, nous avons vérifié si chez le Dromadaire vivant dans les zones désertiques du Sud Marocain, où la photopériode varie peu (03h26 min/an), des variations saisonnières du rythme des concentrations plasmatiques de mélatonine étaient observées. Nous avons démontré que le profil de la sécrétion de cette hormone présente des variations saisonnières parallèles aux changements annuels de la photopériode. Cet animal est donc capable de mesurer et d'intégrer l'information photopériodique, même si celle-ci varie peu au cours de l'année.

Nous avons remarqué que les concentrations plasmatiques en mélatonine présentent des réponses rapides à la lumière et que leur augmentation se fait rapidement dans les 30min après le coucher du soleil. Cette monté rapide est probablement, comme chez d'autres espèces, dont le mouton, le résultat d'un état constitutif de la synthèse de l'AA-NAT dont la régulation et l'activation serait induite par un mécanisme post-transcriptionel. Afin de tester cette hypothèse, les niveaux des ARNm du gène Aa-nat ont été comparés dans des glandes pinéales prélevées pendant le jour et pendant la nuit. Le résultat obtenu démontre que les niveaux d'ARNm de l'AA-NAT de jour est égale à celui de la nuit et ce malgré les concentrations différentes de la mélatonine (6 fois plus élevées la nuit). Ceci démontre que dans la glande pinéale du Dromadaire, comme chez beaucoup de ruminants, l'expression des ARNm de l'AA-NAT est constitutive et que la libération nocturne de la noradrénaline, responsable de la synthèse de la mélatonine, agit par une régulation post transcriptionelle de l'enzyme.

Dans un troisième temps, nous avons voulu étudier chez cette espèce, les phénotypes neurochimiques des neurones des noyaux suprachiasmatiques (SCN), lieu de l'horloge biologique. Nous avons également analysé les différentes innervations, voies possibles pour l'entraînement photique et non photique. Un référentiel anatomique ainsi qu'une cartographie de l'hypothalamus du Dro-

THÈSE
Présentée à

UNIVERSITÉ DE STRASBOURG

L'Université Abdelmalek Essaadi
Faculté des Sciences, Tétouan, Maroc
En cotutelle
Avec L'Université de Strasbourg
Faculté des Sciences de la vie
En collaboration et avec le soutient de l'Institut Agronomique et Vétérinaire Hassan II, Rabat, Maroc

En vue de l'obtention du titre de
DOCTEUR DE L'UNIVERSITE
Discipline : *Sciences du vivant*, Spécialité : *Neurosciences*

Par Khalid EL ALLALI

Mélatonine, Rythme de la Température Corporelle et Organisation de l'Hypothalamus et des Noyaux Suprachiasmatiques chez le Dromadaire (*Camelus dromedarius*) : Démonstration de l'Entraînement de l'Horloge Circadienne par la Photopériode et par la Température Ambiante.

23 novembre, 14h

Soutenance le 2009 à la Faculté des Sciences-Université Abdelmalek Essaâdi, Tétouan-Maroc, devant la commission d'examen :

Pr. Paul Pévet (DR CNRS, UDS, Strasbourg)	Directeur de thèse
Pr. Mohammed Errami (Professeur à l'UAE, Tétouan)	Directeur de thèse
Pr. Nouria Lakhdar-Ghazal (Professeur à l'UM5, Rabat)	Directeur de thèse
Pr. Marie-José Freund-Mercier (Professeur à l'UDS)	Rapporteur
Pr. Ali Ouarour (Professeur à l'UAE, Tétouan)	Rapporteur
Pr. Mohamed Ouassat (Professeur à l'IAV, Rabat)	Rapporteur
Pr. André Calas (Professeur à l'U-Bordeaux 2)	Examinateur

UDS : Université de Strasbourg, France
UAE : Université Abdelmalek Essaâdi, Tétouan-Maroc
UM5 : Université Mohammed V, Agdal Rabat-Maroc
IAV : Institut Agronomique et Vétérinaire Hassan II, Rabat-Maroc
U-Bordeaux 2 : Université Bordeaux 2, France

(Suite page 119)

Age-related impairment in thermoregulatory capacities in a non-human primate species, the gray mouse lemur (Microcebus murinus)

Jérémy Terrien

Résumé

De nombreuses études épidémiologiques rapportent une prévalence accrue de la mortalité causée par hypo- ou hyperthermie chez les populations âgées. Bien que des ajustements comportementaux soient possibles pour compenser les déficits autonomiques, le maintien de l'homéostasie chez l'individu âgé apparaît néanmoins clairement altéré. En particulier, la détérioration avec l'âge des mécanismes impliqués dans la régulation de la temperature corporelle (Tc), des systèmes endocriniens sous-jacents ou même des changements de composition corporelle pourrait contribuer à altérer la gestion énergétique des réponses thermorégulatrices.

Cette hypothèse a été testée chez une espèce primate qui utilise l'hétérothermie journalière comme alternative énergétique aux coûts élevés imputés au maintien de l'endothermie. En effet, le Microcèbe (*Microcebus murinus*) est une espèce nocturne originaire de Madagascar dont le métabolisme énergétique est fortement dépendant des variations saisonnières de la photopériode. Ainsi, en photopériode hivernale, le Microcèbe est peu actif, privilégiant l'utilisation de la torpeur journalière afin de réaliser de grandes économies d'énergie et d'eau. Pendant l'été, l'animal s'active et entre en période de reproduction. Ces variations saisonnières du métabolisme sont également accompagnées de très fortes modulations de la balance énergétique, de la composition corporelle et des facteurs endocriniens, impliquant une forte saisonnalité dans les capacités thermorégulatrices. Néanmoins, de nombreuses altérations des fonctions métaboliques ont été démontrées avec l'âge chez cette espèce, faisant du Microcèbe un modèle de choix pour l'étude des effets du vieillissement. Le but de cette thèse était donc de déterminer l'impact du vieillissement sur les capacités thermorégulatrices du microcèbe mâle face à des variations de la température ambiante, d'en évaluer les implications énergétiques, et de relier les déficiences observées à divers facteurs métaboliques, endocriniens et cérébraux.

UNIVERSITÉ PARIS DESCARTES
FACULTÉ DE MÉDECINE

C2iD

Age-related impairment in thermoregulatory capacities in a non-human primate species, the gray mouse lemur (*Microcebus murinus*)

THÈSE

présentée et soutenue publiquement le 25 Novembre 2009 par

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pour obtenir le grade de

DOCTEUR

Sciences de la Vie et de la Santé

Spécialité : Biologie du Vieillissement

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Lors d'un test de choix spontané de température ambiante, les animaux âgés ont clairement exprimé leur préférence pour des nichoirs chauds, bien qu'aucune déficience autonomique n'ait pu être mise en évidence en conditions thermoneutres. L'adaptation à la photopériode estivale semble induire des changements métaboliques et hormonaux suffisamment préservés chez l'animal âgé pour le prévenir contre des variations excessives de sa température interne. En effet, au cours de l'exposition à des températures froides ou chaudes, les animaux testés en été n'ont pas montré de variations majeures du niveau de Tc, maintenant une balance énergétique positive en ajustant efficacement leur dépense énergétique. En revanche, chez des animaux âgés acclimatés à une photopériode hivernale, le défaut de maintien de la normothermie était associé à une balance énergétique négative lors de l'exposition au froid, probablement liée à une trop grande dépense énergétique. Les mécanismes de thermogenèse impliqués dans la réponse au froid, et notamment la thermogenèse de non-frisson lors de la sortie de la

(Suite page 119)

(Suite de la page 117)

madaire a été ainsi été réalisé. Comparé aux autres Mammifères, les SCN du dromadaire présentent plusieurs caractéristiques. 1) Ils sont très longs avec une extension rostrale et post chiasmatique. 2) Une présence massive de neurones à la tyrosine hydroxylase formant deux populations est observée 3) La présence d'ocytocine dans les parvoneurones de ces noyaux ce qui implique pour ce neuropeptide un rôle modulateur 4) plusieurs types d'innervation (NPY, 5-HT, Met-enk…). Elles témoignent de la présence de voies d'entrainement photique et non photique de l'horloge. La distribution de la vasopressine et de l'ocytocine dans les magnoneurones de l'hypothalamus, montre que le système hypothalamo-neurohypophysaire est large et comprend en plus des noyaux supra-optiques et paraventriculaires, les noyaux : dorsomédians, ventromédians, arqué, le noyau magnocellulaire de l'hypothalamus latéral et surtout le noyau tubérale qui semble jouer un rôle particulier chez cette espèce.

Finalement, nous avons examiné la possibilité d'un entraînement non photique de l'horloge par le cycle de la température ambiante. La température ambiante en effet est un facteur environnemental cyclique particulièrement important pour cette espèce. Pour cette étude nous choisi le rythme de la température corporelle comme marqueur des activités de l'horloge. Après avoir démontré le contrôle de ce paramètre par une horloge circadienne et sa dépendance de la photopériode nous avons pu établir que le rythme de la température corporelle (donc l'horloge) pouvait être influencé par le cycle de la température ambiante. En étudiant l'effet du cycle de la température ambiante sur le rythme de la mélatonine nous avons pu montrer qu'il s'agissait bien d'un entrainement par le cycle de température de l'horloge. Chez le Dromadaire, le cycle de la température ambiante est donc un véritable zeitgeber. Toutefois, nous avons aussi montré, dans certaines conditions expérimentales précises, que le rythme de température corporelle suivait passivement le cycle de la température ambiante. Cette dernière observation nous a permis de poser les limites dans l'utilisation de certains paramètres (e.g ; température corporelle) pour étudier les propriétés de l'horloge circadienne

Il est maintenant évident, en terme de Zootechnie vétérinaire, qu'à côté de la photopériode, la prise en compte de la température ambiante comme un facteur environnemental, est nécessaire pour aborder la régulation des rythmes saisonniers (e.g ; reproduction) sur cette espèce.

Mots clés : Dromadaire, Mélatonine, AA-NAT, Horloge biologique, noyaux suprachiasmatiques, hypothalamus, rythme de la température ambiante, cycle de la température ambiante, entraînement photique et non photique.

(Suite de la page 118)

torpeur journalière, étaient efficacement activés chez le Microcèbe âgé. En revanche, une déficience dans le maintien énergétique de cette activité thermogénique soutenue est apparue. De plus, lors de l'exposition au chaud, la robustesse de la rythmicité journalière de la Tc était largement perturbée, ceci étant également associé à une balance énergétique négative. Ces déficiences pourraient premièrement être liées à un déclin de la balance vasoconstriction/ vasodilatation, entrainant une isolation moins efficace de l'organisme vis-à-vis des variations environnementales. En effet, l'étude des capacités de dissipation de la chaleur corporelle ont révélé des difficultés chez l'individu âgé à évacuer l'excédent de chaleur corporelle produit suite à l'induction pharmacologique d'une hyperthermie. Puis dans un second temps, les efforts métaboliques fournis pour rétablir la normothermie seraient plus grands, induisant ainsi le déséquilibre de la balance énergétique. L'hypothèse d'une contrainte énergétique a été ensuite testée expérimentalement par l'exposition d'animaux adultes à une restriction calorique chronique modérée. Les animaux restreints ont alors exprimé des variations saisonnières des paramètres métaboliques et endocriniens similaires aux individus nourris *ad libitum*. A l'âge adulte, le Microcèbe serait donc capable d'activer des voies métaboliques compensatoires au déficit énergétique qui ne seraient plus disponibles au cours du vieillissement. Finalement, aucune déficience thermorégulatrice n'a pu être mise en relation avec les altérations endocriniennes (taux plasmatiques d'IGF-1) ou cérébrales (mesures d'atrophie cérébrale), suggérant la nécessité d'effectuer des investigations plus approfondies dans ces domaines.

En conclusion, le vieillissement est fortement associé chez le Microcèbe au déclin des fonctions impliquées dans la gestion énergétique des réponses thermorégulatrices. Bien que l'hétérothermie de cette espèce implique des mécanismes thermorégulateurs différents de ceux activés chez l'Homme, on peut spéculer que les déficiences impliquées dans la fragilité des individus âgés lors d'épisodes prolongés de froid ou de chaud pourraient également être reliées à une altération de la balance énergétique et des voies métaboliques sous-jacentes. L'activation de ces voies métaboliques matérialisée par certaines interventions similaires à la restriction calorique chronique modérée pourrait induire une prédisposition à répondre efficacement à des variations brusques de la Ta, réduisant ainsi les risques de mort par hypo- ou hyperthermie.

Mots clés : Vieillissement – capacités thermorégulatrices – balance énergétique – primate non-humain – *Microcebus murinus*.

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Rythmes est édité par la Société Francophone de Chronobiologie, Siège Social : Faculté des Sciences et Techniques. Laboratoire de Biologie Animale et Appliquée, 23 rue du Dr Paul Michelon, 42023 Saint-Étienne Cedex 2. Directeur de la publication : Bruno Claustrat. Rédactrice en chef : Fabienne Aujard. Comité de rédaction : Fabienne Aujard, Jacques Beau, Jean-François Vibert. Réalisation : Jacques Beau et Jean-François Vibert. Impression : Muséum National d'Histoire Naturelle, Paris.

Site Web : http://www.sf-chronobiologie.org Numéro ISSN 0154-0238.