Université de Montréal

Composantes électrophysiologiques et circadiennes de la sensibilité à la lumière

par

Marianne Rufiange

Département de Psychiatrie

Faculté de Médecine

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Université de Montréal

Faculté des études supérieures

Cette thèse intitulée :

Composantes électrophysiologiques et circadiennes de la sensibilité à la lumière

présentée par :

Marianne Rufiange

a été évaluée par un jury composé des personnes suivantes :

Dr Julie Carrier, PhD, président-rapporteur

Dr Marie Dumont, PhD, directeur de recherche

Dr Pierre Lachapelle, PhD, co-directeur

Dr Franco Lepore, PhD, membre du jury

Dr Marc Hébert, PhD, examinateur externe

?, représentant du doyen de la FES

Résumé

L'objectif de cette thèse était d'approfondir les connaissances sur la sensibilité à la lumière chez l'humain. Nous avons d'abord étudié la sensibilité rétinienne à l'aide de l'électrorétinogramme (ERG) sous diverses conditions expérimentales. Nous avons élaboré une méthode d'analyse approfondie de la courbe luminance-réponse photopique, aussi appelée « photopic hill », afin d'en faire un outil efficace pour l'étude des variations de la fonction des cônes. Nous avons examiné les changements du « photopic hill » chez des sujets normaux en modulant l'éclairage ambiant et la longueur d'onde des stimuli, ainsi que chez quatres patients atteints de différentes pathologies rétiniennes.

Nous avons ensuite étudié la relation entre le moment de sécrétion de mélatonine et les variations diurnes de l'ERG scotopique et photopique. L'enregistrement des ERG s'est tenu aux mêmes heures, en fin de soirée et en début de matinée, chez deux groupes de sujets maintenus sur le même horaire éveil-sommeil mais dont le moment de sécrétion de mélatonine différait; ceci permettait de distinguer l'effet de la mélatonine de celui du moment de la journée. Les résultats ont démontré que les variations diurnes de l'ERG scotopique ne semblent pas influencées par la mélatonine, mais bien par le renouvellement des disques des bâtonnets qui a lieu le matin. L'ERG photopique, quant à lui, paraît affecté par la mélatonine; une forte concentration de mélatonine étant associée à une baisse de l'amplitude de l'ERG photopique.

Finalement, nous avons examiné l'effet de l'histoire lumineuse sur la sensibilité à la lumière du système circadien, évaluée par la suppression de sécrétion de mélatonine, et de la rétine, évaluée par l'ERG. Nous avons donc étudié des populations se situant aux deux extrêmes : un groupe travaillant à temps plein dans un milieu sombre, sans fenêtre et un groupe travaillant à l'extérieur. L'exposition lumineuse a été mesurée 24h sur 24 pendant deux semaines à l'aide d'un moniteur ambulatoire. Une histoire lumineuse de faible intensité était associée à une plus grande sensibilité circadienne, une phase circadienne tardive, une adaptation à la noirceur plus rapide, une plus grande sensibilité rétinienne en condition scotopique et une plus faible, en condition photopique.

Mots-clés : vision, électrorétinogramme (ERG), sensibilité rétinienne, photorécepteur, chronobiologie, rythme circadien, mélatonine, exposition lumineuse, milieu de travail, humain.

Abstract

The aim of this thesis was to better understand the sensitivity to light in humans. First, we studied the retinal sensitivity using the electroretinogram (ERG) under different experimental conditions. We developed a method of extensive analysis of the photopic luminance-response curve, also called "photopic hill", in order to have a useful tool for studies on variations of the cone function. We examined the changes in the "photopic hill" of normal subjects with variations in the background light and in the wavelength of stimuli, as well as of four patients affected with different retinal diseases.

We also looked at the relationship between the timing of melatonin secretion and the diurnal variations in the scotopic and photopic ERG. ERG recordings took place at the same clock times, in the late evening and in the early morning, in two groups of subjects maintained on the same sleep-wake schedule but differing in their timing of melatonin secretion; thus permitting a distinction between the effect of melatonin and that of the time of day. Results indicate that the diurnal variations in scotopic ERG do not seem to be affected by melatonin, but rather by the rod disk shedding which takes place in the morning. On the contrary, the photopic ERG appears to be affected by melatonin, high concentrations of melatonin being associated with a decrease in the amplitude of the photopic ERG.

Finally, we examined the effect of light history on the sensitivity to light of the circadian system, evaluated with the suppression of melatonin secretion, and of the retina, evaluated with the ERG. We therefore studied populations exposed to the two extremes : one group working full time indoors, without any windows and one group working outdoors. The light exposure was measured 24 hours a day for two weeks with an ambulatory monitor. Low light history was associated with a higher circadian sensitivity, a later circadian phase, a faster dark adaptation, a higher retinal sensitivity in scotopic conditions and a lower one in photopic conditions.

Keywords : vision, electroretinogram (ERG), retinal sensitivity, photoreceptor, chronobiology, circadian rhythm, melatonin, light exposure, work place, human.

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Liste des abréviations

en français :

cônes-L :	cônes à longues longueurs d'onde
cônes-M :	cônes à moyennes longueurs d'onde
cônes-S :	cônes à courtes longueurs d'onde
CRY :	cryptochrome
DLMO :	début de la période de sécrétion de mélatonine
ERG :	électrorétinogramme
GCS :	ganglion cervical supérieur
MT :	mélatonine
NPV :	noyau paraventriculaire
NSC :	noyaux suprachiasmatiques
OP :	potentiel oscillatoire
SOP :	somme des potentiels oscillatoires
VRH :	voie rétino-hypothalamique

en anglais :

ANOVA :	analysis of variance
CREB :	cAMP response element binding protein
CSNB :	congenital stationary night blindness
CV :	coefficient of variation
DLMO :	dim-light melatonin onset
ERG :	electroretinogram
E-type :	evening-type
GRP :	gastrin-releasing peptide

HB :	habitual bedtime
ipRGC :	intrinsically photosensitive retinal ganglion cell
ISCEV :	International Society for Clinical Electrophysiology of Vision
L-cones :	long-wavelength sensitive cones
M-cones :	middle-wavelength sensitive cones
M/E :	morningness-eveningness
M-type :	morning-type
NMDA :	*N*-methyl-*D*-aspartate
NSAID :	non-steroidal anti-inflammatory drug
OD :	right eye
OFF-HBC :	OFF-hyperpolarizing bipolar cell
ON-DBC :	ON-depolarizing bipolar cell
ONL :	outer nuclear layer
OP :	oscillatory potential
OS :	left eye
RGC :	retinal ganglion cell
RHT :	retino-hypothalamic tract
SCN :	suprachiasmatic nucleus
S-cones :	short-wavelength sensitive cones
SD :	standard deviation
SEM :	standard error of the mean
SF :	standard flash
SOP :	sum of oscillatory potentials
VIP :	vasoactive intestinal polypeptide

À ce qui a commencé par un feu douteux et qui dure encore!

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1. Introduction

1.1. Lumière et système circadien

L'organisation temporelle d'un individu provient du système circadien. Celui-ci est constitué de trois grandes composantes : un oscillateur central ou horloge biologique qui génère une impulsion rythmique endogène d'environ 24 heures, des voies d'entraînement permettant à l'oscillateur de se synchroniser avec les cycles environnementaux, et des voies efférentes transmettant le rythme endogène aux différentes fonctions physiologiques et psychologiques de l'organisme (Moore, 1995). Chez les mammifères, l'oscillateur circadien central est situé dans les noyaux suprachiasmatiques (NSC) de l'hypothalamus. La périodicité qu'il génère n'est pas tout à fait égale à 24 heures. Chez l'humain, la périodicité endogène serait en moyenne de 24,2 heures (Czeisler et al., 1999). Des facteurs externes appelés synchroniseurs agissent sur l'oscillateur circadien et le synchronisent avec le rythme d'exactement 24 heures présent dans l'environnement.

1.1.1. Entraînement circadien par le cycle lumière-obscurité

Le principal synchroniseur est le cycle lumière-obscurité. Ainsi, la lumière a la propriété de modifier la phase et la périodicité de l'oscillateur circadien en fonction de la phase de l'oscillateur au moment de l'exposition lumineuse (Minors et al., 1991; Beersma et al., 1999). De façon générale, la lumière reçue en fin de journée ou en début de nuit produit un délai de la phase circadienne, alors que la lumière reçue le matin ou en fin de nuit produit

une avance de la phase de l'oscillateur. La lumière reçue au milieu de la journée n'a que peu ou pas d'effet sur la phase circadienne. Ce principe est à la base du mécanisme d'entraînement et permet de maintenir l'individu dans une relation de phase stable avec le cycle lumière-obscurité de 24 heures. Cette phase circadienne peut être relativement avancée ou retardée chez certains individus selon leur chronotype (Horne & Ostberg, 1976). Un type du matin présente une phase hâtive : il se couche tôt, se lève tôt et préfère les activités matinales. Un type du soir présente, quant à lui, une phase tardive : il se couche et se lève tard et choisit des activités vespérales. La phase circadienne d'un type intermédiaire se situe entre ces deux extrêmes.

Le signal lumineux capté par la rétine se rend aux NSC essentiellement via une projection directe des cellules ganglionnaires : la voie rétino-hypothalamique (VRH). Cette voie projette aussi, en moindre proportion, aux aires hypothalamiques latérale et antérieure ainsi qu'à l'aire rétrochiasmatique (Johnson et al., 1988a). Les cellules ganglionnaires formant la VRH constituent seulement 1% de la quantité totale de ces cellules chez le rat (Moore et al., 1995). La VRH est à la fois nécessaire et suffisante pour maintenir l'entraînement circadien. En effet, le sectionnement de toutes les voies visuelles autres que la VRH, après le chiasma optique, amène une cécité complète mais préserve l'entraînement des fonctions circadiennes (Klein & Moore, 1979), alors que le sectionnement sélectif de la VRH abolit cet entraînement (Johnson et al., 1988b). Les axones de la VRH forment également certaines collatérales qui innervent le feuillet intergéniculé du corps genouillé latéral

(Pickard, 1985), le noyau thalamique constituant le principal relais des voies visuelles entre la rétine et le cortex visuel. L'information rétinienne parvient aux feuillets intergéniculés par la voie rétino-intergéniculée alors que l'information venant de ces feuillets vers les NSC passe par la voie géniculé-hypothalamique (Miller et al., 1996). Les feuillets intergéniculés reçoivent de l'information des NSC, des noyaux à la noradrénaline, à la sérotonine et à l'acétylcholine du tronc cérébral ainsi que de l'aire rétrochiasmatique (Moore & Card, 1994). Cette innervation provenant à la fois de la rétine, du tronc cérébral et de l'hypothalamus laisse envisager que le feuillet intergéniculé soit un site d'intégration de l'information lumineuse et non-lumineuse qui modulerait la fonction circadienne d'un organisme (Moore, 1992; Moore & Card, 1994).

Des études chez le rat (Tanaka et al., 2003) et le hamster (Aïoun et al., 1998) ont montré que les cellules des NSC qui reçoivent les projections rétiniennes sont des neurones qui contiennent principalement le polypeptide intestinal vasoactif (en anglais : VIP) et le peptide de libération de la gastrine (en anglais : GRP). Plus de 30% des neurones des NSC sont sensibles à la lumière dans ces deux espèces; une augmentation d'intensité lumineuse peut soit élever le niveau de décharge neuronale, soit l'abaisser selon le type de cellule. Le seuil de réponse à la lumière se situe autour d'un lux, la courbe d'intensité-réponse est ensuite linéaire pour finalement saturer entre 100 et 1000 lux. Plusieurs études affirment que le principal neurotransmetteur de la VRH est l'acide aminé excitateur glutamate (Meijer & Schwartz, 2003). Ainsi, un stimulus lumineux mènerait à un influx de calcium

dans les cellules des NSC par une liaison du glutamate aux récepteurs NMDA (en anglais : « *N*-methyl-*D*-aspartate ») et par une dépolarisation membranaire. Il s'en suivrait une phosphorylation de CREB (en anglais : « cAMP response element binding protein ») et finalement, une cascade de transcription de plusieurs gènes, dont les plus connus sont *c-fos*, *per1* et *per2* (Meijer & Schwartz, 2003).

1.1.2. Mélatonine et sensibilité à la lumière

L'information sur le cycle lumière-obscurité est aussi transmise sous forme hormonale par la mélatonine. La mélatonine est une hormone qui est sécrétée principalement par la glande pinéale. Le moment de son épisode de sécrétion est déterminé directement par les NSC. Ainsi, l'exposition à la lumière, en permettant l'ajustement de la phase de l'oscillateur circadien, détermine aussi la phase de la sécrétion de mélatonine. De façon générale, la sécrétion de mélatonine a lieu la nuit, d'environ 21h00 à 08h00 et atteint sa concentration plasmatique maximale vers 03h00 (Geoffriau et al., 1998). Le niveau de mélatonine durant la journée est presque indétectable (<3 pg/ml). La phase du rythme de sécrétion de la mélatonine, souvent définie par le moment du début de la sécrétion (en anglais : « Dim Light Melatonin Onset » ou DLMO), est un bon marqueur de la phase de l'oscillateur circadien (Lewy et al., 1999).

La lumière a aussi un effet suppresseur direct sur la sécrétion de mélatonine. Ici encore, le signal lumineux est transmis à la glande pinéale en provenance des NSC, par les mêmes voies anatomiques que pour l'entraînement du rythme de la sécrétion. Les fibres en provenance des NSC se terminent dans le noyau paraventriculaire de l'hypothalamus (NPV). Le NPV innerve à son tour les cellules pré-ganglionnaires de la moelle épinière dont les axones s'étendent jusqu'au ganglion cervical supérieur (GCS). Les fibres sympathiques post-ganglionnaires du GCS se rendent finalement à la glande pinéale où l'illumination rétinienne bloquera la libération de noradrénaline, produisant une inhibition de l'activité de la *N*-acetyltransférase, enzyme nécessaire à la synthèse de la mélatonine à partir de la *N*-acetylsérotonine (Moore, 1996).

Le degré de suppression de la sécrétion de mélatonine varie selon les caractéristiques du signal lumineux, en particulier de son intensité. Il en est de même pour les changements de phase. Une étude chez l'humain a d'ailleurs mesuré l'effet de 6.5h d'exposition (de 23h00 à 05h30) à diverses intensités lumineuses sur la suppression de mélatonine et le changement de phase de son épisode de sécrétion (Zeitzer et al., 2000). Les courbes de réponse à la lumière suivaient une fonction non-linéaire; une plus forte intensité lumineuse produisant une plus grande suppression et un changement de phase plus important. Il est intéressant de constater que la dose efficace à 50% était d'environ 120 lux dans les deux cas. Par contre, la saturation survenait à plus faible intensité pour la suppression (environ 200 lux) que pour le changement de phase (environ 550 lux). Comme le signal lumineux produisant ces deux

effets physiologiques provient directement des cellules des NSC, leur amplitude à une intensité donnée peut être considérée comme une bonne mesure de la sensibilité du système circadien à la lumière. La mesure la plus couramment utilisée est la suppression de sécrétion de mélatonine (Lewy et al., 1981, 1985). Une variation dans la sensibilité peut refléter des changements soit au niveau des cellules réceptrices de l'information lumineuse des NSC, soit au niveau des récepteurs rétiniens responsables de la transmission du signal lumineux aux NSC. Ces deux hypothèses n'étant pas mutuellement exclusives.

1.1.3. Effet de l'histoire lumineuse sur la sensibilité circadienne

Suite à une série de cycles lumière-obscurité de différentes intensités, le système rétine-NSC-pinéale semble capable de comparer l'intensité relative de la lumière pour distinguer le jour de la nuit. Ainsi, il a été montré chez l'animal qu'une faible intensité lumineuse était considérée soit comme une intensité diurne, soit comme une intensité nocturne selon que l'autre partie du cycle lumière-obscurité était d'une intensité plus faible ou plus forte (Lynch et al., 1981, 1985). Dans chaque cas, la sécrétion de mélatonine se produisait durant la partie du cycle ayant la plus faible intensité lumineuse. Chez les animaux qui avaient été maintenus dans le cycle obscurité / lumière tamisée, la lumière tamisée avait la propriété de bloquer la sécrétion nocturne de mélatonine, alors qu'elle n'avait aucun effet chez les animaux habitués au cycle lumière tamisée / lumière forte. Il semble donc que la sensibilité circadienne à la lumière était devenue beaucoup plus élevée chez les animaux habitués à un

cycle obscurité / lumière tamisée. Dans un même ordre d'idée, des expériences chez des écureuils diurnes ont montré que le seuil d'intensité lumineuse pour la suppression de la mélatonine était plus élevé chez les animaux sauvages habitués à la lumière extérieure que chez les animaux élevés dans le milieu plus sombre du laboratoire (Reiter et al., 1983). Le passé lumineux (ou histoire lumineuse) de l'organisme semble donc influencer la sensibilité du système circadien à la lumière.

Peu d'études sur le sujet ont été réalisées chez l'humain et on ignore encore si le système circadien humain modifie sa sensibilité à la lumière en fonction des intensités lumineuses reçues auparavant. Quelques indications suggèrent que ce pourrait être le cas. Une étude récente a révélé que la plupart (75%) des sujets exposés successivement à une semaine de lumière tamisée et une semaine de lumière vive montrait une suppression de mélatonine plus importante suite à la semaine en lumière tamisée que celle en lumière vive (Hébert et al., 2002). Les sujets devaient porter des lunettes d'atténuation lorsqu'ils étaient à l'extérieur pendant la semaine en lumière tamisée alors qu'ils devaient aller dehors et/ou s'exposer à des boîtes lumineuses au moins 4 heures par jour durant la semaine en lumière vive. La moyenne de suppression de mélatonine passait ainsi de 53% à 41%. Toutefois, on notait une importante variation inter-individuelle que l'on pourrait possiblement expliquer par la courte période d'exposition et par l'absence d'un certain laps de temps entre les deux régimes lumineux qui aurait permis un retour à la condition de base.

À l'exception d'une étude de cas où une augmentation du seuil pour la suppression de mélatonine a été observée après deux semaines de photothérapie chez un patient souffrant de dépression saisonnière (McIntyre et al., 1990), toutes les autres études chez l'humain ont utilisé le modèle des variations saisonnières. En effet, comme à certaines latitudes la durée de la photopériode varie beaucoup entre l'été et l'hiver, la différence dans la quantité de lumière naturelle disponible entre les deux saisons peut être assez importante. Une de ces études a été menée auprès du personnel d'une base de recherche en Antarctique, où l'illumination maximale en hiver est de 500 lux durant les 3 mois où le soleil ne se lève pas, et est de plus de 100 000 lux en été, durant les 3 mois où le soleil ne se couche pas (Owen & Arendt, 1992). Les résultats suggèrent une plus forte suppression de la mélatonine en hiver, que ce soit en réponse à de la lumière de faible (300 lux) ou de forte (2200 lux) intensité, ce qui concorde avec une augmentation de la sensibilité circadienne après une exposition chronique à une faible illumination. Les sujets montraient en outre un délai de phase circadienne d'environ une heure en hiver comparé à l'été. Toutefois, ces résultats doivent être considérés comme préliminaires en raison du petit nombre de participants (n=4).

Deux études additionnelles ont comparé la suppression de mélatonine en été et en hiver chez des sujets normaux. La première n'a trouvé aucune différence entre les deux saisons, que ce soit en réponse à un stimulus de faible (300 lux) ou forte (2000 lux) intensité (Thompson et al., 1990), et la deuxième rapporte une suppression plus grande en hiver avec

un stimulus de 500 lux, mais chez les femmes seulement (Lewy et al., 1997). Tel que souligné par Boulos et Terman (1998), le modèle saisonnier a ses lacunes puisque les habitudes de vie des sujets peuvent produire des variations d'exposition qui ont peu à voir avec le cycle saisonnier de la disponibilité de lumière naturelle et qui pourraient avoir un impact important sur les mesures de sensibilité à la lumière. Finalement, il est à noter qu'aucune de ces études n'a rapporté la quantité de lumière à laquelle les sujets étaient exposés au cours des journées précédant les mesures.

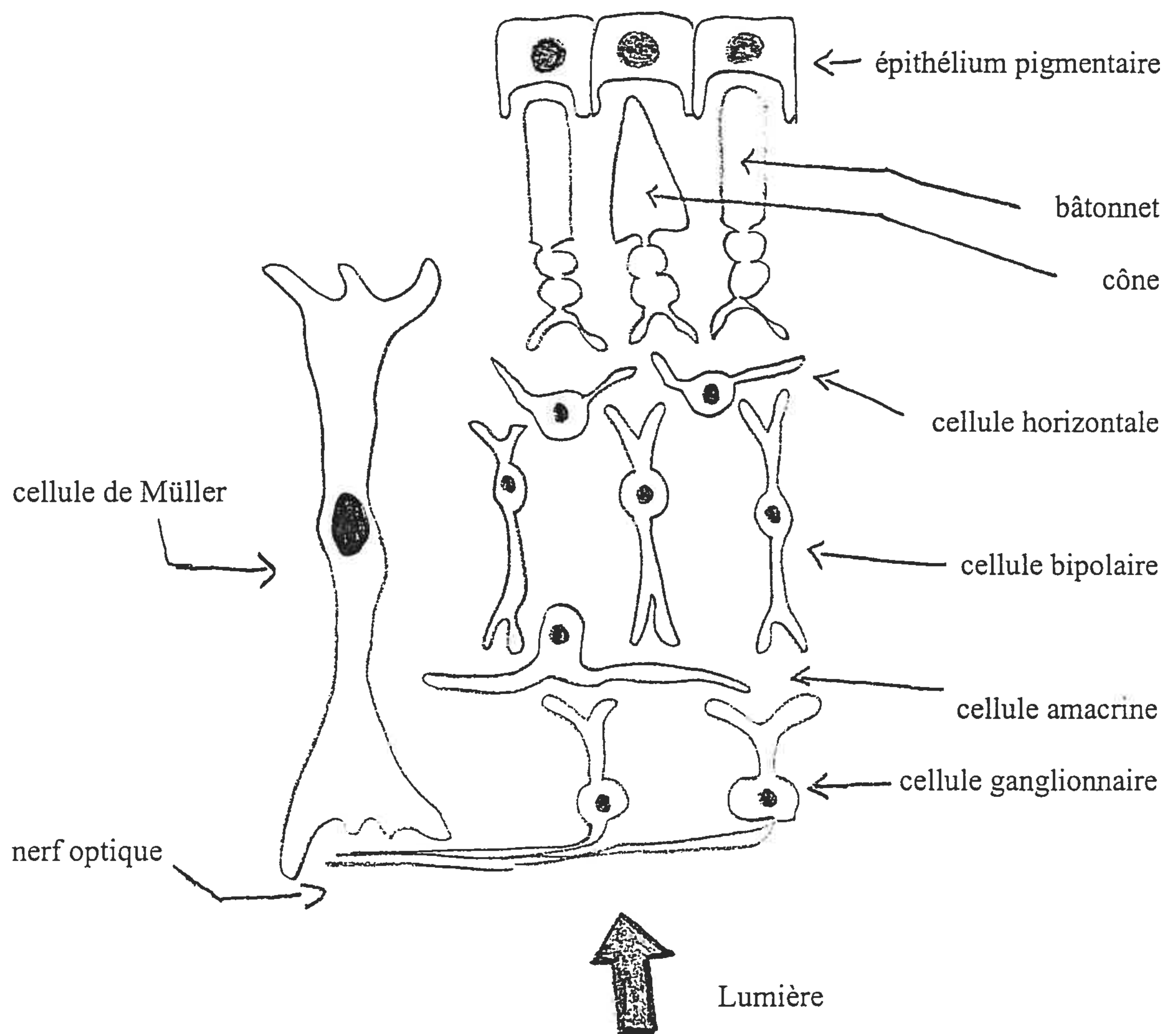
1.2. Rétine et photoréception circadienne

Depuis quelques années, l'oeil n'est plus seulement perçu comme l'organe de la vue mais également comme l'instrument essentiel à l'entraînement du système circadien. En effet, on a d'abord appris qu'une énucléation abolissait toute réponse circadienne à la lumière chez le rat (Foster et al., 1991; Foster, 1998) ainsi que chez l'humain (Czeisler et al., 1995). Une équipe de chercheurs (Campbell & Murphy, 1998) a bien avancé qu'une réponse du système circadien était possible avec une exposition à la lumière dans la région poplitée (arrière des genoux), mais ce résultat a été démenti par plusieurs études subséquentes (Hébert et al., 1999; Eastman et al., 2000; Lindblom et al., 2000; Wright & Czeisler, 2002). Après plus d'une décennie de recherche, on sait maintenant que la rétine agit comme senseur de la lumière ambiante et transmet ainsi l'information sur la quantité de lumière dans l'environnement aux NSC (Van Gelder, 2003).

1.2.1. Notions d'anatomie et de physiologie de la rétine

La rétine est le tissu oculaire responsable de la réception lumineuse (voir schéma de la rétine qui suit ce paragraphe). La rétine humaine est inversée, c'est-à-dire que la lumière traverse la rétine en entier avant de stimuler les photorécepteurs, nommément les cônes et les bâtonnets. Les cônes sont les photorécepteurs responsables de la vision diurne (photopique), c'est-à-dire de la vision en milieu éclairé. Ils couvrent l'ensemble de la rétine mais leur densité est maximale dans la macula, c'est-à-dire dans la rétine centrale. Les cônes permettent la vision des couleurs, il en existe donc trois types : les cônes-S (en anglais : « short-wavelength ») sont sensibles aux courtes longueurs d'onde (i.e. bleu), les cônes-M (en anglais : « middle-wavelength ») sont sensibles aux moyennes longueurs d'onde (i.e. vert) et les cônes-L (en anglais : « long-wavelength ») sont sensibles aux longues longueurs d'onde (i.e. rouge). Les bâtonnets sont spécialisés dans la vision nocturne (scotopique), c'est-à-dire la vision en milieu sombre. Ils sont essentiellement localisés dans la rétine périphérique et sont absents de la fovéa (centre de la macula). Les bâtonnets ont une meilleure sensibilité à la lumière que les cônes et elle se concentre dans les courtes longueurs d'onde. Leur signal est également plus amplifié que celui des cônes, c'est-à-dire qu'il converge pour augmenter davantage la sensibilité du système. La voie des cônes, quant à elle, transmet le signal lumineux avec peu de convergence ce qui favorise une meilleure acuité. Le signal lumineux en provenance des deux types de photorécepteurs passe par les cellules bipolaires puis par les cellules ganglionnaires pour se rendre au cortex

visuel, et peut également voyager latéralement dans la rétine par les cellules horizontales et amacrines. Finalement, les cellules de Müller sont des cellules gliales, elles ont un rôle de soutien mais peuvent également participer au transfert d'ions suite à une stimulation lumineuse. Ce transfert d'ions est le constituant majeur de l'électrorétinogramme, il génère l'onde-b (Risse, 1999).



1.2.2. Les photorécepteurs classiques : les cônes et les bâtonnets

Étant donné que l'entraînement circadien passe par l'œil chez les mammifères, il était tentant pour plusieurs de penser que les photorécepteurs responsables de la vision, c'est-à-dire les cônes et les bâtonnets, soient également responsables de la photoréception circadienne. Les premiers mammifères qui ont été utilisés pour vérifier cette hypothèse furent les souris *rd/rd* atteintes de dégénérescence rétinienne. Ces animaux qui n'avaient plus de bâtonnets et un nombre très réduit de cônes ont démontré des réponses circadiennes à la lumière (suppression de la sécrétion de mélatonine) identiques à celles des normaux (Foster et al., 1991; Provencio et al., 1994). Des souris *rdta cl* et *rd/rd cl* présentant une perte complète des bâtonnets et des cônes ont par la suite été étudiées (Freedman et al., 1999; Lucas & Foster, 1999a; Lucas et al., 1999). Ces deux génotypes ont maintenu leur capacité à s'entraîner avec le cycle lumière-obscurité, ce qui démontre une sensibilité inaltérée du système circadien à la lumière, et ce, malgré l'absence de photorécepteurs. De plus, suite à l'énucléation de ces souris mutantes, les réponses circadiennes à la lumière étaient abolies. Ces études ont été suffisantes pour signifier clairement que la photoréception circadienne n'est pas effectuée principalement par les photorécepteurs conventionnels mais bien par un nouveau récepteur oculaire.

Une étude subséquente effectuée sur des patients ayant perdu la vue suite à différentes pathologies a démontré une suppression de la sécrétion de mélatonine par la lumière chez

certains d'entre eux (Czeisler et al., 1995). Ces mêmes patients n'ont montré aucune suppression de mélatonine lorsqu'on les a testé avec les yeux bandés. Toutefois, on est en droit de se demander si ces patients avaient vraiment perdu toute fonction de leurs photorécepteurs. En effet, l'absence de réponse à l'électrorétinogramme, un des critères utilisés par les auteurs de cette étude pour diagnostiquer une cécité totale, ne signifie pas nécessairement une perte complète de la vision. Certains patients souffrant, par exemple, de rétinite pigmentaire peuvent montrer un ERG plat en condition scotopique et photopique tout en conservant une acuité quasi-normale et, donc, une certaine fonction visuelle (Lachapelle, 1990). Par ailleurs, une autre étude a montré que la presque totalité des patients « non-voyants capables d'une perception lumineuse » avait un rythme de sécrétion de mélatonine entraîné sur le cycle lumière-obscurité alors que la majorité des gens incapables d'une telle perception n'était pas entraînée (Lockley et al., 1997).

Dans une étude chez des sujets normaux, on a procédé à une exposition nocturne à de la lumière de couleur bleue, verte et rouge (Morita et al., 1995). Des effets inhibiteurs sur la sécrétion de mélatonine et sur la baisse nocturne de température corporelle se sont manifestés avec le bleu et le vert mais non avec le rouge, qui était comparable à la situation contrôle. Ces mêmes auteurs (Morita & Tokura, 1998) ont par la suite suggéré que les cônes-M (« middle wavelengths ») puissent être responsables de l'entraînement circadien chez l'humain étant donné que l'amplitude de la réponse suivait la sensibilité spectrale de ce type de cône. Plus récemment, une autre équipe (Brainard et al., 2001a) a mesuré la

sensibilité du système circadien par une suppression de mélatonine en réponse à une lumière de 555 nm, qui représente la sensibilité combinée des trois types de cônes chez l'humain, et l'a comparée à une lumière de 505 nm. Cette dernière longueur d'onde a engendré une réponse quatre fois plus importante que celle de 555 nm, soit une suppression de 60% et de 15%, respectivement. Les auteurs avaient alors conclu que la rhodopsine demeurait un photopigment candidat pour la photoréception circadienne chez l'humain.

Dernièrement, deux études (Thapan et al., 2001; Brainard et al., 2001b) ont utilisé une nouvelle technique très efficace dans l'identification d'un photopigment responsable d'une réponse biologique : le spectre d'action. On détermine le spectre d'action en comparant la quantité de lumière requise pour obtenir le même effet biologique, ici la suppression de la sécrétion de mélatonine, à différentes longueurs d'onde. En comparant le spectre d'action de cette suppression aux spectres d'absorption des différents types de photopigments, on obtient celui qui est le plus probable d'engendrer une telle réponse. Les deux études sont unanimes pour dire que le photopigment responsable de la réponse circadienne n'est ni la rhodopsine des bâtonnets (λ_{max}= 496 nm), ni les différentes opsines des trois types de cônes (cônes-S, λ_{max}= 419 nm; cônes-M, λ_{max}= 531 nm; cônes-L, λ_{max}= 558 nm) chez l'humain. En effet, la réponse maximale est observée à 459 nm (Thapan et al., 2001) et à 464 nm (Brainard et al., 2001b), respectivement. Ceci laisse envisager que le photopigment responsable de la photoréception circadienne chez l'humain en soit un nouveau.

1.2.3. Les cryptochromes

Parmi les photopigments suggérés comme étant responsables de l'entraînement du système circadien chez les mammifères, on retrouve des protéines ressemblant aux photorécepteurs des plantes et qui sont sensibles à la lumière bleue, soient les cryptochromes. En effet, des gènes qui encodent deux protéines ressemblant aux cryptochromes existent chez les mammifères, incluant l'humain, et sont exprimés dans presque tous les tissus étudiés (Van der Spek et al., 1996). Ces cryptochromes 1 et 2 (CRY1 et 2) sont faits à partir de la vitamine B_2 contrairement aux opsines des cônes et des bâtonnets qui sont fabriquées à base de vitamine A. CRY1 et 2 ont récemment été identifiés dans les couches nucléaire interne et ganglionnaire de la rétine humaine, CRY2 y étant sur-représenté par rapport à CRY1 (Miyamoto & Sancar, 1998; Thompson et al., 2003). L'inverse est par contre observé dans les NSC. Par ailleurs, il a été découvert que l'absence de CRY2 chez la souris n'affecte pas l'entraînement du système circadien au cycle lumière-obscurité mais change légèrement le phénotype circadien, par exemple, en augmentant l'amplitude des changements de phase circadienne induits par la lumière (Thresher et al., 1998). CRY2 ne semble donc pas être une composante essentielle à la photoréception circadienne.

Il est également important de noter que la distribution des cellules ganglionnaires qui expriment CRY1 et 2 n'est pas la même que celle des cellules ganglionnaires formant la VRH (Lucas & Foster, 1999b). En fait, la proportion de cellules exprimant les

cryptochromes est de beaucoup supérieure à celle composant la VRH. De plus, un autre fait qui va à l'encontre d'une implication des cryptochromes dans la photoréception circadienne est la quasi omniprésence des CRY1 et 2 dans les tissus mammifères (Van der Spek et al., 1996). En effet, l'observation que l'entraînement circadien soit interrompu par l'énucléation va à l'encontre de la présence de photorécepteurs circadiens au niveau extraoculaire (Lucas & Foster, 1999b). Finalement, le spectre d'absorption des cryptochromes obtient une très mauvaise corrélation avec les spectres d'action de la réponse circadienne à la lumière (Lucas & Foster, 1999c; Thapan et al., 2001; Brainard et al., 2001b).

Néanmoins, un nouveau rôle est de plus en plus évoqué pour les cryptochromes des mammifères. En effet, des souris dépourvues de l'un ou l'autre des cryptochromes demeurent capables d'entraînement circadien alors que l'absence des deux cryptochromes rend l'oscillateur arythmique (Van der Horst et al., 1999). D'autres équipes (Griffin et al., 1999; Kume et al., 1999) ont récemment validé l'hypothèse que les cryptochromes soient en fait des composantes essentielles à l'oscillateur circadien et fassent ainsi partie de la machinerie moléculaire de l'horloge comme telle (Provencio et al., 2000).

1.2.4. La mélanopsine

Avec l'information provenant des spectres d'action de la réponse circadienne à la lumière (Lucas & Foster, 1999c; Thapan et al., 2001; Brainard et al., 2001b), il est devenu de plus en plus évident que les photopigments responsables de l'entraînement circadien chez les mammifères soient des photopigments faits à base de vitamine A, tels que les opsines. La principale opsine qui est reconnue à l'heure actuelle pour ses fonctions dans le système circadien est la mélanopsine. Elle a d'abord été découverte chez la grenouille et le poisson (Provencio et al., 1998; Soni et al., 1998), entre autres dans des cellules de la rétine. La mélanopsine a plus récemment été observée chez l'humain où elle n'est présente que dans la rétine interne soit dans les couches nucléaire interne et ganglionnaire (Provencio et al., 2000). De façon étonnante, la mélanopsine des mammifères ressemble davantage aux opsines des invertébrés qu'à celle des vertébrés en ce qu'elle retient son chromophore rétinaldéhyde après la photo-isomérisation de la configuration 11-*cis* à all-*trans* (Provencio et al., 2000). Ainsi, elle ne requiert pas la présence d'un tissus régénérateur de chromophore tel que l'épithélium pigmentaire rétinien. La distribution et le nombre des cellules à mélanopsine chez la souris laissaient entrevoir la possibilité que celles-ci correspondent aux cellules formant la VRH (Provencio et al., 2000). Ceci s'est confirmé par la suite dans deux études effectuées chez le rat où la grande majorité des cellules ganglionnaires formant la VRH contenaient de la mélanopsine, et vice-versa (Gooley et al., 2001; Hannibal et al., 2002). Le 20% de cellules de la VRH n'exprimant pas de mélanopsine pourrait recevoir

exclusivement de l'information des photorécepteurs visuels ou posséder d'autres types de photopigments (Belenky et al., 2003).

La mélanopsine semble donc être une candidate de choix pour la photoréception circadienne chez les mammifères étant donné que les cellules ganglionnaires de la VRH paraissent être les seules à en posséder. Ceci amène une nouvelle notion, c'est-à-dire la possibilité de phototransduction par une certaine catégorie de cellules ganglionnaires rétiniennes. Hattar et collaborateurs (2002) ont en effet montré que, chez le rat, les cellules ganglionnaires capables d'une photosensibilité intrinsèque sont celles qui contiennent de la mélanopsine, et vice versa. Ils ont également confirmé que ces cellules à mélanopsine projettent aux NSC mais également aux feuillets intergeniculés. Ces cellules ganglionnaires ont la particularité de dépolariser en réponse à la lumière même lorsque les synapses les reliant aux cônes et aux bâtonnets sont bloquées ou encore lorsqu'elles sont complètement isolées (Berson et al., 2002). Le spectre d'action de ces cellules démontre une sensibilité maximale à 484 nm ce qui se rapproche beaucoup du maximum observé pour la suppression de mélatonine chez l'humain, soit entre 446 et 477 nm (Brainard et al., 2001b). Berson et collaborateurs (2002) précisent d'ailleurs à ce sujet qu'une différence entre les courbes physiologique et comportementale peut être due à un certain filtrage spectral par le cristallin ou par toute autre structure.

1.2.5. Le réseau neuronal de la photoréception circadienne

Même si la mélanopsine est actuellement considérée comme étant le principal photopigment circadien, les photorécepteurs conventionnels, soit les cônes et les bâtonnets, ne devraient pas d'emblée être éliminés du réseau neuronal circadien (Hattar et al., 2003; Berson, 2003; Van Gelder, 2003). D'une part, les souris sans bâtonnets et sans cônes ont besoin d'une intensité lumineuse plus forte pour s'entraîner au cycle lumière-obscurité et présentent des changements de phase plus modestes que les animaux contrôle (Yoshimura et al., 1994; Mrosovsky, 2003). D'autre part, les souris sans mélanopsine conservent un entraînement circadien normal quoiqu'elles démontrent une diminution de changements de phase par rapport aux normaux (Panda et al., 2002; Ruby et al., 2002). Finalement, on apprenait tout récemment que lorsque l'on combine l'absence de bâtonnets et de cônes à l'absence de mélanopsine, l'entraînement et la suppression de la synthèse de mélatonine sont abolis (Hattar et al., 2003; Panda et al., 2003).

Par ailleurs, il n'est pas encore clair si l'information provenant des photorécepteurs classiques passe par les cellules ganglionnaires photosensibles (Panda et al., 2003). Une étude récente a rapporté que la sensibilité spectrale, les seuils de détection et les temps d'adaptation à la noirceur et à la lumière des neurones des NSC chez le rat en conditions scotopiques et photopiques correspondaient aux propriétés des réponses des bâtonnets et des cônes (Aggelopoulos & Meissl, 2000). Il semble donc que les photorécepteurs

classiques modulent, en partie du moins, la réponse des NSC à la lumière. De plus, on a récemment démontré que les cellules ganglionnaires photosensibles possèdent des liens synaptiques avec des cellules bipolaires et amacrines et, donc, seraient reliées aux voies visuelles conventionnelles (Belenky et al., 2003). Ces cellules bipolaires paraissent reliées à la voie des cônes plutôt qu'à celle des bâtonnets. Ainsi, une réponse normale du système circadien à la lumière semble nécessiter l'interaction de la voie visuelle classique et de celle des cellules ganglionnaires photosensibles et de la mélanopsine (Van Gelder, 2003). En conséquence, il est pertinent, dans le cadre d'études sur les modalités de l'entraînement circadien par la lumière, d'utiliser des mesures électrophysiologiques comme l'électrorétinogramme (ERG) qui ont l'avantage d'être objectives et d'évaluer de façon préférentielle la réponse de certains types de cellules rétiniennes à la lumière.

1.3. Mesures électrophysiologiques de la fonction rétinienne

1.3.1. L'électrorétinogramme (ERG)

L'ERG est un biopotentiel représentant l'activité électrique générée par la rétine en réponse à des stimuli lumineux. Ce signal est composé de plusieurs ondes distinctes qui proviennent de diverses parties de la rétine. La réponse ERG typique consiste en une déflection négative identifiée comme l'onde-a suivie d'une large déflection positive appelée l'onde-b. L'onde-a reflète l'activité des photorécepteurs et l'onde-b, celle des cellules bipolaires et de Müller.

L'amplitude de l'onde-b, qui est le plus souvent utilisée comme indicateur de la fonction rétinienne, est mesurée du creux de l'onde-a au sommet de l'onde-b. Il existe deux types d'ERG : l'ERG scotopique qui mesure la vision nocturne, c'est-à-dire la réponse des bâtonnets, et l'ERG photopique qui mesure la vision diurne, c'est-à-dire la réponse des cônes.

1.3.2. Fonction luminance-réponse

À l'origine, la technique d'ERG consistait à mesurer une onde en réponse à une intensité lumineuse standard et unique dans le but de poser un diagnostic de normalité ou d'anormalité (Marmor & Zrenner, 1998; Lachapelle et al., 2001). L'évaluation électrophysiologique était alors basée sur l'amplitude, le temps de culmination et, plus rarement, sur la morphologie de l'onde ERG. Malheureusement, ces mesures sont ponctuelles et statiques. Elles ne représentent qu'une partie d'un ensemble complexe et reflètent peu le processus dynamique qu'est la vision. De plus, l'amplitude de l'ERG est très sensible aux variations intra-individuelles comme la grandeur de la pupille et la position des électrodes et aux variations inter-individuelles telles que la dimension de l'œil, la réfraction ainsi que le sexe et l'âge du sujet. La technique d'enregistrement de l'ERG scotopique s'est développée au fil des années de façon à obtenir un diagnostic plus précis, basé non seulement sur l'amplitude mais aussi sur l'intensité lumineuse nécessaire pour enregistrer une réponse donnée. En effet, l'enregistrement d'une courbe luminance-réponse,

c'est-à-dire l'utilisation d'éclairs lumineux de différentes intensités, permet une mesure relative de la sensibilité rétinienne qui, contrairement à une mesure statique, facilite la comparaison des résultats obtenus d'un sujet à l'autre ou d'une condition à l'autre. On utilise généralement la courbe de Naka-Rushton pour modéliser les données brutes d'amplitude de l'onde-b en fonction de l'intensité des stimuli (Naka & Rushton, 1966; Peachey et al., 1989; Evans et al., 1993; Hébert et al., 1996). Un déplacement de la courbe vers des intensités plus fortes signifie une baisse de sensibilité et un déplacement vers des intensités plus faibles signifie une augmentation de celle-ci. De façon plus précise, la sensibilité rétinienne (K) est habituellement déterminée par l'intensité nécessaire pour obtenir une réponse équivalente à la moitié de l'amplitude maximale de l'onde-b (V_{max}).

En ce qui a trait à l'ERG photopique, l'enregistrement de la courbe luminance-réponse est beaucoup moins répandu (Peachey et al., 1992). En effet, ce n'est que récemment que le terme « photopic hill » a été utilisé pour la première fois par Wali et Leguire (1992, 1993) pour décrire la fonction luminance-réponse de l'ERG photopique chez l'humain. Le terme « hill » ou « colline » illustre le fait que l'amplitude de l'onde-b augmente d'abord graduellement avec l'intensité du stimulus lumineux puis, en continuant d'augmenter l'intensité, une diminution de l'amplitude de l'onde-b est observée. L'enregistrement d'un ERG photopique avec une seule intensité de stimulus pourrait donc facilement mener à un faux diagnostic ou à de fausses interprétations. En effet, un ERG ayant une amplitude réduite par rapport au critère de normalité n'a pas la même signification selon qu'il soit en

début ou en fin de photopic hill. Dans la portion ascendante du photopic hill, une amplitude réduite signifie une baisse de sensibilité alors que dans la portion descendante, elle peut indiquer une augmentation de sensibilité, la « colline » étant alors déplacée vers les intensités plus faibles. Somme toute, l'utilisation de courbes luminance-réponse est donc avantageuse dans le cadre d'études sur les modalités de la fonction visuelle en permettant une évaluation précise de la sensibilité rétinienne en condition scotopique et photopique.

1.3.3. Variations diurnes de l'ERG

Étant donné que la rétine est la porte d'entrée de l'entraînement circadien, il est intéressant d'étudier ses changements physiologiques au cours de la journée. Des variations diurnes de la fonction rétinienne telle que mesurée par l'ERG ont déjà été rapportées chez l'humain. Nozaki et coll. (1983) ont observé un rythme diurne de l'amplitude de l'onde-b en condition scotopique mixte (bâtonnet-cône) chez plus de 60% de leurs sujets, l'amplitude minimale étant enregistrée tôt le matin. Birch et coll. (1984, 1986) ont rapporté un rythme de 24 heures dans l'amplitude de l'onde-b scotopique suite à un entraînement sur un cycle de 14 h lumière / 10 h obscurité. La plus faible amplitude était observée à 09h30, un moment associé par les auteurs au pic diurne du renouvellement des disques des bâtonnets. Récemment, Hankins et coll. (1998, 2001) rapportaient une augmentation du temps de culmination de l'onde-b photopique la nuit comparée au jour chez des sujets gardés en

condition de lumière naturelle. Ceci concorde également avec une variation diurne de l'ERG chez l'humain.

Ces variations d'ERG pourraient être la conséquence du cycle lumière-obscurité ou celle du rythme éveil-sommeil. Toutefois, plusieurs études ont montré des variations diurnes de l'ERG chez des animaux maintenus en conditions environnementales constantes (White & Hock, 1992; Shaw et al., 1993; Lu et al., 1995; McGoogan & Cassone, 1999; Miranda-Anaya et al., 2000). Il est donc possible que les variations diurnes des fonctions rétiniennes soient sous le contrôle d'un oscillateur circadien endogène. A cet effet, certains chercheurs avancent l'hypothèse de la présence d'un oscillateur circadien rétinien chez les mammifères car plusieurs rythmes endogènes, entre autres un rythme de sécrétion de mélatonine, ont été observés dans des rétines isolées et chez des animaux avec lésion des NSC (Remé et al., 1991; Terman et al., 1993; Tosini & Menaker, 1996, 1998; Tosini, 2000). Il semble donc que la sécrétion rythmique de mélatonine par la rétine soit d'origine endogène, ce qui en fait un candidat plausible pour expliquer les variations diurnes de la fonction rétinienne. A ce propos, Dubocovich (1988) a déjà suggéré que les interactions entre la dopamine et la mélatonine rétiniennes puissent contrôler la sensibilité rétinienne à la lumière.

1.4. Rôle de la mélatonine dans la rétine

Selon la plupart des études, la sécrétion de mélatonine dans la rétine des mammifères aurait lieu dans la couche des photorécepteurs (Niki et al., 1998; Tosini & al., 1998), les cônes étant les candidats les plus probables (Tosini, 2000; Tosini & Fukuhara, 2003). Par ailleurs, la présence de récepteurs à mélatonine de type 1 (MT_1) dans des rétines humaines a tout récemment été démontrée. Le marquage se situait principalement dans les segments internes des photorécepteurs et dans les cellules horizontales, amacrines et ganglionnaires (Meyer et al., 2002; Scher et al., 2002, 2003). La mélatonine rétinienne a été impliquée dans une multitude de phénomènes physiologiques pouvant favoriser la voie des bâtonnets ou vision scotopique au détriment de celle des cônes ou vision photopique. Ces phénomènes incluent l'élongation des cônes (Pierce & Besharse, 1985), l'agrégation des pigments de mélanine dans l'épithélium pigmentaire (Pang & Yew, 1979; Kemali et al., 1986), la suppression de la formation des spinules des cellules horizontales lors de l'adaptation à la lumière (Behrens et al., 2000), l'amorçage du renouvellement des disques des bâtonnets lors d'une exposition lumineuse (Besharse & Dunis, 1983; White & Fisher, 1989) et la protection des bâtonnets contre un stress oxidatif (Marchiafava & Longoni, 1999). De façon générale, la mélatonine est associée à l'adaptation à la noirceur et à la portion nocturne du nycthémère alors que la dopamine est associée à l'adaptation à la lumière et à la période diurne (Remé et al., 1991; Zawilska, 1994; Tosini, 2000; Berhens et al., 2000; Doyle et al., 2002; Tosini & Fukuhara, 2003). Plusieurs études ont démontré cette

dualité mélatonine / dopamine dans la rétine. Ainsi, il a été établi que la mélatonine inhibe la sécrétion de dopamine (Dubocovich, 1983; Nowak et al., 1989; Boatright et al., 1994) et participe à la régulation du rythme circadien de cette dernière (Doyle et al., 2002). Étant donné ces nombreuses fonctions rythmiques et/ou périodiques attribuées à la mélatonine rétinienne, il est possible d'envisager qu'elle puisse également être impliquée dans les variations diurnes de l'ERG.

Chez l'humain, il est impossible de mesurer directement la sécrétion de mélatonine rétinienne. Toutefois, avec le développement de techniques de radio-immunologie hautement sensibles, il est possible de déterminer de façon répétée et non-invasive la concentration de mélatonine circulante dans la salive. Cette mesure reflète adéquatement la concentration plasmatique de la mélatonine pinéale (Voultsios et al., 1997). Même si la relation de phase entre la mélatonine pinéale et rétinienne est toujours incertaine chez l'humain, les deux épisodes de sécrétion sont essentiellement nocturnes. De plus, comme pour la mélatonine pinéale, la sécrétion de mélatonine par la rétine est fortement supprimée par une exposition à la lumière chez la grande majorité des animaux étudiés. Finalement, les deux rythmes circadiens sont entraînés de façon semblable par le cycle lumière-obscurité (Tosini, 2000; Tosini & Fukuhara, 2003). Il est donc raisonnable d'assumer que les deux rythmes de sécrétion de mélatonine aient la même phase circadienne sous un cycle lumière-obscurité normal et que le rythme de mélatonine circulante puisse être utilisé pour estimer le rythme de sécrétion de mélatonine rétinienne.

1.5. La photostasie

En plus de présenter des rythmes diurnes et circadiens, la rétine montre également des changements physiologiques à plus long terme. En effet, il a été montré chez le rat que la structure et la composition des photorécepteurs sont modifiées en réponse aux changements de luminosité ambiante. Ce processus est appelé photostasie (en anglais : « photostasis »). Ainsi, la physiologie rétinienne changerait selon l'environnement lumineux pour assurer l'absorption d'une quantité constante de photons par jour (Boulos & Terman, 1998). En effet, plus le milieu est illuminé, plus la longueur des segments externes des bâtonnets et leur contenu en rhodopsine diminuent (Battelle & LaVail, 1978; Penn & Williams, 1986). Ces changements sont observables dès la deuxième semaine d'exposition à un nouveau régime lumineux. En outre, les mêmes modifications rétiniennes ont été observées lorsque la variation s'effectue au niveau de la durée de la photopériode plutôt que de l'intensité lumineuse comme telle (Parker & Williams, 1995). Schremser et Williams (1995a et b) ont montré que le renouvellement des segments externes serait le mécanisme par lequel la photostasie s'opère; les disques qui sont fabriqués lorsque la rétine est exposée à une lumière tamisée contiennent davantage de rhodopsine que les disques synthétisés sous lumière vive. Chez le rat, le renouvellement des disques et l'adaptation au nouvel environnement lumineux seraient complétés en une dizaine de jours.

Par ailleurs, des études récentes chez le rat albinos ont montré un impact direct du régime lumineux sur l'ERG (Li et al., 2001, 2003). En effet, comparés aux animaux maintenus dans un cycle 5 lux / obscurité, les rats placés dans un cycle 400 lux / obscurité montraient une diminution graduelle de l'épaisseur de la couche nucléaire externe (celle des photorécepteurs) et de l'amplitude de l'onde-b scotopique (Li et al., 2003). A ces observations, probablement causées par le phénomène de photostasie, s'ajoutait une protection de la rétine contre une dégénérescence causée par une exposition ultérieure à une lumière très vive (24 heures à 1700 lux). Cette dégénérescence se traduit, tout comme le processus de photostasie, par une diminution de la couche des photorécepteurs et de l'amplitude de l'onde-b. La dégénérescence causée par la lumière vive se distingue de la photostasie par son irréversibilité. Ainsi, une augmentation du temps passé à 400 lux permettait de préserver une plus grande proportion de la structure et de la fonction rétinienne suite à l'exposition à cette lumière néfaste. Ces effets de photostasie et de protection contre la lumière vive étaient perceptibles après un seul jour passé à 400 lux, puis devenaient significatifs après deux jours. Un effet aussi rapide a également été observé dans une étude où la quantité de vacuoles autophagiques contenant des molécules de rhodopsine augmentait significativement dès le premier jour dans un milieu plus lumineux (3 lux vers 200 lux; Remé et al., 1999). Ces vacuoles étant un indice de l'élimination de la rhodopsine, une telle augmentation peut être considérée comme un phénomène de photostasie. Par ailleurs, il est important de rappeler que la susceptibilité rétinienne à une exposition prolongée à de la lumière vive présente une variation circadienne chez le rat

albinos; le dommage étant plus important pendant la nuit subjective que durant le jour (Organisciak et al., 2000). Finalement, le mécanisme de photostasie n'a été démontré que chez l'animal, et uniquement pour les bâtonnets jusqu'à maintenant. Il n'est toutefois pas exclu qu'un tel mécanisme existe aussi chez l'humain et pour les cônes, avec possiblement une vitesse d'ajustement plus ou moins longue (Boulos & Terman, 1998).

2. Objectifs et hypothèses

L'objectif général de cette thèse était d'approfondir les connaissances sur la sensibilité à la lumière chez l'humain. Dans un premier temps, nous avons étudié la sensibilité rétinienne à l'aide de l'électrorétinogramme (ERG) enregistré sous diverses conditions expérimentales pour en comprendre davantage les différents mécanismes. Dans un deuxième temps, nous avons examiné les modalités d'entraînement du système circadien au cycle lumière-obscurité en y appliquant la technique d'ERG et une mesure de la sensibilité circadienne à la lumière.

2.1. Perfectionnement du protocole d'ERG photopique

Dans cette série d'études, nous souhaitions améliorer et raffiner l'utilisation et la compréhension de l'ERG photopique en enregistrant des courbes luminance-réponse dans

diverses conditions expérimentales. Nous avons également examiné la pertinence clinique de notre protocole d'enregistrement.

2.1.1. Effet de l'intensité de la lumière de fond

Dans cette étude, nous avons enregistré des courbes luminance-réponse en condition photopique, ou « photopic hills », sous différentes conditions d'éclairage ambiant. Les ERG ont été obtenus avec des lumières de fond (en anglais : « background ») allant de 18 à 525 $cd.m^{-2}$. Le but de cette étude était de déterminer si l'amplitude maximale de l'onde-b variait chez un individu donné selon la condition d'éclairement. Nous supposions que cette amplitude et possiblement d'autres paramètres du « photopic hill » soient modulés par la lumière ambiante. Ces résultats sont présentés dans le premier article (section 3.1.) intitulé : « *Cone-dominated ERG luminance-response function: The Photopic Hill revisited.* »

2.1.2. Définition des paramètres et signification clinique

Dans le cadre de cette recherche, nous avons mis au point une série de paramètres qui permettent une analyse approfondie du « photopic hill ». Nous avons également appliqué cette technique à quatre patients atteints d'une héméralopie congénitale, d'une anomalie de cônes, d'une rétinopathie pigmentaire et d'une micro-ophtalmie unilatérale. Le but de cette étude était d'évaluer les variations normales et anormales des différents paramètres du

« photopic hill ». Ces résultats sont présentés dans le deuxième article (section 3.2.) intitulé : « *The photopic ERG luminance-response function (Photopic Hill): Method of analysis and clinical application.* »

2.1.3. Effet de la longueur d'onde du stimulus

Pour ce projet, nous avons enregistré des « photopic hills » avec différents stimuli lumineux. Nous avons utilisé des éclairs de trois longueurs d'onde, c'est-à-dire bleu, vert et rouge, et les avons comparés à des éclairs blancs. L'objectif de cette recherche était de déterminer si les différents paramètres du « photopic hill » variaient en fonction de la longueur d'onde des stimuli. En se basant sur les résultats obtenus dans l'étude mentionnée plus haut portant sur l'effet de la lumière de fond sur l'ERG photopique, nous supposions que la plupart de ces paramètres resteraient constants, indépendamment de la longueur d'onde des stimuli. Ces résultats sont présentés dans le troisième article (section 3.3.) intitulé : « *Modulation of the human photopic ERG luminance-response function with the use of chromatic stimuli.* »

2.2. Effet de la mélatonine sur l'ERG

Le but de cette étude était de déterminer la relation entre le moment de sécrétion de mélatonine et les variations diurnes de la fonction rétinienne telle que mesurée par l'ERG.

L'hypothèse principale était que l'épisode de sécrétion de mélatonine serait associé à une hausse de la réponse des bâtonnets (amplitude et/ou sensibilité) et à une baisse de celle des cônes étant donné que la mélatonine rétinienne est associée à la période nocturne du nycthémère. Nous avons également examiné si les variations dans la réponse des cônes pouvaient dépendre de la longueur d'onde du stimulus étant donné que nous avions précédemment découvert lors d'une étude préliminaire que l'amplitude maximale de l'onde-b variait avec la couleur du stimulus. La fonction rétinienne en condition scotopique et photopique a donc été évaluée à la même heure, en fin de soirée et en début de matinée, chez deux groupes de volontaires sains maintenus sur le même horaire éveil-sommeil mais dont le moment de sécrétion de mélatonine différait. Ceci nous offrait la possibilité de différencier l'effet potentiel de la mélatonine sur l'ERG de l'effet du moment de la journée. Ces résultats sont présentés dans le quatrième article (section 3.4.) intitulé : « *Correlating retinal function with melatonin secretion in subjects with an early or late circadian phase.*»

2.3. Effet de l'histoire lumineuse sur la sensibilité circadienne et rétinienne

Dans le troisième volet de ce programme de recherche, nous voulions examiner l'effet de l'environnement lumineux à long terme sur la sensibilité à la lumière du système circadien et de la rétine. Nous avons choisi d'étudier des populations de travailleurs se situant aux deux extrêmes : un groupe travaillant à plein temps en milieu à très faible luminosité

(milieu sans fenêtre, avec luminosité < 300 lux) et un groupe travaillant principalement à l'extérieur, où la luminosité est toujours supérieure à 1000 lux pendant la journée.

La quantité de lumière reçue était contrôlée par enregistrement ambulatoire de l'illumination pour une durée de deux semaines. La mesure principale était le pourcentage de suppression de la sécrétion de mélatonine suite à l'exposition nocturne à un stimulus lumineux de 500 lux. La sensibilité de la rétine était également mesurée, en condition scotopique et en photopique, de même que la vitesse d'adaptation à l'obscurité. Ces mesures rétiniennes devaient nous permettre d'interpréter la présence éventuelle d'une adaptation du système circadien, afin de déterminer si celle-ci pouvait être due à une modification de la sensibilité à la lumière au niveau de la rétine même. Finalement, la phase circadienne était aussi mesurée dans les deux groupes. En effet, une différence de sensibilité du système circadien à la lumière pourrait modifier le patron lumineux perçu par l'horloge circadienne et produire un changement d'angle de phase entre l'oscillateur circadien et le cycle éveil-sommeil. Un tel changement peut avoir un impact direct sur la qualité du sommeil et de la vigilance des travailleurs concernés (Dumont & Carrier, 1997).

Nos hypothèses étaient les suivantes : 1- Les travailleurs en milieu sombre auront une plus grande sensibilité circadienne que les travailleurs en milieu éclairé. Cela se traduira par un pourcentage de suppression de la sécrétion de mélatonine plus élevé lors de la présentation nocturne d'un stimulus lumineux. 2- Les travailleurs en milieu sombre auront une plus

grande sensibilité rétinienne (scotopique et/ou photopique) que les travailleurs en milieu éclairé. Cela se traduira par l'obtention d'une réponse d'amplitude maximale à l'aide d'une intensité lumineuse plus faible. Nous croyons qu'ils montreront aussi une adaptation plus rapide à la noirceur tel que démontré par la vitesse de l'augmentation de l'amplitude de l'onde-b. 3- La phase circadienne des travailleurs en milieu sombre sera plus tardive que celle des travailleurs en milieu éclairé. En effet, si la sensibilité à la lumière est augmentée et que la lumière reçue durant la journée est de très faible intensité, la lumière en soirée aura comparativement un plus grand effet sur l'oscillateur et tendra à produire un délai de la phase circadienne. Ceci se traduira par un début de sécrétion de mélatonine (DLMO) plus tardif chez les travailleurs en milieu sombre. Ces résultats sont présentés dans le cinquième article (section 3.5.) intitulé : « *Circadian and retinal sensitivity to light in association with light exposure in the work environment.* »

N.B. Une description détaillée des protocoles utilisés dans les différents volets de ce programme de recherche se trouve dans chacun des articles présentés au chapitre suivant.

3. Méthodologie et résultats : articles de recherche

3.1. Premier article

Cone-dominated ERG luminance-response function:

The Photopic Hill revisited.

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Cone-dominated ERG luminance-response function: The Photopic Hill revisited.

Marianne Rufiange, B.Sc.[1]; Sophie Rousseau, M.Sc., M.D.[2]; Olga Dembinska, Ph.D.[1,2] and Pierre Lachapelle, Ph.D.[1,2]

Departments of Ophthalmology [1] and Neurology-Neurosurgery [2], McGill University-Montreal Children's Hospital Research Institute

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Corresponding author:

Pierre Lachapelle, PhD

Department of Ophthalmology (D-164)

McGill University- Montreal Children's Hospital Research Institute

2300 Tupper Street

Montreal, Quebec, CANADA. H3H 1P3

TEL:(514) 934-4400, ext 3890

FAX:(514) 934-4331

email: pierre.lachapelle@mcgill.ca

Abstract

PURPOSE: In response to progressively brighter stimuli, the b-wave of the photopic ERG gradually augments in amplitude, reaches a plateau for a narrow range of intensities and then rapidly decreases with further increments in the luminance of the flash. This unique luminance-response function was originally introduced as the Photopic Hill. The purpose of this study was to further characterize this unique feature of the cone ERG, investigate if it was only limited to b-wave measurements and if it could be obtained under different photopic background luminances. METHODS: Photopic ERGs and oscillatory potentials were generated in response to flashes of light ranging from 0.5 to 16 cd.m^{-2}.sec in intensity and presented against photopic backgrounds varying from 18-525 cd.m^{-2} in luminance. RESULTS: All but the brightest background yielded a clear Photopic Hill like luminance-response function which could only be evidenced with the b-wave, the i-wave and OP_4 amplitude measurements. Interestingly, the maximal amplitude reached remained almost identical irrespective of the background luminance. CONCLUSIONS: Our results suggest that the retinal mechanisms at the origin of the Photopic Hill effect could represent a voltage limitation mechanism, intimately tied to the OFF pathway. The latter would however be intrinsic to the cone system only and not to the entire retinal network since significantly higher peak amplitudes are reached with dark adaptation.

Introduction

In response to progressively brighter stimuli, the b-wave of the photopic ERG gradually augments in amplitude, reaches a plateau for a narrow range of intensities and then rapidly decreases with further increments in the luminance of the flash. This unique luminance-response function was originally introduced as the Photopic Hill[1,2]. While it might be intuitively simple to suggest possible retinal mechanisms, such as recruitment and response saturation, at the origin of the first two phases of the Photopic Hill, the third segment (e.g. the decay phase) cannot be explained with similar ease. What causes the gradual decline in the ERG response? Does it reflect the activation of some postreceptoral retinal mechanism whose purpose is to limit the retinal output or does it result from the gradual depletion, as a consequence of overstimulation, of retinal elements (such as: photopigment, neuromodulators or neurotransmitters, to name a few) which are implicated in the genesis of the b-wave. The work of Wali and Leguire[1,2] did provide us with some key information in order to help in understanding this unique phenomenon. For example, they showed that identical Photopic Hills could be obtained wether the flash stimuli were presented in incremental or decremental order; results which could challenge the depletion hypothesis suggested above.

In order to further understand this unique feature of the cone ERG and possibly postulate on its origin, we obtained photopic ERG luminance-response functions generated in the presence of rod-desensitizing backgrounds varying from 18-525 cd.m^{-2}. These were compared to the luminance-response function obtained at the onset of dark-adaptation; a time where there is already some demonstrable rod contribution[3]. Data analysis included all the photopic flash ERG components, namely: a-, b- and i-waves as well as the oscillatory potentials (OPs). Our results show that: 1- a Photopic Hill like luminance-response function could only be evidenced with the b-wave i-wave and OP_4 amplitude measurements; and 2- all but the brightest background yielded a clear Photopic Hill. To our surprise however, the maximal amplitude, reached at the peak of the Hill, was identical for all backgrounds suggesting that the decay phase could represent a voltage limitation mechanism. The latter would however be intrinsic to the cone system only and not to the entire retinal network since significantly higher peak amplitudes were reached within the first minute of dark adaptation.

Methods

Preparation of subjects:

Experiments were performed on five normal subjects (aged 18-25 years old). An informed consent was obtained from all subjects after the nature and possible consequences of the

study were explained. Electroretinographic signals were recorded from both eyes, as previously reported, with a DTL electrode (X-Static® conductive yarn, Sauquoit Industries, Scranton, PA, USA) positioned deep into the conjunctival bag[4]. In order to avoid contact with the skin of the subjects, the DTL fibre was secured at the external and internal canthi with double adhesive tape. Ground and reference electrodes were pasted on the forehead and external canthi respectively. All recordings were obtained with fully dilated pupils (Tropicamide 1%).

The subjects were positioned on a head and chin rests in front of a Ganzfeld of 60 cm in diameter. The Ganzfeld also housed the rod-desensitizing background lights whose luminance could be fixed at precalibrated values of: 18, 32, 52, 90, 150 and 525 $cd.m^{-2}$. The flash stimuli were delivered with two Grass PS-22 photostimulators, also housed in the Ganzfeld, and triggered simultaneously to yield, at maximal setting, a flash of white light of 16 $cd.m^{-2}.sec$ in energy (measured with a IL 1700 Research Radiometer, International Light, Newburyport, MA, USA). ERGs (Grass P511 preamplifiers, amplification 10,000x, bandwidth 1-1000 Hz, 6dB) and oscillatory potentials (OPs: 50,000X, 100-1000 Hz, 6dB) were recorded simultaneously with the use of the Acknowledge Data Acquisition System (Biopac MP 100 WS, Biopac Systems Inc., Goleta, CA, USA) and stored on floppy disks for off-line analysis.

Experimental procedure:

In order to generate the Photopic Hills, the subjects were first light adapted for five minutes to one of the above-mentioned photopic background light, following which 30 ERG/OP responses were averaged to each of the following flash intensities: 0.5, 1, 2, 4, 8 and 16 $cd.m^{-2}.sec$ (interstimulus interval: 1.024 sec) and stored individually. The above procedure (including the pre-adaptation time) was repeated for each of the 6 predetermined photopic backgrounds. Only one recording session per subject was needed in order to secure the data.

Data analysis:

The amplitude of the a-wave was measured from baseline to the most negative trough, while the amplitude of the b-wave was measured from the trough of the a-wave to the most positive peak. The amplitude of each OP was measured from the preceding trough to the peak and reported either individually (i.e. OP_2, OP_3 and OP_4) or collectively ($SOPs=OP_2+OP_3+OP_4$). Peak times were measured from flash onset to peak. For each background luminance, the amplitude and peak time data (a-wave, b-wave and OPs) were then plotted against the flash intensities in order to examine if the previously described Photopic Hill only applies to the b-wave luminance-response function. Statistical analysis was performed with ANOVAs for paired data and Dunnet's post-hoc comparison test. The differences were considered statistically significant at $p<0.05$.

Results

The Photopic Hill of the broadband (1-1000Hz) ERG components:

At figure 1 are shown representative broadband 1-1000 Hz ERG luminance-response functions (flash intensities indicated at the left of the first set of tracings), evoked in the presence of progressively brighter backgrounds (the luminance of the background is indicated at the top, above the vertical arrows, of each set of tracings). All tracings were obtained from the same normal subject. Irrespective of background luminance one notes that, at least initially, there is a gradual increase in ERG amplitudes with progressively brighter stimuli. This is best visualised at figure 2 where the data obtained from all our subjects is presented. With progressively brighter stimuli, there is a linear increase in amplitude of the a-wave which is also seen at all backgrounds. Also of interest, one notices that dimmer backgrounds yield larger a-waves than brighter ones. Similarly, apart from the measurements obtained at the brightest background, as a rule the peak time of the a-wave shortens as the flash luminance increases (figure 3).

In contrast, and as previously reported by others[1,2], the amplitude of the b-wave behaved quite differently. As shown at figure 2, the amplitude of the b-wave first increases, then reaches a plateau which is followed by a gradual decline, a function that fits well the terminology Photopic Hill previously suggested. The unusual morphology of the

luminance-response function of the photopic ERG is best illustrated with responses obtained in the presence of the dimmer backgrounds (i.e.18 to 90 $cd.m^{-2}$). Although a peak amplitude also appears to be reached with the 150 $cd.m^{-2}$ background, the decay phase is not as well delineated. Similarly, the energy output of our flash unit was insufficient to generate a true Photopic Hill in the presence of the brightest background (i.e. 525 $cd.m^{-2}$). The intensity of the flash necessary to reach the peak of the Hill was 4 $cd.m^{-2}.sec$ at backgrounds 18 (5/5 subjects), 32 (4/5 subjects) and 52 (4/5 subjects). At background 90 $cd.m^{-2}$, it increased to 8 $cd.m^{-2}.sec$ for 3/5 subjects and, at background 150 $cd.m^{-2}$, to 16 $cd.m^{-2}.sec$ for 3/5 subjects. Notwithstanding the above, what appears to us as the most fascinating aspect of our results pertains to the maximal (V_{max}) b-wave amplitude which is reached at the peak of the Photopic Hill. Despite the fact that with the gradual increase in the luminance of the rod desensitising background there is a progressive reduction in amplitude of the b-waves which compose ascending phase of the Photopic Hill (i.e. from threshold to peak), the amplitude of V_{max}, when reached, remains, as seen at figure 2, almost identical at approximately 130 μvolts and that irrespective of the luminance of the backgrounds. This is best exemplified with the following comparisons. In response to the dimmest flash, the amplitude of the b-wave is of 63.4±18 μvolts with background 18 $cd.m^{-2}$ and 36±9 μvolts with background 90 $cd.m^{-2}$; the two values being significantly ($p<.05$) different from each other. In contrast, there is no significant ($p>.05$) difference in the amplitudes of the b-waves reached at the peak of the Photopic Hill (V_{max}); the values being 128±29 μvolts (background 18 $cd.m^{-2}$) and 135±33 μvolts (background 90 $cd.m^{-2}$)

respectively. There is however a difference in the peak time of the corresponding b-waves. As seen at figure 3, as the intensity of the flash stimulus increases, there is a progressive increase in the peak time of the b-wave which is observed at all backgrounds. However, for the same intensity of stimulation, the timing measured in presence of the dimmer backgrounds are slower than those measured with the brighter backgrounds. As a result of this relationship, the peak time of the b-wave which forms the peak of the 18 $cd.m^{-2}$ Photopic Hill (i.e. evoked to a flash of 4 $cd.m^{-2}.sec$) is slightly, but significantly (30.4±0.7 msec, 28.7±0.9 msec; $p<.05$) slower than that measured at the peak of the 90 $cd.m^{-2}$ background (evoked to a flash of 8 $cd.m^{-2}.sec$).

A similar, although not as well characterised Photopic Hill can also be evidenced with measurements of the i-wave, which is the smaller positive wave that follows the b-wave. It is interesting to note that bright flashes, delivered in the presence of the brighter photopic backgrounds (150 and 525 $cd.m^{-2}$) include another post b-wave potential that we have identified as wave i_2. Similar to the b-wave, the i-wave luminance-response function also yields a Photopic Hill presentation which is most obvious with the use of the dimmer backgrounds. However unlike the b-wave, the intensity necessary to reach the peak does not appear to vary in a predictable fashion. This could be due to the greater difficulty in getting an accurate amplitude measurement of the i-wave as a result of its proximity to the b-wave. Also of interest is to note that the decay phase of the Photopic Hill indicates that,

in the presence of the dimmest background, brighter flashes bring the amplitude of the i-wave close to extinction (7.3±3.8 μvolts). Finally, as shown with the b-wave, the maximal i-wave amplitude (V_{max}) does not appear to be influenced by the luminance of the background. This is again best exemplified with the following comparisons. In response to the dimmest flash, the amplitude of the i-wave if of 23.±11 μvolts with background 18 $cd.m^{-2}$ and 9±4 μvolts with background 90 $cd.m^{-2}$; the two values being significantly ($p<.05$) different from each other. In contrast, there is no significant ($p>.05$) difference in the amplitudes of the i-waves reached at the peak of the Photopic Hill (V_{max}); the values being 35±13 μvolts (background 18 $cd.m^{-2}$) and 29±9 μvolts (background 90 $cd.m^{-2}$) respectively.

The Photopic Hill of the oscillatory potentials:

Analysis of the luminance-response function of the oscillatory potentials was also performed. As illustrated at figure 4, irrespective of the luminance of the background, progressively brighter flashes will, at least initially, augment the number of OPs evoked. However, unlike the other ERG components (a-, b- and i-waves) the luminance-amplitude relationship of the different OPs is not as readily obvious. It would appear, as previously reported elsewhere[5], that the original OP (i.e. that with the lowest threshold: OP_2) initially grows in amplitude up to a point where a new OP is added almost as if it was sprouting from it. This new OP will then grow until it subdivides into two distinct OPs. Thus the OP

with the lowest threshold (OP_1 is not considered here) is OP_2 (for example, see response to flash 0.5 given against background 90). With progressively brighter stimuli another OP, of a slightly longer latency, sprouts from OP_2, such as seen in responses evoked to flashes of 1 and 2 $cd.m^{-2}.sec$ given against background 90. This new OP continues to grow before splitting into two distinct OPs (OP_3 and OP_4: see the sequence intensity 2, 4 and 8 at background 32 for example). It is difficult to state if, at the intermediate flash intensities (for example intensity 2 at background 32, intensity 4 at background 90, or intensity 8 at background 150), the large OP which follows OP_2 is OP_3 or OP_4. Therefore, we normally refer to it as the OP_3-OP_4 complex. Needless to say that this behaviour does complicate the analysis of the luminance-response function of individual OPs. It is for that reason that the OPs were first analyzed collectively with the SOP variable (figure 2).

When considered collectively (i.e. SOP variable) the OP luminance-response function did not yield a typical Photopic Hill similar to that found for the b- and i-waves of the broadband ERG (figure 2). Although we do note, especially in the presence of the brighter backgrounds (52, 90, 150 $cd.m^{-2}$), that with progressively brighter flashes there is a gradual increase in the amplitude of the SOP variable which appears to be followed by a plateau phase, the latter is not replaced by the decay phase which characterizes the Photopic Hills of the b- and i-waves. However a closer analysis of the waveforms shown at figure 4 suggest that, once the final splitting into the three major OPs (i.e. OP_2, OP_3, OP_4) has

occurred, individual OPs might be affected differently by the strength of the stimulus and the luminance of the background. This is best illustrated at figure 5 where the amplitudes and peak times of the three major photopic OPs (i.e. OP_2, OP_3 and OP_4) are plotted against the intensity of stimulation which produced, at backgrounds 18 and 32 $cd.m^{-2}$, a complete splitting. One can readily observe that while the amplitudes of OP_2 and OP_3 grow as the intensity of the stimulus augments, that of OP_4 is either progressively reduced (at background 18 $cd.m^{-2}$) or demonstrates a typical Photopic Hill (at background 32 $cd.m^{-2}$). Similarly, while the timing of OP_2 and OP_3, like the a-wave, shortens with brighter flashes, that of OP_4, like the b-wave, augments at both backgrounds.

The Photopic Hills described above were evidenced by plotting, for a given background luminance, the amplitude of the ERG/OP components against the intensity of the stimulus. What type of luminance-response relationship do we get if, for a given intensity of stimulation, the amplitude and peak time of the ERG components are plotted against the luminance of the background? The result of this data manipulation is shown at figures 6 and 7. Irrespective of the intensity of the stimulus, the amplitude of the a-wave decreases gradually as the luminance of the background increases. Interestingly while in response to the dimmer flashes (.5 and 1 $cd.m^{-2}.sec$), the peak time of the a-wave shortens with brighter backgrounds, the reverse is seen with brighter (4 and 8 $cd.m^{-2}.sec$) stimuli. The most interesting, and almost equivalent, luminance-response relationship is seen with amplitude

data of the b- and i-waves and SOPs. In responses evoked to the dimmer flashes there is, as expected from figure 2, a gradual decrease in amplitude of the b-wave, i-wave and SOP measurements as the luminance of the background is augmented. However as the intensity of the flash is increased, the relationship is progressively shifted so that, at intensity 8 $cd.m^{-2}.sec$ for example, the amplitude of the b-wave grows up to background 90 $cd.m^{-2}$ and then decreases. A similar shift is also seen with i-wave and SOP measurements. Finally, as shown at figure 7, irrespective of flash intensity the peak time of the b-wave always shortens as the luminance of the background brightens.

Discussion

The results presented in this study not only confirm, as previously documented elsewhere[1,2], the uniqueness of the luminance-response function of the photopic ERG b-wave, but also demonstrate that a Photopic Hill-like luminance-response function is also obtained with i-wave and OP_4 measurements. In contrast, a more linear luminance-response relationship describes the remaining ERG parameters (a-wave, OP_2 and OP_3). It should be noted that the linear relationship between the amplitude of OP_2 and the relative intensity of the stimulus (i.e. intensity of flash irrespective of background luminance), a feature which earned it the name intensity-coding oscillatory potential, was previously reported elsewhere[6]. Our study also showed that a gradual increase in the luminance of the photopic background had a minimal impact on the shape of the luminance-response, as long as the

intensity of the flash was bright enough to cause the decay phase which is characteristic of the Photopic Hill. What this manipulation did reveal however was that there was no significant difference ($p>.05$) in the amplitude of the b-wave V_{max} (which is reached at the peak of the Hill) irrespective of background luminance. In all instances where a Photopic Hill could be clearly evidenced (i.e. peak amplitude followed by a decay phase), the amplitude of the b-wave reached at V_{max} varied between 128±29 μvolts (background 18 $cd.m^{-2}$) and 135±33 μvolts (background 90 $cd.m^{-2}$). A similar consistency in peak amplitude measurements was also observed with the i-wave.

Our b-wave V_{max} amplitudes compare with the 190 μvolts reported by Wali and Leguire[1], since their ERGs were recorded with corneal contact lens electrodes while ours were obtained with DTL fibre electrodes. In a previous study we showed that ERGs obtained with the DTL fibre electrode were approximately 70% of those recorded with a contact lens electrode[6]. Thus 70% of 190 μvolts equals to 133 μvolts, a value well within the range of that reported in the present study. Their V_{max} was obtained in response to flashes of white light of 7 $cd.m^{-2}.sec$ delivered against a rod desensitizing background of 30 $cd.m^{-2}$, while we obtained the same value of V_{max} for a range of backgrounds. The only difference being that, as the luminance increased, brighter flashes were needed in order to reach the peak of the Hill. The similarity in b-wave V_{max} between our results (obtained over a wide range of backgrounds) and that of Wali and Leguire[1] could suggest the existence of some intraretinal

voltage limiting network whose purpose would be to limit the b-wave V_{max} amplitude to approximately 130 μvolts. However, it is well documented in the literature that b-wave voltages often exceeds the 130 μvolts limit reported above. For example, significantly larger ERG voltages are obtained in dark-adaptation conditions (i.e. retina dark-adapted for at least 30 minutes) as a result of the massive contribution of the rods to the genesis of the b-wave. Scotopic luminance-response functions do not however have the unusual Hill shape shown to characterize the photopic luminance-response function. We could however demonstrate a Hill shape for b-wave measurements obtained within the first minute of dark-adaptation; that is at a time when cones and rods almost equally contribute to the genesis of the ERG response[3]. As shown at figures 8 and 9, a Photopic Hill-like luminance- response function can still be evidenced for the b-wave, i-wave and to a lesser extent for the SOP variable. However a close inspection of the OP tracings reveals that, similar to what we reported for recordings obtained in a photopic environment, a gradual increase in the brightness of the flash stimulus augments the amplitude of the first two major OPs (OP_2 and OP_3) and split the last one into two smaller ones. Following this split however, unlike the results obtained in a photopic environment, brighter flashes do not yield further increase in amplitude of the last OPs. On the contrary, the amplitude of OP_4 is almost completely abolished. Also of interest is to note that, in the absence of a photopic environment the i-wave is more ill defined, with a V_{max} limited to about 15 μvolts; less then half of what is measured in photopic environment. The latter would strongly suggest a photopic nature for this post-b-wave potential.

Two important ERG features are illustrated at figure 9. First, unlike results obtained in photopic conditions, there is evidence of an early saturation of the a-wave measurements with brighter flashes. The amplitude of the a-wave evoked to the 4 $cd.m^{-2}.sec$ flash not being different from that evoked to the 16 $cd.m^{-2}.sec$ flash (81±26 μvolts, 81±19 μvolts; $p>.05$). This contrasts with measurements obtained in photopic condition (figure 2) where signs of a-wave saturation were not as readily obvious, especially in the presence of the brighter backgrounds. Also of interest is to note that the b-wave V_{max} is 198±42 μvolts (reached with a flash of 2 $cd.m^{-2}$) a value which represents a 50% increment over measurements obtained in photopic conditions. This result would suggest that the 130 μvolts b-wave maximum measured in photopic conditions, irrespective of the brightness of the background would represent a voltage limitation intrinsic to the cone pathway and not to the entire retinal network. Is there a physiological reason for this to occur and how is it achieved? As previously shown by Wali and Leguire[1], it most probably does not result from a light adaptation effect, an habituation or fatigue phenomenon, or as a consequence (due to overstimulation) of the depletion of retinal elements (such as photopigments, neuromediators,neuromodulator, etc.) so critical to a flawless transcription of the visual stimulus. They showed that an identical b-wave Photopic Hill could be obtained 1-whether the stimuli were presented in incremental or decremental order and 2- with the interflash intervals varying between 15 seconds and 5 minutes. Fundus pigmentation did however

impact on the resulting b-wave V_{max}; darker fundi yielding smaller responses[2]. What then could have such a powerful impact on the amplitude of ERG components?

In a previous study, Sieving et al.[7] introduced the Push-Pull concept to explain the genesis of the b-wave. Briefly, according to their concept the ascending phase of the b-wave would be under the control of the ON-depolarizing bipolar cells (ON-DBC) whose activity would push the b-wave to its peak amplitude and the OFF-hyperpolarizing bipolar cells (OFF-HBC) whose function would be to limit the b-wave amplitude by pulling the depolarisation back to a baseline value. We believe that our results would support a similar concept to explain the decay phase of the Photopic Hill. OP_2 and OP_3, that is those OPs which have been previously associated with the retinal ON pathway[8] do not appear to show any deterioration as a result of progressively brighter flashes. In contrast, the b-wave, OP_4 and the i-wave, are the three most severely affected ERG potentials. If as previously suggested, OP_4 (i.e. the last major OP of the photopic response) and the i-wave are generated by the retinal OFF pathway[8-10] and, as suggested by Sieving et al.[7] the OFF pathway is responsible for bringing down the b-wave potential down, then how can we explain that a progressively more energized OFF pathway yields gradually smaller responses? Although we do not yet have an adequate answer to the above question it is of interest to note that previous studies have shown that increase or decrease in amplitude of the photopic b-wave are often accompanied by a similar increase or decrease in amplitude of OP_4 and of i-wave[11-13]. It

could be that the alleged OFF contribution would in fact be of longer latency than the ON contribution and of negative polarity. The actual OFF contribution would not be visible on the ERG, but its negativity would summate with the more positive potentials. Thus a more active (and consequently more negative) OFF response would have a larger pulling effect on the ERG components and thus explain why the "OFF" ERG components are more attenuated despite an alleged enhancement of the OFF activity. Supportive of the latter explanation is the recent evidence, in the macaque ERG, of the photopic negative response; a large negativity seen immediately after the peak of the b-wave[14]. Clearly more research will be needed in order to better understand this unique feature of the cone ERG.

Acknowledgments

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Figure legends

Figure 1: Representative broadband ERG luminance-response function. The intensity of the flash stimuli are indicated at the left of the tracings (in $cd.m^{-2}.sec$) in the first group of recordings. The luminance of the rod-desensitizing background light is indicated (in $cd.m^{-2}$) at the top of each set of recordings (above the vertical arrows). a=a-wave, b=b-wave, 2, 3 identify the peak of OP_2 and OP_3 as they are normally seen on the ascending limb of the b-wave. Note that the b-wave is often followed by one or two i-waves. Vertical arrows indicate flash onset. Calibration: Vertical, 100 μvolts; horizontal, 20 milliseconds. All tracings were obtained from the same normal subject.

Figure 2: Group data for amplitude measurements (ordinate in μvolts) for a-wave, b-wave, i-wave and SOP plotted against flash intensity (abscissa in $cd.m^{-2}.sec$). The luminance of the rod desensitizing background is shown (in $cd.m^{-2}$) at the left of each row. At the bottom of each column, the curves are superposed to ease comparisons.

Figure 3: Group data of peak time measurements (ordinate in milliseconds) for a-wave and b-wave plotted against flash intensity (abscissa in $cd.m^{-2}.sec$). The luminance of the rod desensitizing background is shown (in $cd.m^{-2}$) at the left of each row. At the bottom of each column, the curves are superposed to ease comparisons.

Figure 4: Representative oscillatory potentials luminance-response function. The intensity of the flash stimuli are indicated at the left of the tracings (in cd.m^{-2}.sec) in the first group of recordings. The luminance of the rod-desensitizing background light is indicated (in cd.m^{-2}) at the top of each set of recordings (above the vertical arrows). The OPs are identified in numerical order, with OP_1 being that with the shortest latency. However given that it is often difficult to identify, data analysis will be limited to the first three major OPs namely OP_2, OP_3 and OP_4. Numerals at the right of some tracings identify the responses used at figure 5. Vertical arrows indicate flash onset. Calibration: Vertical, 30 μvolts; horizontal, 20 milliseconds. All tracings were obtained from the same subject as in figure 1.

Figure 5: Group data for amplitude (A,C; ordinate in μvolts) and peak time (B,D; ordinate in milliseconds) of the three major photopic OPs (OP_2, OP_3, OP_4) plotted against flash intensity (abscissa in cd.m^{-2}.sec). The luminance of the rod desensitizing background is shown (in cd.m^{-2}) at the top of each plot (18 cd.m^{-2}: A,B and 32 cd.m^{-2}: C,D).

Figure 6: Group data for amplitude measurements (ordinate in μvolts) for a-wave, b-wave, i-wave and SOP plotted against background luminance (abscissa in cd.m^{-2}). The intensity of the flash stimulus is shown (in cd.m^{-2}.sec) at the left of each row. At the bottom of each column, the curves are superposed to ease comparisons.

Figure 7: Group data of peak time measurements (ordinate in milliseconds) for a-wave and b-wave plotted against background luminance (abscissa in $cd.m^{-2}$). The intensity of the flash stimulus is shown (in $cd.m^{-2}$) at the left of each row. At the bottom of each column, the curves are superposed to ease comparisons.

Figure 8: Representative broadband ERG (left) and oscillatory potentials (right) luminance-response function recorded within the first minute of dark-adaptation. The tracings are presented in order of flash intensity as in figures 1 and 4 (i.e. dimmest flash at the bottom). Vertical arrows indicate flash onset. Calibration: vertical, 100µvolts (ERG) and 30µvolts (OPs); horizontal, 20 milliseconds.

Figure 9: Group data of a-wave (A), b-wave (B), i-wave (C) and SOP (D) amplitudes (ordinate in µvolts) and peak time measurements (E: a-wave, F: b-wave; ordinate in milliseconds) plotted against flash intensity (abscissa in $cd.m^{-2}.sec$). All measurements were taken at the onset of dark-adaptation (i.e. within the first minute of dark-adaptation).

Figure 1

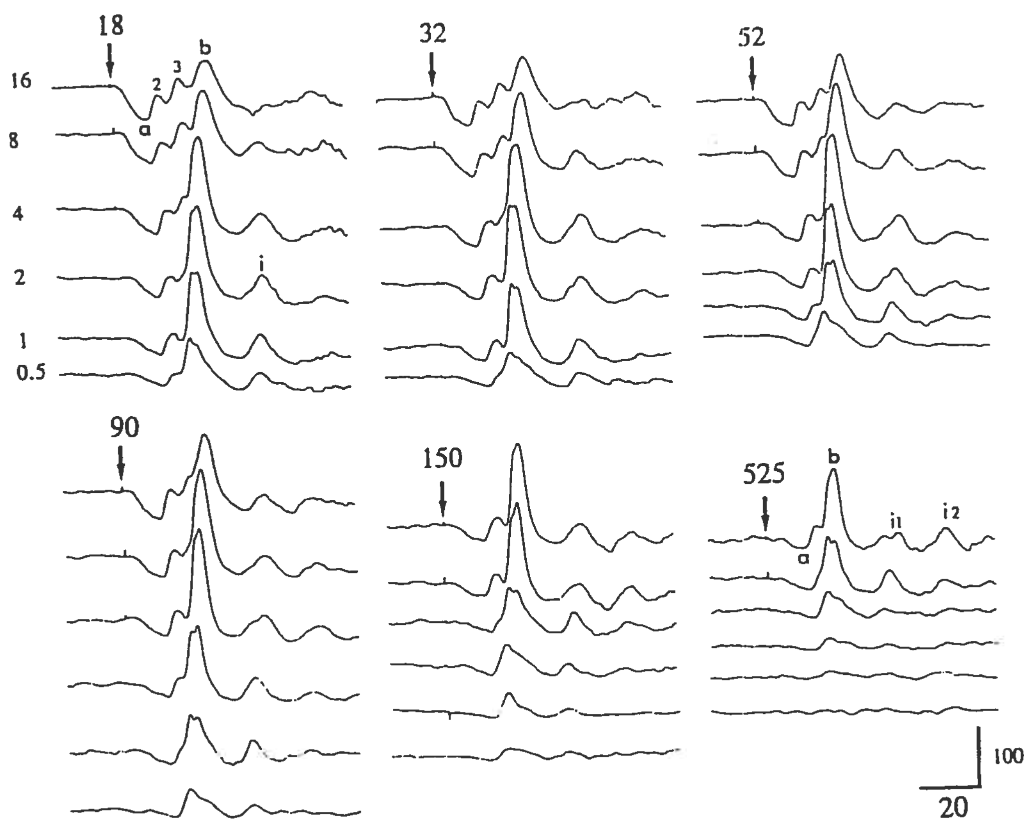


Figure 2

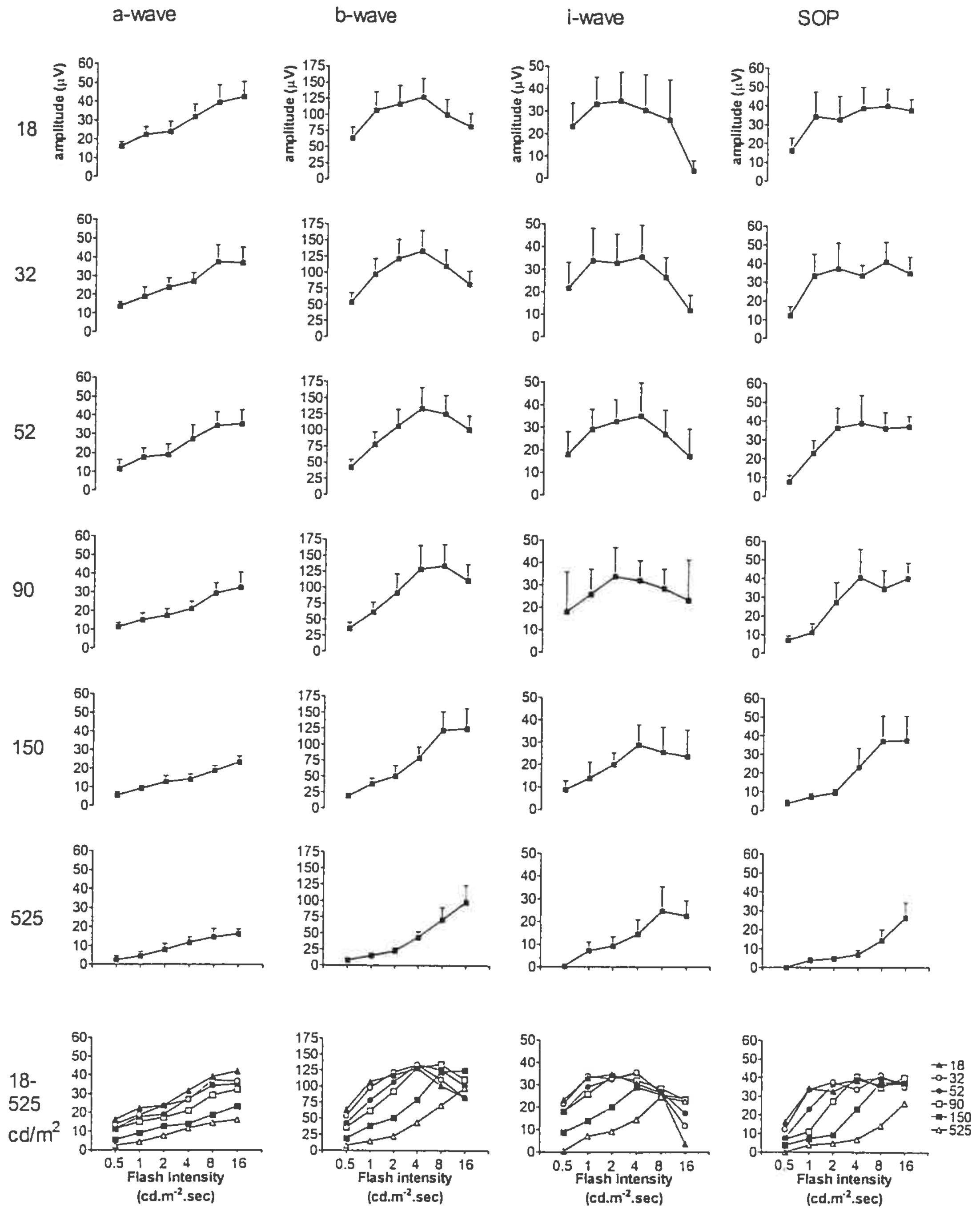


Figure 3

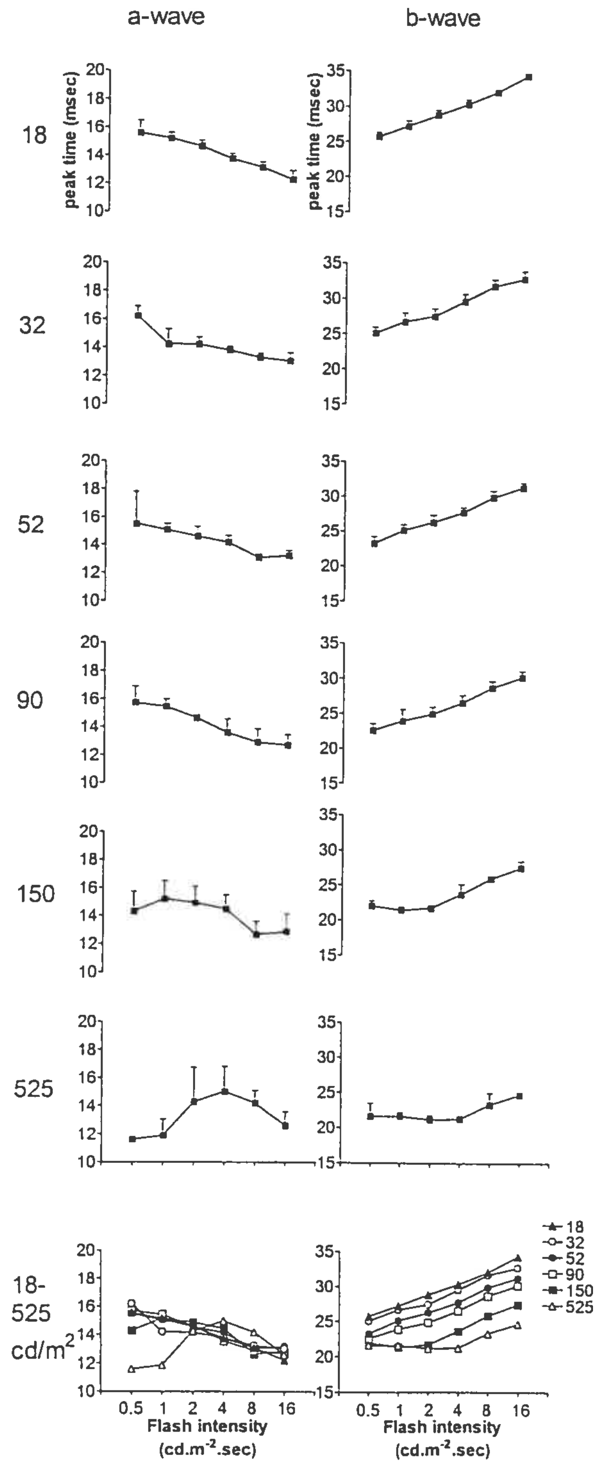


Figure 4

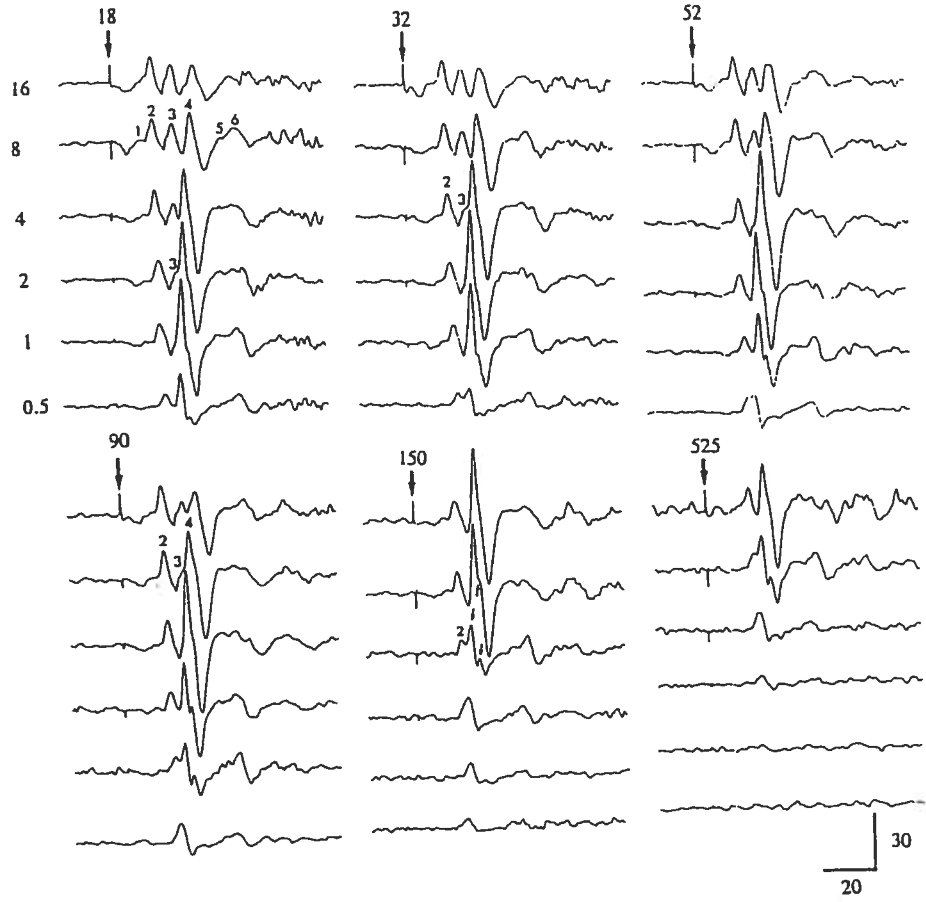


Figure 5

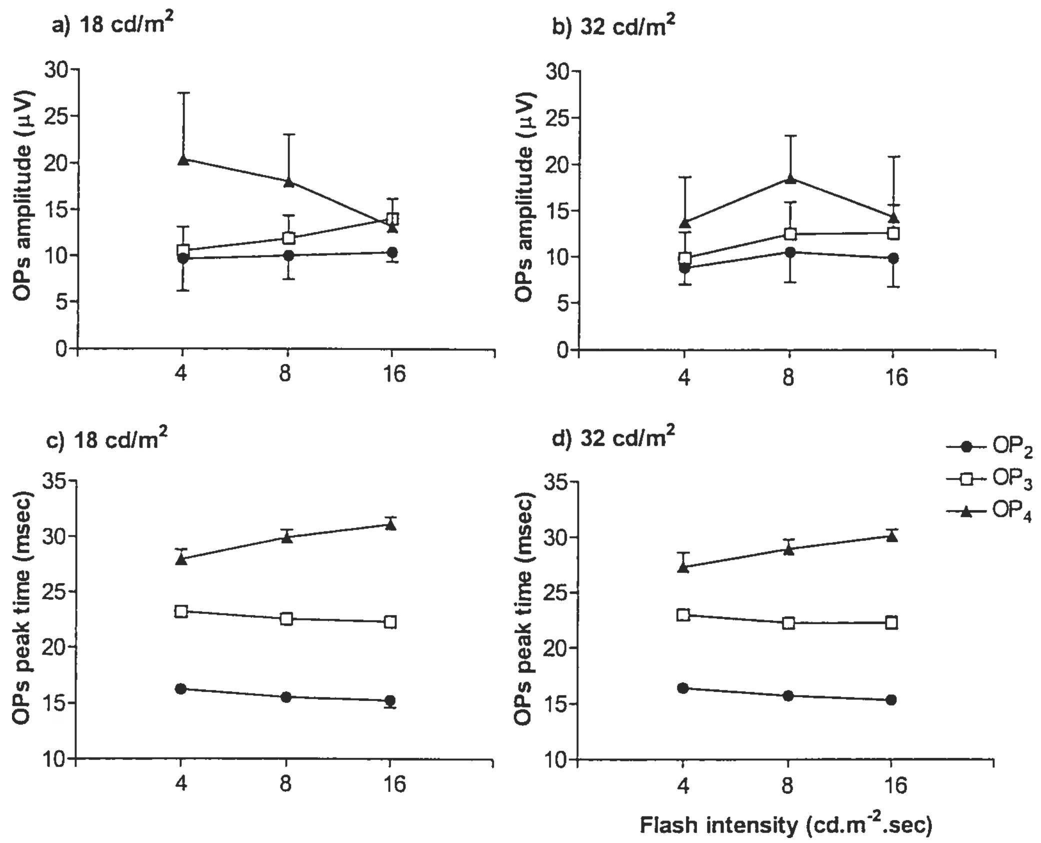


Figure 6

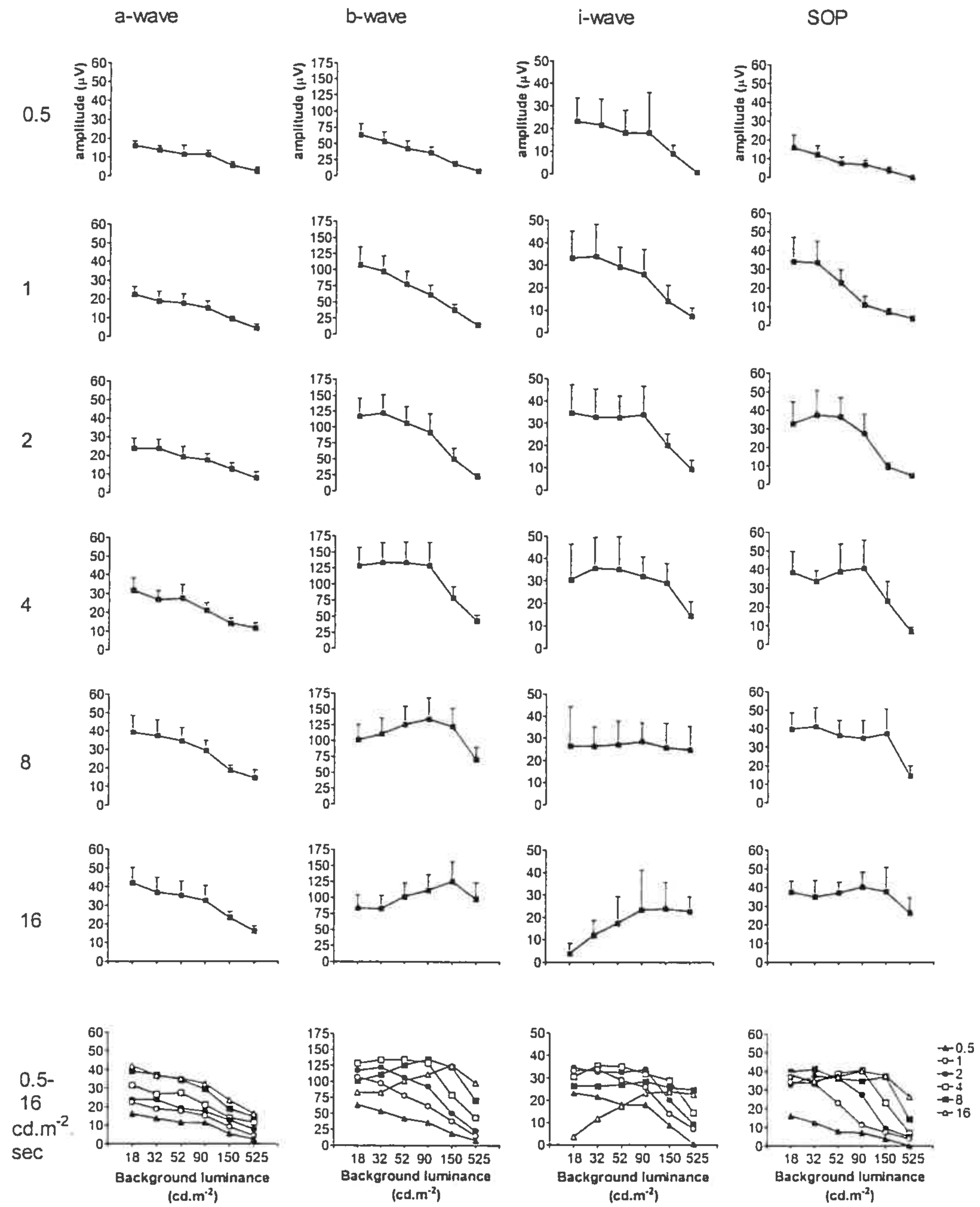


Figure 7

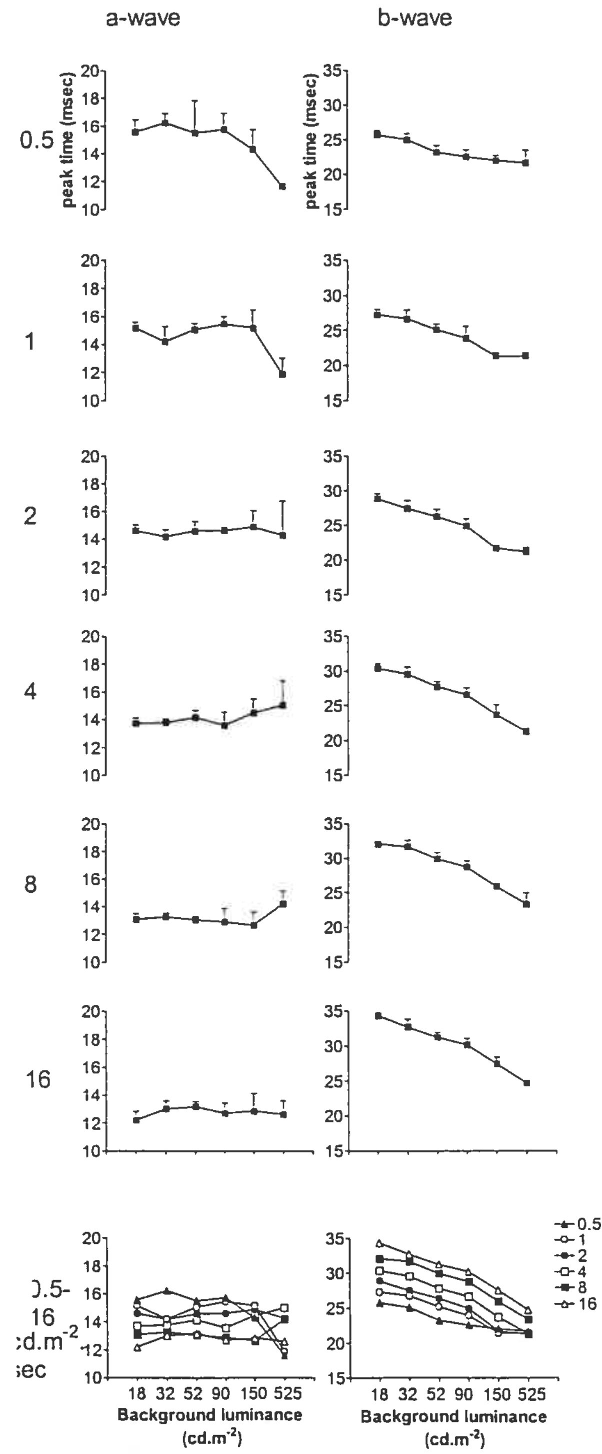


Figure 8

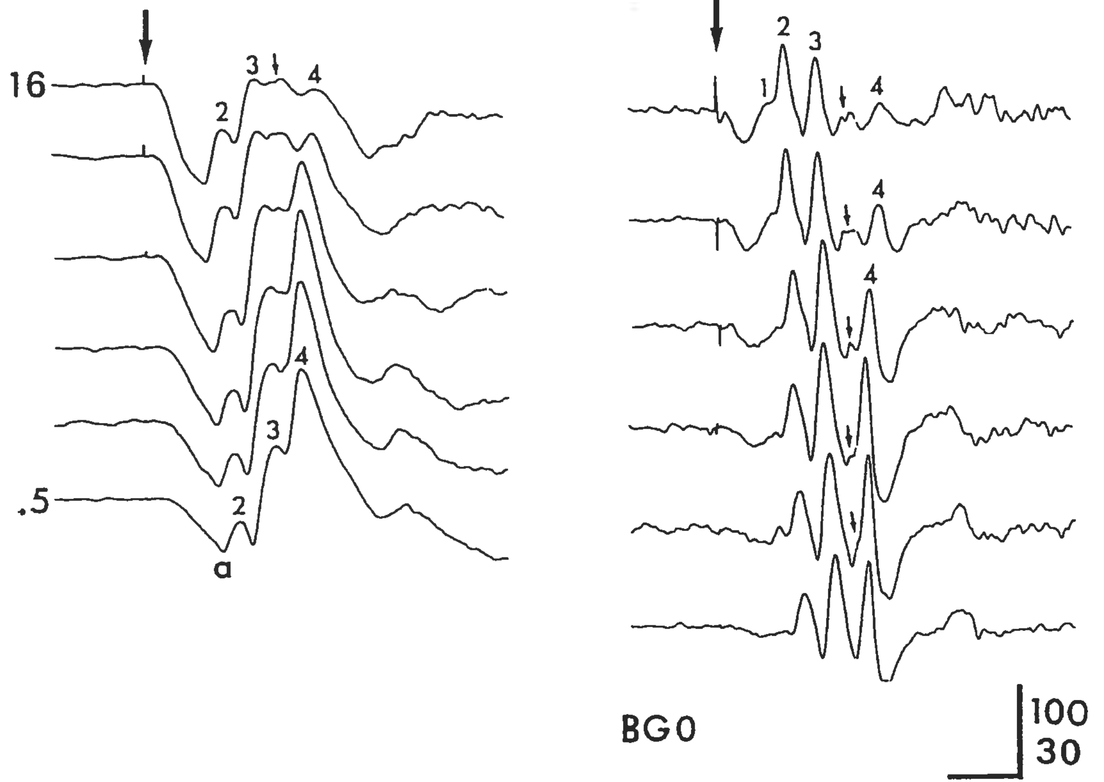
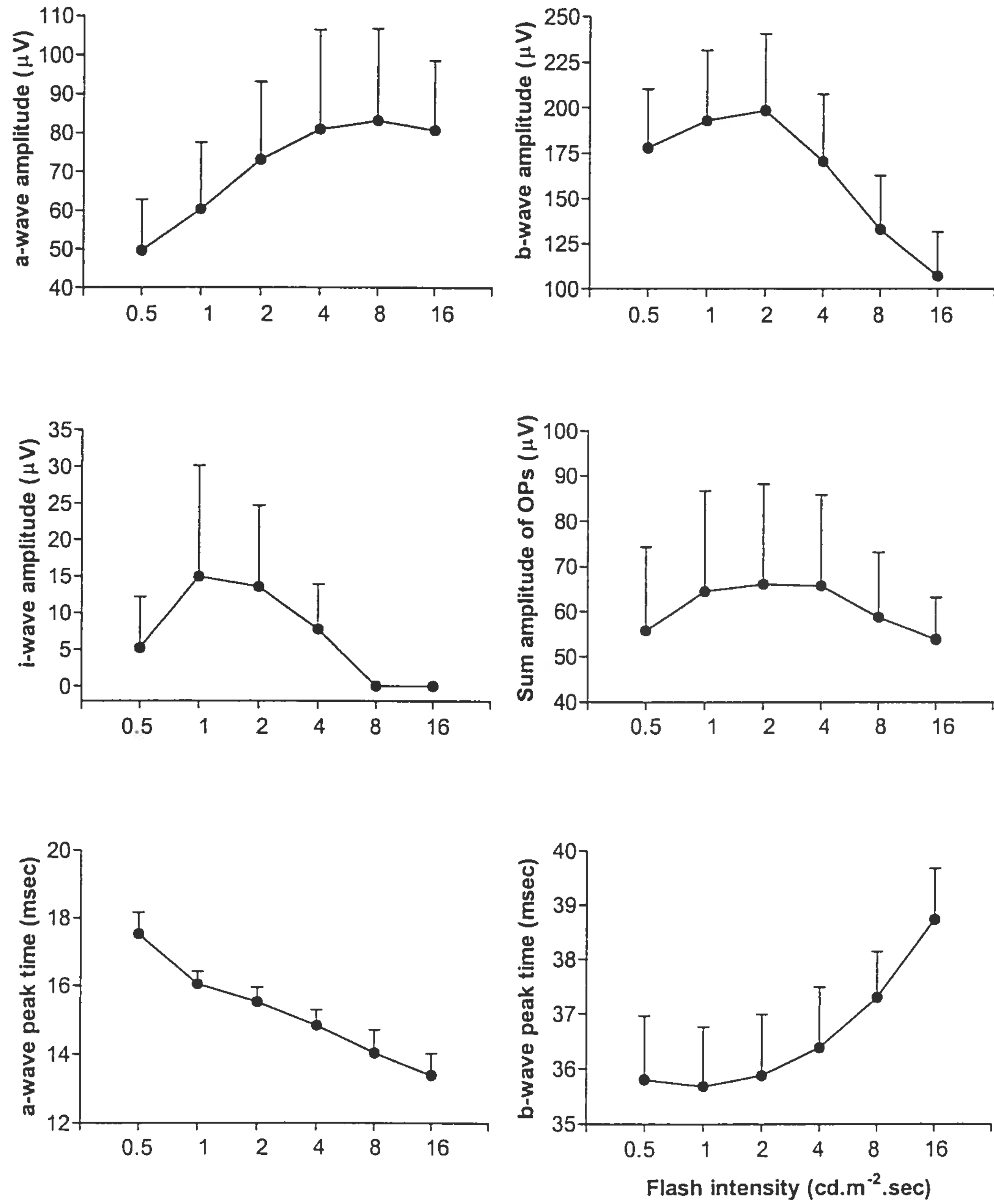


Figure 9



3.2. Deuxième article

The photopic ERG luminance-response function (Photopic Hill):

Method of analysis and clinical application.

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The photopic ERG luminance-response function (Photopic Hill): Method of analysis and clinical application.

Marianne Rufiange, M.Sc.[1,2], Justine Dassa, B.Sc.[1], Olga Dembinska, Ph.D.[1], Robert K. Koenekoop, M.D., Ph.D.[1], John M. Little, M.D.[1], Robert C. Polomeno, M.D.[1], Marie Dumont, Ph.D.[2], Sylvain Chemtob, M.D., Ph.D.[3], Pierre Lachapelle, Ph.D.[1]

[1] Departments of Ophthalmology & Neurology-Neurosurgery, McGill University, Montreal Children's Hospital Research Institute; [2] Department of Psychiatry, University of Montreal & Chronobiology Laboratory, Sacré-Coeur Hospital; [3] Department of pediatrics, University of Montreal

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Corresponding author:

Pierre Lachapelle, PhD

Department of Ophthalmology (D-164)

McGill University - Montreal Children's Hospital Research Institute

2300 Tupper Street

Montreal, Quebec, Canada, H3H 1P3

TEL: (514) 412-4400, ext 23890

FAX: (514) 412-4331

Email: pierre.lachapelle@mcgill.ca

Abstract

With progressively brighter stimuli, the amplitude of the photopic b-wave first increases, briefly saturates and then decreases gradually to reach a plateau, where the amplitude of the b-wave equals that of the a-wave; a phenomenon previously presented as the photopic hill. The unique presentation of this luminance-response function seriously complicates its analysis with curve fitting equations such as that of Naka-Rushton used for scotopic electroretinogram. We report a method of analysis of the photopic hill based on easily identifiable and reproducible features of the ascending and descending limbs of this function. The clinical usefulness of these parameters is illustrated with selected cases of retinal disorders.

Keywords: electroretinogram (ERG), luminance-response function, photopic, visual sensitivity, b-wave.

Introduction

The luminance-response function of the photopic electroretinogram (ERG) b-wave was previously shown to adopt a unique shape where the amplitude of the b-wave first increases, then saturates briefly following which it decreases to reach a final plateau where the amplitude of the b-wave equals approximately that of the a-wave (Peachey, Alexander, Derlacki, & Fishman, 1992; Wali & Leguire, 1992, 1993; Kondo, Piao, Tanikawa, Horiguchi, Terasaki, & Miyake, 2000; Lachapelle, Rufiange & Dembinska, 2001; Rufiange, Dumont & Lachapelle, 2002; Rufiange, Rousseau, Dembinska, & Lachapelle, 2002). Wali and Leguire (1992, 1993) coined the term photopic hill to describe this unusual ERG function. The latter contrasts with the scotopic ERG b-wave luminance-response function which is normally fitted to a sigmoidal curve known as the Naka-Rushton equation (Naka & Rushton, 1966). Given that the particular configuration of the photopic hill function would hardly allow any curve fitting, the purpose of this paper is to suggest an analysis of the ERG b-wave luminance-response function based on seven easily identifiable function descriptors. We believe that their use could permit a more complete and objective evaluation of the photopic ERG function of the normal and pathological retina.

Methods

Subjects:

A total of 48 subjects participated in this study. In a first group of 30 subjects (age 18 to 25, mean: 21.1; 23 women, 7 men), the photopic ERGs were recorded against a rod-desensitizing white-light background of 17 $cd.m^{-2}$ (lower limit of ISCEV standard photopic background, Marmor & Zrenner, 1998) while in a second group of 18 subjects (age 21 to 43, mean: 30.1; 8 women, 10 men), the photopic ERGs were recorded against a photopic background of 30 $cd.m^{-2}$ (upper limit of ISCEV standard photopic background, Marmor & Zrenner, 1998). A complete ophthalmological examination was performed prior to the experiment in order to rule out any retinal disorders. All the participants signed an informed consent approved by the Institutional Review Board of the Montreal Children's Hospital and received a financial compensation. In this study we also report the photopic hills obtained from four patients namely: a 10 year-old male diagnosed with Congenital Stationary Night Blindness (CSNB) with normal visual acuity, normal visual fields, normal fundi, no myopia, no rod ERGs and nyctalopia since birth; a 12 year-old female suspected of a cone related anomaly based on best corrected visual acuity of 20/40, myopia of –4.00 OU, low voltage photopic ERGs at ISCEV standard intensities, normal rod ERGs and a normal fundus examination; an 82 year-old male with a pigmentary retinopathy of unknown etiology with reduced visual acuity, reduced amplitude of cone and rod ERGs and white dots at fundus examination; and a 50 year-old female with unilateral

microophthalmia and dense cataract (OD). This study followed the tenets of the Declaration of Helsinki.

Procedures:

The electrophysiological recordings were performed as previously reported (Hébert, Lachapelle, & Dumont, 1996; Hébert, Vaegan, & Lachapelle, 1999; Lachapelle et al., 2001; Rufiange et al., 2002a,b) and in accord with the ISCEV ERG standards (Marmor & Zrenner, 1998). Briefly, the pupils were maximally (8-9 mm) dilated with drops of Tropicamide 1% and the pupil size was measured at the beginning and at the end of the recording procedure. No significant pupil size differences were noted. DTL fiber electrodes (27/7 X-Static silver coated nylon conductive yarn; Sauquoit Industries, Scranton, PA, USA) were positioned deep into the inferior conjunctival bag and secured with double-sided adhesive tape at the external and internal canthi of both eyes. Reference and ground electrodes (Grass gold cup electrodes filled with Grass EC2 electrode cream) were pasted at the external canthi and forehead respectively. ERGs (bandwidth: 0.3-500Hz; amplification: 20000X; attenuation: 6dB) were recorded from both eyes simultaneously with a LKC UTAS-E-3000 system (LKC Systems Inc., Gaithersburg, MD, USA), which included a Ganzfeld of 30 centimeters in diameter.

To avoid the light adaptation effect previously reported (Lachapelle, 1987), the subjects were first light adapted for 10 minutes to a white light rod-desensitizing background of 17 or 30 $cd.m^{-2}$. The ERGs were evoked to flashes of white light of -0.8 to 2.84 log $cd.sec.m^{-2}$ in intensity presented against one of the above-mentioned backgrounds. Each flash had a duration of 20 μsec and the interstimulus interval was fixed at 2.3 seconds. Ten responses were averaged for each flash intensity and each tracings included a 40 msec pre-stimulus baseline. Background luminance and flash intensities were measured with a research radiometer (IL 1700; International Light, Newburyport, MA, USA).

Data analysis:

Analysis of the ERG included peak time and amplitude measurements of the a- and b-waves. The data from both eyes were averaged to yield a single data point. The amplitude of the a-wave was measured from baseline to trough and that of the b-wave from the trough of the a-wave to peak of the b-wave. Peak times were measured from flash onset to the peak of each wave.

Results

Figure 1 illustrates typical photopic ERGs evoked to progressively brighter stimuli (from bottom to top) presented against a background light of 17 cd.m^{-2} (left) and 30 cd.m^{-2} (right). In this example, both intensity-response series were obtained from the same subject and on the same recording session. It can be seen that the two recording conditions yield ERG responses of similar amplitude, timing and morphology. In both sets of recording, the amplitude of the a-wave augments regularly with the gradual increase in intensity of the stimulus while that of the b-wave first increases, reaches a maximum (V_{max}) and then decreases with progressively brighter stimuli. The only obvious difference being that for the same intensity of stimulation, the amplitude of the ERG is usually larger if evoked in the presence of the dimmest background. The latter is most obvious with responses evoked to the weaker flashes (i.e. -0.41 and -0.23 log cd.sec.m^{-2}).

At Figure 2 are shown the mean (±1 SD) luminance-response functions of the ERG a-wave and b-wave obtained with the use of the two backgrounds (obtained from two different groups of subjects: see method section). At the bottom, where the two luminance-response functions are superposed, one notices that while for the same flash luminance, the amplitude of the a-wave is always larger in responses obtained against the dimmer background, that of the b-wave follows a different pattern. For data generated against the brighter background, the entire b-wave luminance-response function is shifted to the right

so that for the dimmer flash intensities (dimmer than 0.17 log cd.sec.m^{-2}), the amplitude of the b-wave is larger in responses collected against the weaker background while the reverse is observed with b-waves evoked to flashes of intensities comprised between 0.17 and 1.40 log cd.sec.m^{-2}. Finally for flash luminances brighter than 1.40 log cd.sec.m^{-2}, the relationship becomes more ill-defined, reflecting most probably the near absence of a positive b-wave (i.e. above the baseline) in responses evoked at these intensities (see Figure 1). Also of interest, the shape of the ascending limb of the photopic hill differs depending on the brightness of the background light: the rise adopting a concave-like progression with the 30 cd.m^{-2} background compared to a more linear shape with the 17 cd.m^{-2} background.

Notwithstanding the above-mentioned differences, both backgrounds generated b-wave luminance-response functions with comparable key features that can be used to ease comparisons, namely: an initial ascent, a peak or V_{max}, a descent and a final plateau phase where the amplitude of the a- and b-waves are equal. From this, we have identified the following seven function descriptors: 1- the maximal b-wave amplitude or V_{max}; 2- the amplitude of the a-wave at the V_{max} intensity or a_{max}; 3- the flash intensity needed to generate the V_{max} response or I_{max}; 4- the ratio of the amplitude of the b-wave over that of the a-wave measured at V_{max} intensity or b/a_{max}; 5- K_a and 6- K_d which represent the intensity of stimulation that will generate a b-wave half the amplitude of V_{max} on the ascending (K_a) or descending (K_d) portion of the photopic hill, and finally 7- $K_{a=b}$ which

represents the intensity of stimulation needed to generate an ERG where the amplitude of the b-wave equals that of the a-wave. These descriptors are illustrated at Figure 3 and their values are given at Table 1.

As shown at Table 1 (and Figure 3), while both backgrounds yielded ERGs of near identical ($p>.10$) a- and b-wave amplitudes at V_{max}, the flash intensities necessary to reach V_{max} ($p=.06$) along with K_a ($p<.0001$) and $K_{a=b}$ ($p=.09$) were dimmer in responses collected against the weakest background. No similar trend was observed with the K_d measurement, this variable being almost identical in both conditions ($p>.10$). Finally, the b/a_{max} descriptor which more or less defines the morphology of the resulting ERG waveform indicates that there is a trend towards a lower b-/a-wave ratio in responses evoked against the 17 $cd.m^{-2}$ background ($p=.08$).

In order to examine if use of the above function descriptors might be useful in interpreting clinical ERGs, we examined the photopic luminance-response functions of selected patients. Representative examples, taken from patients affected with Congenital Stationary Night Blindness (CSNB: Figure 4B), a suspected cone anomaly (Figure 4C), and a pigmentary retinopathy of unknown etiology (Figure 4D), are illustrated at Figure 4 and the data graphically reported at Figure 5. As shown at Figure 4, a simple comparison of waveforms with the normal responses (Figure 4A) already allows one to extract significant

diagnostic information such as: the truncated or square-wave morphology of the ERG signal (most obvious in responses evoked to the 0.90 or 1.13 log cd.sec.m^{-2} stimuli) which is almost pathognomonic for CSNB (Lachapelle, Little, & Polomeno, 1983; Heckenlively, Martin, & Rosenbaum, 1983) or the elevated threshold observed in the responses obtained from the other two patients. Moreover, while the ERG obtained from the patient affected with the suspected cone anomaly rapidly reaches a normal b-wave V_{max}, that obtained from the patient affected with the pigmentary retinopathy never does. There are however more subtle differences which are best visualized with the photopic luminance-response functions shown at Figure 5. For each patient, the amplitudes of both the a- and b-waves are presented as a percentage (%) of the maximal amplitude reached by each individual and are compared to the mean (±1 SD: dash line) of the photopic hill obtained from the 30 control subjects (also expressed in % of maximum). The absolute values of the key descriptors of the photopic hill of these patients are reported at Table 1. As shown at Figure 5, analysis of the luminance-response function of the a-wave (Figure 5A, C and E) measured in all three patients indicates a normal growth pattern with progressively brighter flashes. However, as indicated at Table 1, while the maximal amplitude (a_{max}) reached is within the normal range for the CSNB and pigmentary retinopathy patients it is definitively larger than normal in ERG responses obtained from the patient affected with the suspected cone anomaly. It should be noted however that the latter was evoked in response to a significantly brighter flash than that used in normals to generate the a_{max} response (see I_{max} values at Table 1). The latter would thus suggest that while the amplitude of the a-wave progressed normally

with gradually brighter flashes that of the b-wave augmented at a much slower pace, which explains for the shift of the I_{max}.

Similarly, while the shape of the b-wave's photopic hill is also abnormal in all three patients, the pattern of anomaly differs enough that they can be easily distinguished from one another. For example, the data obtained from the patient affected with CSNB (Figure 5B) reveals a normal ascension of the photopic hill where the K_a, V_{max} and I_{max} all fall within the normal range (Table 1). Of interest, the ratio b/a_{max} is higher than normal, showing a somewhat increased b-wave relative to a-wave, a feature which is easily evidenced with the waveforms shown at Figure 4 (tracings 0.17 or 0.39). In contrast, the descending phase of the photopic hill straddles along the lower limit of the normal curve resulting in a smaller but still within the normal range K_d (Table 1), while the $K_{a=b}$ value is higher than normal (Table 1) and the final plateau phase definitively outside the normal range (Figure 5B).

The above results differ from those obtained from the patient suspected to be affected with a cone anomaly (Figure 5D). Here the ascending phase of the photopic hill is definitively abnormal, a feature which is also witnessed with the elevated K_a and I_{max} values (Table 1). This yields cone b–wave amplitudes which are significantly smaller than normal, a finding more consistent with the suspected diagnosis of cone anomaly. However, the amplitude of

V_{max} still falls within the normal range (relative or absolute values). In contrast, albeit borderline, the initial phase of the descent of the photopic hill is accomplished in a near normal fashion as illustrated with the normal K_d value (Table 1), while the $K_{a=b}$ remains somewhat higher than normal range. The b/a_{max} ratio is lower than normal in this patient because of the a-wave amplitude being greatly increased at V_{max} intensity as mentioned earlier. Finally, analysis of the data obtained from the patient affected with pigmentary retinopathy suggests that the entire photopic hill has shifted to the right, resulting in higher values for I_{max}, K_a and $K_{a=b}$. It is also of interest to note that in the latter case, a K_d value could not be measured. Also of interest is the fact that while the absolute amplitude of the a-wave at V_{max} is within the normal range (again most probably due to the significantly brighter flash used), that of the b-wave is significantly smaller than normal thus resulting in a significantly smaller than normal b/a_{max} ratio. The potential clinical usefulness of the photopic hill is probably best illustrated with the results obtained from a patient affected with unilateral microophthalmia (OD) as shown at Figure 6. Comparison of the two photopic hills reveals that for intensities of stimulation up to 1.13 log cd.sec.m^{-2}, the smaller b-waves are measured in responses obtained from the pathological eye while the reverse is seen for responses obtained with brighter flashes; thus mimicking the difference in photopic hills observed when the bright and dim photopic backgrounds are compared (see Figure 2).

Discussion

In response to progressively brighter flashes, the photopic (cone-mediated) ERG adopts a unique luminance-response function curve previously presented as the photopic hill (Wali & Leguire, 1992, 1993). The purpose of this study was to investigate if the use of pre-selected descriptors of key features of this unique function could help extract meaningful functional information that could potentially have some clinical (diagnostic) relevance. To do so, we compared the photopic hills generated against two different adapting background lights. Our results reveal that while the maximal amplitude reached by the b-wave (V_{max}) along with that of the corresponding a-wave (a_{max}) are not significantly modified following a raise in background luminance, there is an increase noted in the intensity of the stimulus (I_{max}) necessary to reach these maximal values. Similarly, the intensity of stimulation needed to generate a b-wave 50% of V_{max} amplitude on the ascending limb of the photopic hill (K_a) as well as the flash intensity needed to generate an ERG where the amplitude of the a-wave equals that of the b-wave ($K_{a=b}$) both demonstrate an increase in responses collected against the brighter background. In view of the above, we believe that use of our functional descriptors do add significant information to the analysis of photopic hills.

From an analytical point of view, the photopic luminance-response function presents itself as an interesting challenge compared to that generated in a scotopic environment, most probably reflecting the distinct nature of the cone ERG. While it is possible to analyze,

using a near linear approach, the growth in rod ERG b-wave amplitude with intensity, it is not possible to do so with the cone ERG due to the decay in amplitude that the brightest flashes will cause. This phenomenon leads to one major distinction between the rod and cone V_{max}, namely that this value represents a prediction in the former (e.g. predicted from the Naka-Rushton equation, Naka & Rushton, 1966) while it represents a true value in the latter. Also, while the amplitude of the scotopic ERG will continue to grow beyond the rod V_{max} value, in part as a result of the added contribution of the cone response, the amplitude of the cone ERG b-wave will decrease despite an increase in flash intensity. As a result of the above physiological differences, analysis of the scotopic luminance-response only includes parameters that describe how the rod V_{max} is reached (e.g. slope, flash intensity at V_{max} and half V_{max} (K), etc) while that of the cone ERG luminance response must also include parameters that will describe how the decay of the cone ERG b-wave occurs as this process appears to be a dynamic one that is as susceptible to physiological (or pathological) manipulation as the ascent (of the cone or rod) luminance-response function is. This finding introduces the concept of finiteness in the cone response i.e. the value of the cone b-wave V_{max} represents the maximum amplitude that the light-adapted retina can yield irrespective of background luminance, a concept that was previously advanced (Lachapelle et al., 2001; Rufiange et al., 2002b).

Based on the above observations, it is tempting to postulate that the limitation of the cone b-wave V_{max} could be governed by the same retinal mechanisms that were previously suggested to limit the amplitude of the cone ERG b-wave, namely: the Push-Pull concept of Sieving, Murayama & Naarendorp (1994). According to this hypothesis, the amplitude of the cone ERG b-wave would result from the combined interaction between the activation of the ON-depolarizing bipolar cells (ON-DBC) that would provide the impetus to push the baseline of the ERG to the peak of the b-wave and the OFF-hyperpolarizing bipolar cells (OFF-HBC) that would pull on the baseline to bring it back to its initial (pre-stimulus) value. What our observation would add to Sieving's original claim is that the Push-Pull interaction is also governed by the intensity of the stimulus. Dimmer flashes will favor more the push while brighter flashes will trigger a pull so strong that it will counteract the effect of the push. The physiological reason for this could be to protect the post-retinal structures from being overwhelmed by non-physiological stimulation. However should the Push-Pull concept explain the rise and fall of the photopic hill, it cannot explain our pathological findings, since previous studies have suggested an anomaly of the ON-pathway in CSNB (Quigley, Roy, Barsoum-Homsy, Chevrette, Jacob, & Milot, 1996; Barnes, Alexander, & Fishman, 2002) and an anomaly of the OFF-pathway in cone pathologies (Sieving, 1993). This should yield, according to our explanation of how the building and dismantling of the photopic hill is achieved, to an anomaly of the rising phase in CSNB and decay phase in cone related pathologies while the exact reverse was observed. It could be that what is reflected in these pathological photopic hills is what is left of the

contribution of one of the two retinal pathways to the making of the ERG b-wave in the absence of the complementary one.

Clearly more research is needed before a complete understanding of the retinal mechanisms that govern the rise and fall of the photopic hill is achieved. For example, experimental manipulation of the ON and OFF retinal pathways in animal models, could help us understand the above-mentioned discrepancies. However, notwithstanding the above, our results strongly demonstrate that: 1- the photopic hill represents a functional characteristic that distinguish the cone function from that of the rod; 2- the photopic hill can be described with easily identifiable function descriptors, and; 3- the use of the descriptors can document functional alterations in the retinal physiology either triggered by experimental manipulation or as a result of a pathological process. However, only time will tell if analysis of the cone function with the photopic hill will add meaningful diagnostic information.

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Figure legends

Figure 1: Representative photopic (cone-mediated) ERG waveforms recorded from the same normal subject exposed to a dim (17 cd.m^{-2}, left) or bright (30 cd.m^{-2}, right) rod-desensitizing background light. Each tracing represents an average of 10 responses evoked to flashes of light of progressively brighter intensities (from bottom to top) as indicated at the left of each response (in log cd.sec.m^{-2}) . The a-, b- and i-waves are indicated as a, b and i, respectively and OP_2, OP_3 and OP_4 as 2, 3 and 4, respectively. Vertical arrows indicate flash onset. Horizontal calibration: 20 milliseconds; vertical calibration: 50 microvolts.

Figure 2: Photopic luminance-response curves for the a- (squares) and b- (circles) waves obtained with exposure to the dimmer (17 cd.m^{-2}; n=30) and brighter (30 cd.m^{-2}; n=18) backgrounds. Each data point represents the mean ± 1 SD. At the bottom, the curves thus generated are superposed to ease comparison. The ordinate represents the amplitude in microvolts while the abscissa represents the intensity of the flash in log.cd.sec.m^{-2}.

Figure 3: Nomograms representing the position of the proposed descriptors. Each function descriptors is represented as a shaded box whose limits are dictated by the mean value ± 1 SD along the X and Y-axis. The mean a- and b-wave curves are also depicted along with their respective SD (dotted lines).

Figure 4: Representative photopic (cone-mediated) ERG waveforms recorded from a normal subject (A) and patients affected with CSNB (B) suspected cone anomaly (C) and pigmentary retinopathy of unknown etiology (D). All responses were collected against the dimmer (17 cd.m^{-2}) photopic background. Each tracing represents an average of 10 responses evoked to flashes of light of progressively brighter intensities (from top to bottom) as indicated at the left of each response (in log cd.sec.m^{-2}). Asterisks represent the b-wave V_{max} waveforms and the letters a and b represent the a- and b-waves. Horizontal calibration: 20 milliseconds; vertical calibration: 50 microvolts.

Figure 5: Photopic luminance-response functions for the a-wave (squares, left) and b-wave (circles, right) of the ERG responses obtained from three patients (solid lines, CSNB: panel A and B, cone anomaly: panel C and D, pigmentary retinopathy: panel E and F). Data are compared to the mean obtained from normal subjects, the upper (+1 SD) and lower (-1 SD) limit of which is represented with the dash lines. The ordinate represents the relative amplitude (% of maximal amplitude) while the abscissa represents the intensity of the stimulus in log cd.sec.m^{-2}.

Figure 6: Representative ERGs obtained from a patient affected with unilateral microophthalmia (OD). Corresponding luminance-response functions for the b-wave (bottom left) and a-wave (bottom right) of the right (open symbols) and left (closed symbols) are also illustrated. The ordinate represents the relative amplitude (% of maximal amplitude) while the abscissa represents the intensity of the stimulus in log cd.sec.m^{-2}.

Table 1: ERG parameters (± 1SD) for normal controls (30 subjects for 17 $cd.m^{-2}$ and 18 subjects for 30 $cd.m^{-2}$) and 3 patients (CSNB: Congenital Stationary Night Blindness). Refer to text for definition of the parameters. V_{max} and a_{max} in μV and I_{max}, K_a, K_d and $K_{a=b}$ in log $cd.sec.m^{-2}$. t-tests compare the two backgrounds in controls. n.s.: $p > 0.10$

	V_{max}	a_{max}	I_{max}	b/a_{max}	K_a	K_d	$K_{a=b}$
17 $cd.m^{-2}$	92.2 (21.3)	26.7 (5.5)	0.35 (0.21)	3.49 (0.70)	-0.47 (0.12)	1.59 (0.48)	1.45 (0.22)
30 $cd.m^{-2}$	97.5 (17.4)	25.6 (7.7)	0.47 (0.20)	4.15 (1.42)	-0.19 (0.11)	1.46 (0.22)	1.55 (0.12)
t-tests	n.s.	n.s.	$p < 0.10$	$p < 0.10$	$p < 0.0001$	n.s.	$p < 0.10$
CSNB	120.5	23.5	0.39	5.13	-0.62	1.09	1.90
suspected cone anomaly	118.5	54.0	0.90	2.19	0.09	1.96	1.90
pigmentary retinopathy	61.5	33.0	0.90	1.86	-0.15	___	1.90

K_d includes 19 subjects for 17 $cd.m^{-2}$ and 16 subjects for 30 $cd.m^{-2}$ while $K_{a=b}$ includes 24 subjects for 17 $cd.m^{-2}$ and 11 subjects for 30 $cd.m^{-2}$.

Figure 1

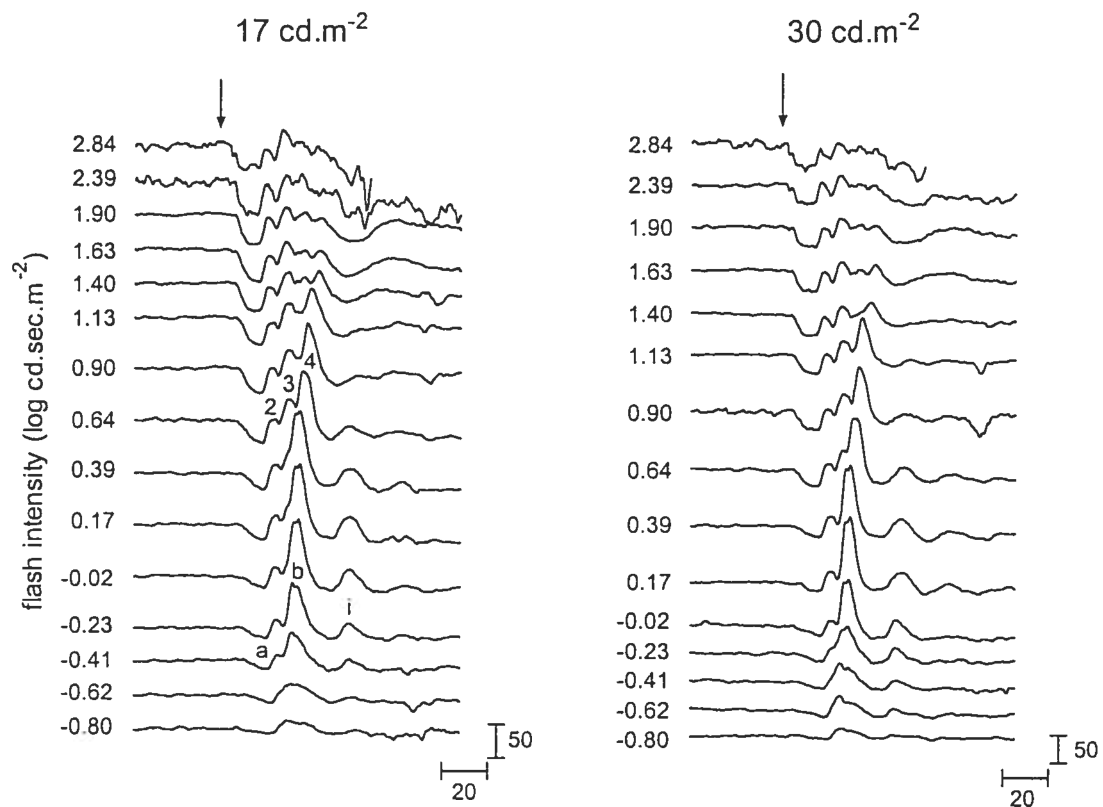


Figure 2

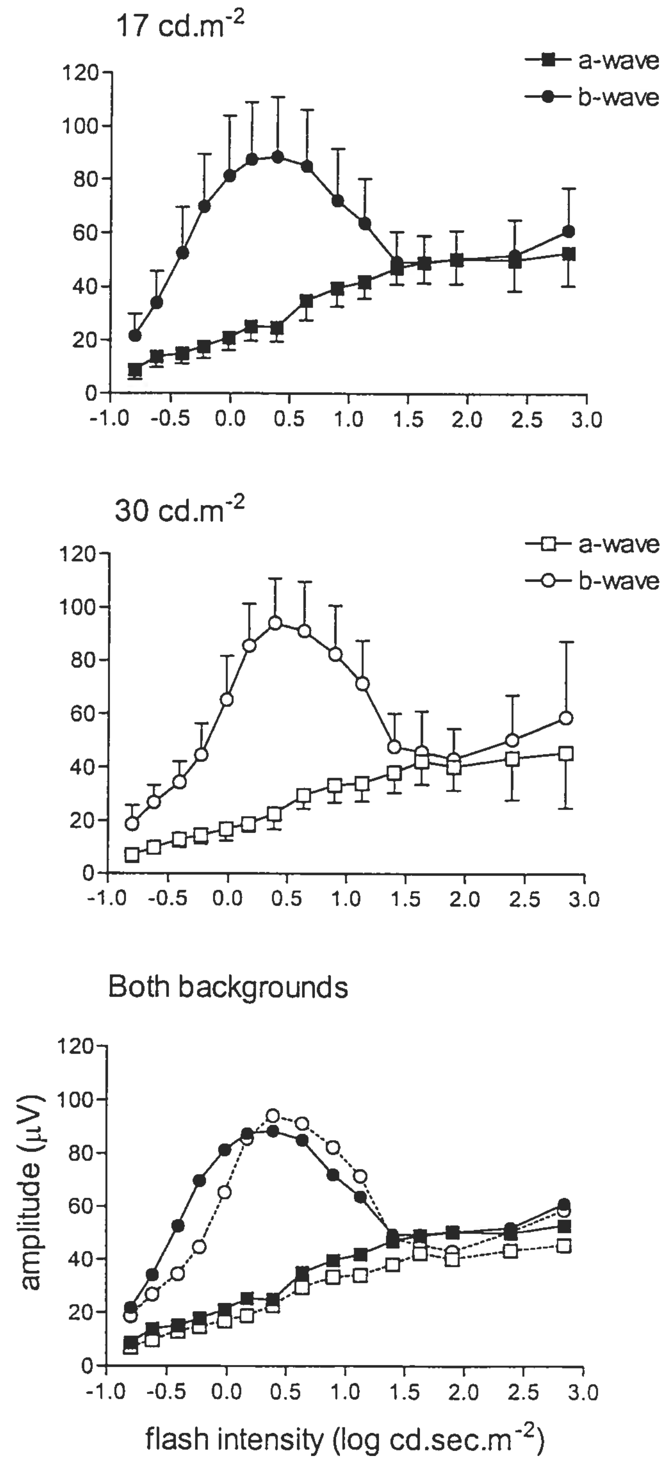


Figure 3

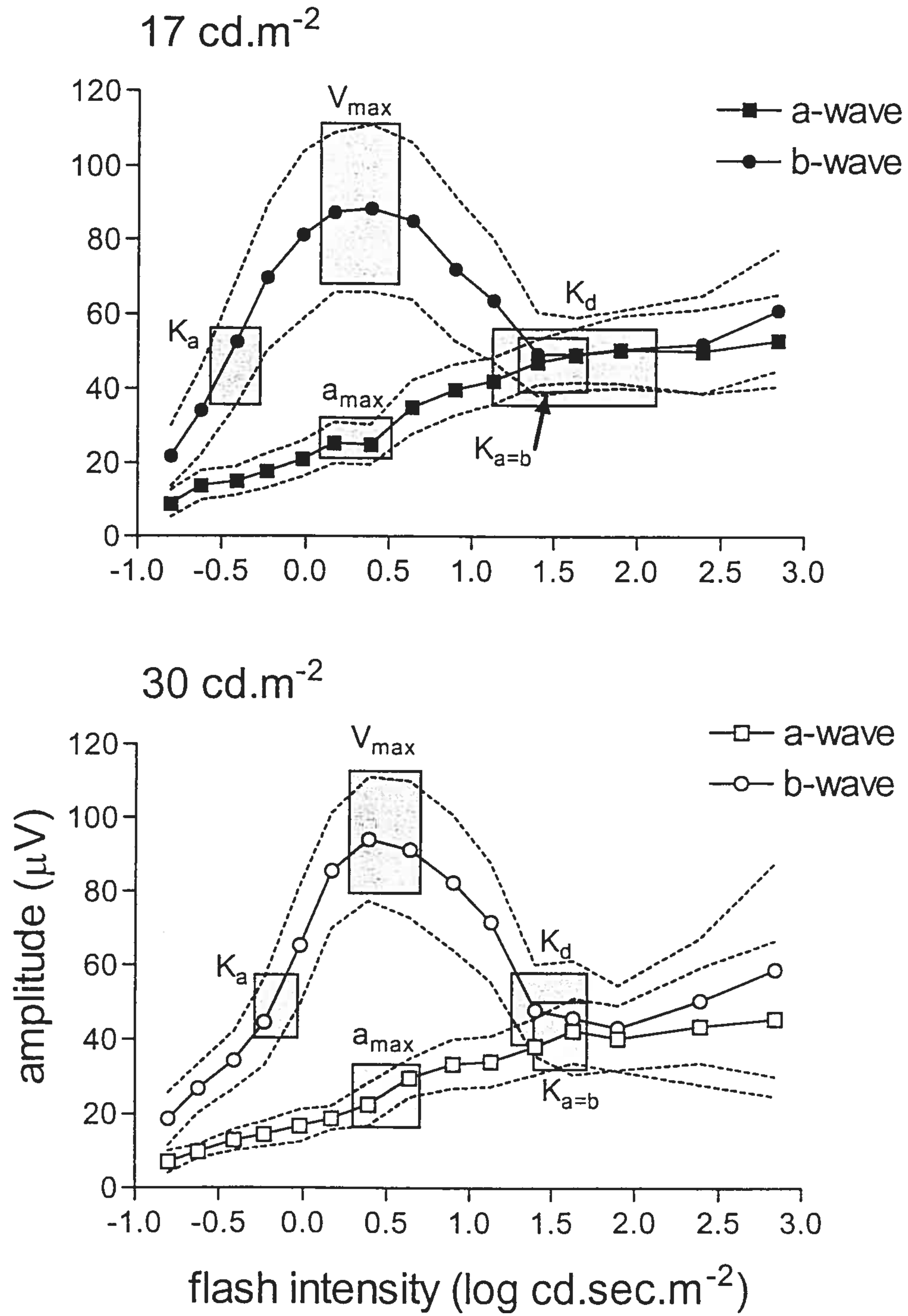


Figure 4

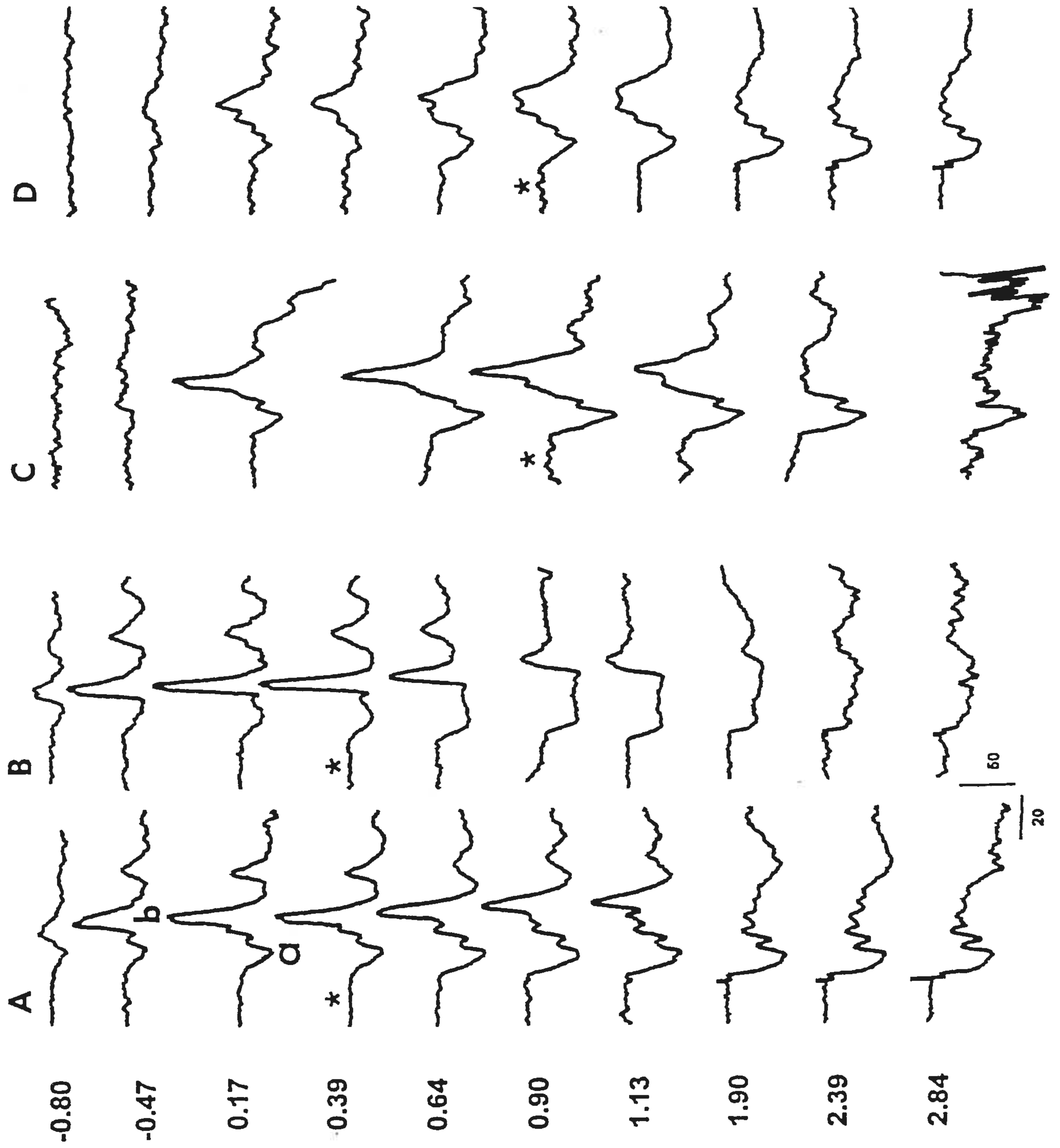


Figure 5

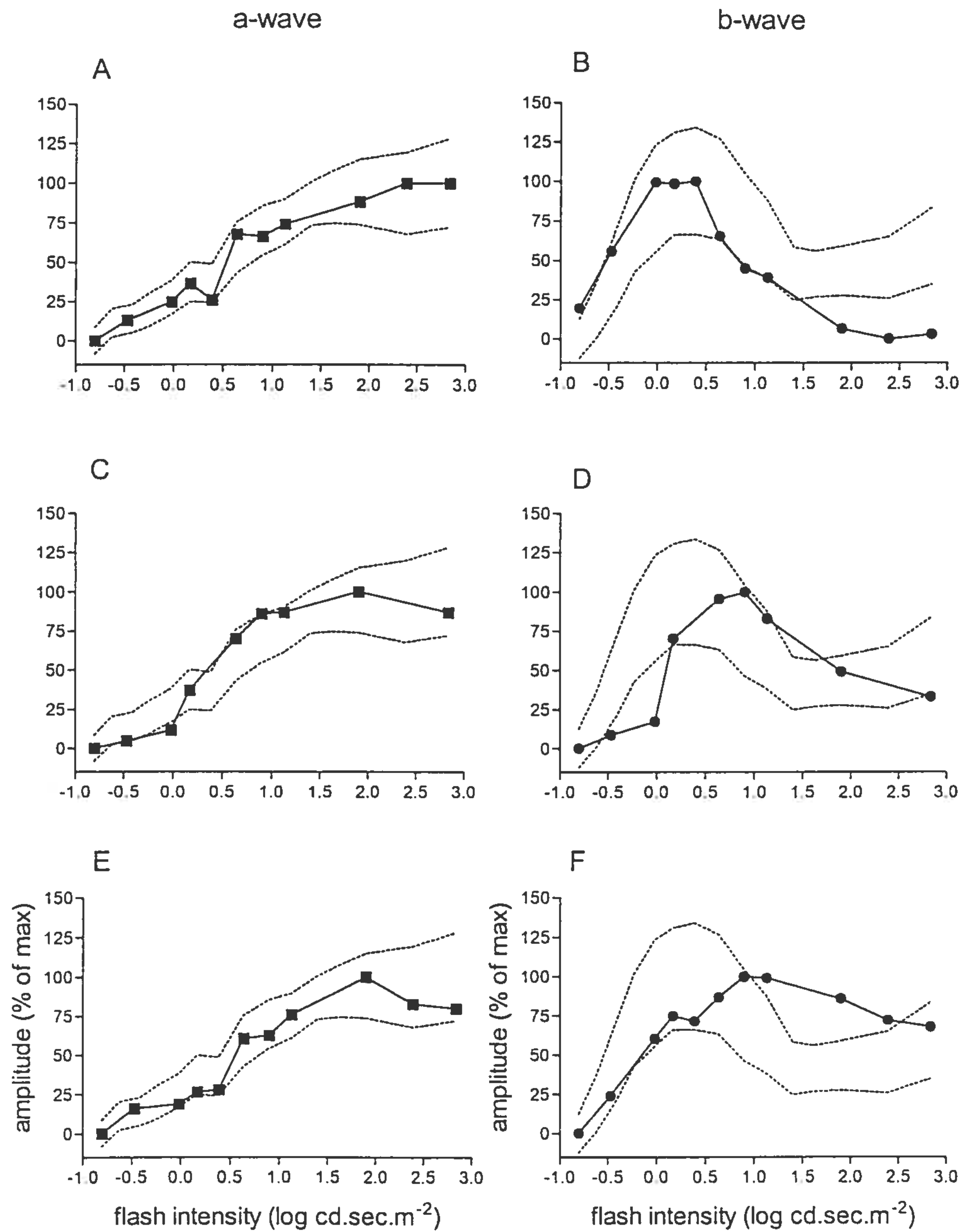
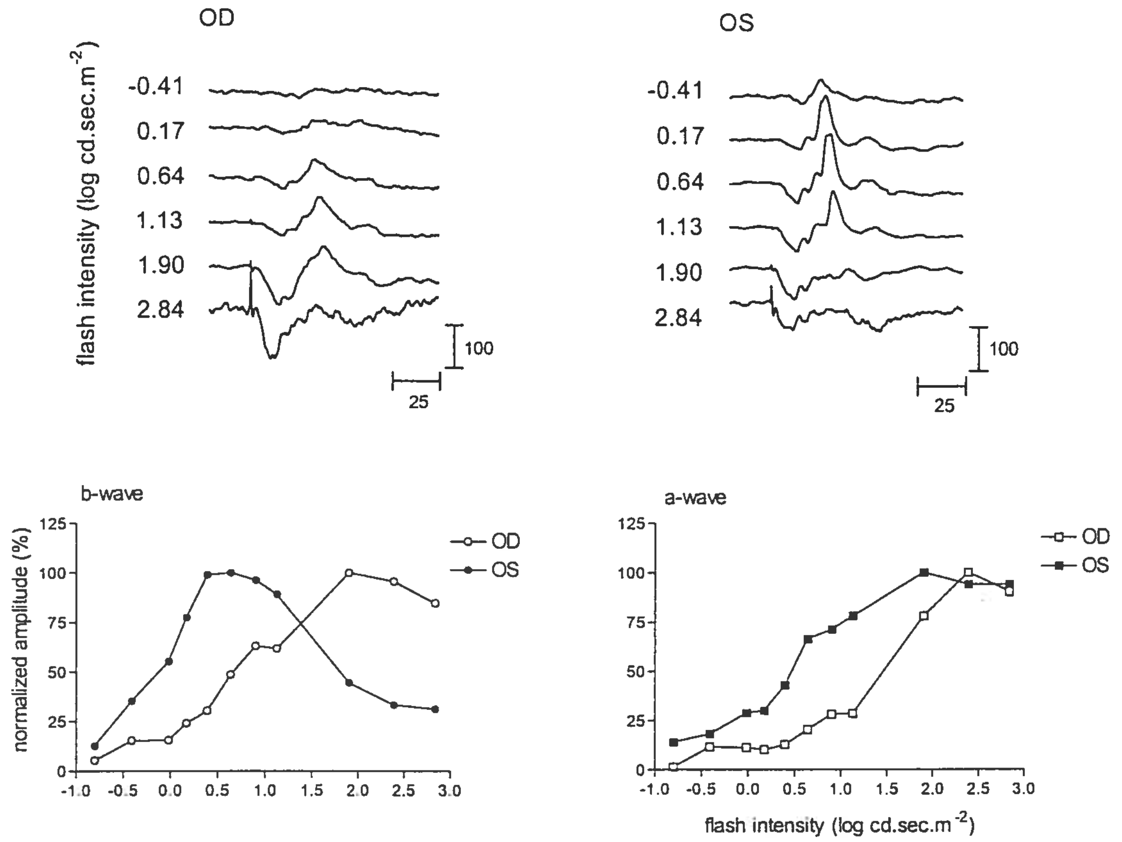


Figure 6



3.3. Troisième article

Modulation of the human photopic ERG luminance-response function with the use of chromatic stimuli.

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Modulation of the human photopic ERG luminance-response function with the use of chromatic stimuli.

Marianne Rufiange, M.Sc.[1,2], Marie Dumont, Ph.D.[2], Pierre Lachapelle, Ph.D.[1]

[1] Departments of Ophthalmology & Neurology-Neurosurgery, McGill University, Montreal Children's Hospital Research Institute; [2] Department of Psychiatry, University of Montreal & Chronobiology Laboratory, Sacre-Coeur Hospital.

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Corresponding author:

Pierre Lachapelle, PhD

Department of Ophthalmology (D-164)

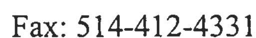
McGill University – Montreal Children's Hospital Research Institute

2300 Tupper Street

Montreal, Quebec, Canada, H3H 1P3

Tel: 514-412-4400, ext 23890

Fax: 514-412-4331



Abstract

In response to progressively brighter flashes, the amplitude of the photopic b-wave of the human electroretinogram (ERG) first increases, then saturates at a maximal value (V_{max}) to finally decrease with the brightest flashes. The purpose of this study was to investigate if this "photopic hill" could be modulated with the use of stimuli of different wavelengths. ERGs were evoked to flashes of white, blue, green and red light presented against a white background in 30 normal subjects. Each chromatic stimulus produced a photopic hill. Findings indicate that the amplitude of V_{max} was essentially identical except for that measured in response to the red stimuli, where it was 20% smaller than the others.

Keywords: human electroretinogram (ERG), photopic hill, cones, chromatic stimulus, luminance-response function.

Introduction

In response to progressively brighter stimuli, the amplitude of the photopic ERG b-wave first increases, then saturates over a narrow range of intensities and, if the flash luminance continues to augment, will demonstrate a gradual deterioration. This unique luminance-response function is referred to as the Photopic Hill, a terminology used by Wali and Leguire (1992, 1993) who were first to describe it. Little is known about the retinal mechanisms at the origin of this ERG phenomenon. It would appear, as its name suggests, to be a feature limited to the cone ERG, given that the luminance-response function of the rod-mediated b-wave is not known to demonstrate a similar amplitude decay with brighter flashes. We know that it most probably does not reflect the gradual depletion of some retinal element (photopigment, neuromediator, neuromodulator, etc) or the progressive inhibition of a response, which could result from an overstimulation caused by the additive effect of a series of progressively brighter flashes, since an identical photopic hill is obtained whether the flashes are delivered in incremental or decremental order (Wali & Leguire, 1992).

Cone ERG responses are normally evoked to flashes of light delivered in the presence of a rod-desensitizing background light whose purpose is to avoid inclusion of a rod contamination to the response. To achieve this goal it is suggested to use a background whose luminance is between 17 and 34 $cd.m^{-2}$ (Marmor & Zrenner, 1998); although

Peachey et al. (1992) have shown that cone specific responses could be isolated with backgrounds as dim as 1.3 $cd.m^{-2}$. In a previous study (Rufiange et al., 2002b), we examined the photopic hills obtained in the presence of a rod-desensitizing background whose luminance varied between 18 and 525 $cd.m^{-2}$ and compared them to one obtained at the onset of dark adaptation. We showed that the maximal amplitude of the b-wave, which is reached at the peak of the photopic hill, was not dependent upon the luminance of the rod-desensitizing background light. There was no significant difference between the V_{max} obtained with the brightest or dimmest photopic backgrounds. The V_{max} of the photopic hill was, however, significantly smaller than that measured within the first minute of dark adaptation. These results suggested to us the concept of finiteness to describe the cone ERG b-wave. In other words, the maximal amplitude which is reached at the peak of the photopic hill, and the gradual decay in amplitude which follows, most probably do not result from the inability of the entire retina to process brighter stimuli, but is more the consequence of a built-in, intrinsic voltage limitation mechanism specific to the retinal cone pathway.

The purpose of this study was to investigate if photopic hills, of the nature described above, could also be evidenced with the use of chromatic stimuli of wavelengths spread along the visible range namely 410 nm (blue), 510 nm (green) and 640 nm (red) stimulus. Results indicate that photopic hills generated with flashes of blue and green stimulus yield almost

identical V_{max} amplitudes which are not significantly different from that obtained with white light stimulus. In contrast, the amplitude of the V_{max} obtained with the red stimulus was significantly smaller than any of the above. The red stimulus also generated smaller oscillatory potentials (OPs), the reduction being most pronounced with the long latency OP_4. Knowledge of the above not only adds to our understanding of the retinal mechanisms underlying this unique feature of the cone ERG response but also further defines its limitation.

Methods

Preparation of Subjects:

Experiments were performed on 30 normal subjects, 23 women and 7 men, aged 18 to 25 years old (mean: 21.1), who all voluntarily agreed to participate and sign an informed consent to that effect. All subjects received a complete ophthalmological examination prior to testing in order to ascertain normalcy. This study followed the tenets of the Declaration of Helsinki. Binocular ERGs were recorded with the use of DTL fiber electrodes (27/7 X-Static silver coated conductive nylon yarn: Sauquoit Industries, Scranton, PA, USA) on fully dilated pupils (Tropicamide 1%) according to a protocol previously reported (Rufiange et al., 2002a, b, 2003). Briefly, the DTL electrodes were positioned deep into the inferior conjunctival bags and secured with double-sided adhesive tape at the external and

internal canthi of each eye. Reference and ground electrodes (Grass gold cup electrodes filled with Grass EC2 electrode cream) were pasted at the external canthi of each eye and on the forehead respectively. In view of the expected inordinate length of the recording sessions (i.e. 90 minutes), the proper positioning of the DTL electrode as well as maximal pupil dilation was verified at regular intervals throughout the recording session.

ERG Procedures:

The subjects were first placed in front of a Ganzfeld of 30 cm in diameter and light-adapted for 10 minutes to a rod-desensitizing white light background of 17 $cd.m^{-2}$ (lower limit recommended by ISCEV; Marmor & Zrenner, 1998) after which photopic electroretinograms (ERGs) (bandwidth: 0.3-500 Hz; amplification: 20000 X; attenuation: 6 dB) and oscillatory potentials (OPs) (bandwidth: 75-500 Hz; amplification: 20000 X; attenuation: 6 dB) were recorded simultaneously with the use of a LKC UTAS-E-3000 system (LKC Systems Inc., Gaitherburg, MD, USA). Each flash had a duration of 20 µsec and the interstimulus interval was fixed at 2.3 seconds. Ten responses were recorded and averaged at each flash intensity and each tracing included a 40 msec pre-stimulus baseline. Luminance-response functions were obtained to white (intensity range: -0.8 to 2.84 log $cd.sec.m^{-2}$), blue (GamColor filter #850, λ_{max}= 410 nm; intensity range: -2.01 to 1.24 log $cd.sec.m^{-2}$), green (GamColor filter #650, λ_{max}= 510 nm; intensity range: -1.31 to 1.14 log $cd.sec.m^{-2}$) and red (GamColor filter #250, λ_{max}= 640 nm; intensity range: -1.43 to 1.02 log

cd.sec.m^{-2}) light. The light transmittance of the three filters are presented in Figure 1. Background luminance and flash intensities were measured with a research radiometer (IL 1700; International Light, Newburyport, MA, USA).

Data Analysis:

Analysis of the ERG included peak time and amplitude measurements of the a-, b- and i-waves and of the OPs. The data from both eyes were averaged to yield a single data point. The amplitude of the a-wave was measured from baseline to trough and that of the b-wave from the trough of the a-wave to peak. The i-wave was measured from the trough following the b-wave to the peak of the i-wave. Analysis of the OPs was restricted to the first three major OPs identified as OP_2, OP_3 and OP_4. The amplitudes of the OPs were measured individually from the preceding trough to the peak, except for OP_2, which was measured from baseline. Peak times were measured from flash onset to the peak of each wave.

Figure 2 identifies the variables that were previously suggested in order to analyze the b-wave photopic hills (Rufiange et al., 2003). The seven variables are: 1) V_{max}: the maximal amplitude of the b-wave; 2) a_{max}: the amplitude of the a-wave of the V_{max} ERG; 3) I_{max}: the intensity of the V_{max}; 4) b/a_{max}: the ratio of the amplitude of the b-wave over that of the a-wave for the V_{max} ERG (not shown at Figure 2); 5) K_a: the flash intensity necessary to

produce a b-wave 50% of V_{max} amplitude on the ascending limb of the photopic hill; 6) K_d: the flash intensity necessary to produce a b-wave 50% of V_{max} amplitude on the descending limb and 7) $K_{a=b}$: the flash intensity at which the a- and b-waves are of equal amplitude.

Statistical comparisons between the results obtained with the different wavelengths of flash were performed with one-way analyses of variance (ANOVAs), with the factor wavelength of flash (white, 410 nm blue, 510 nm green and 640 nm red) as repeated measures. Post Hoc analyses were performed using the Tukey HSD test when the ANOVAs were found significant.

Results

Representative ERG responses (left) with their corresponding OPs (right) evoked to white, 410 nm blue, 510 nm green and 640 nm red stimuli are shown at Figure 3. The a-, b- and i-waves are identified with the corresponding letter and the OP_2, OP_3 and OP_4 with the corresponding number. The overall morphology of the ERG and the OP responses are similar for all four stimuli. In all instances, the amplitude of the a-wave gradually augments with increasing flash intensities while that of the b- and i-waves initially grow in amplitude with progressively brighter flashes, then reach a plateau and finally decrease in amplitude with the brightest stimuli. Moreover, while the amplitudes of the short latency OPs (OP_2

and OP_3) augment regularly in response to progressively brighter flashes, OP_4 splits into two distinct OPs (identified as OP_{4a} and OP_{4b}) with the brightest flashes. Furthermore, it is of interest to note that, unlike OP responses evoked to white, blue and green stimuli, that obtained to red stimulation does not include a fully developed OP_4, that is one which is clearly separated from OP_3, and whose amplitude is significantly larger (almost double in white light responses; Lachapelle, 1994) than that reached by OP_2 and OP_3. This last point will be further discussed later on.

Illustrated at Figure 4 are photopic a- and b-wave luminance-response functions (mean ± SD) obtained to white, blue, green and red stimuli. In the top four graphs, the a-wave is represented by the filled squares and the b-wave by the filled circles. Irrespective of the wavelength of the stimulus, the amplitude of the b-wave increases, reaches a maximal value (V_{max}) and then decreases in response to progressively brighter flashes. Hence, each chromatic stimulus produced a photopic hill. There were however some differences in the corresponding photopic hills as shown at the bottom of Figure 4 where they are superposed to ease comparison. The seven variables measured to describe the photopic hill are reported at Table 1.

The lowest ($p< 0.0001$) maximal amplitude (V_{max}) of the b-wave was obtained in response to the red stimulus, while the V_{max} obtained to the blue and green flashes did not differ from that measured in responses evoked to the white stimuli. Similarly, the amplitude of a_{max} also varied with the wavelength of the stimulus, being largest in response to the blue stimulus ($p< 0.0001$) and smallest in response to the red stimulus while the green and white stimuli yielded almost identical a-wave luminance-response functions. Interestingly, the b/a_{max} ratio was highest in responses evoked to the white stimulus. The dimmest and brightest I_{max} were those used to generate the blue and red V_{max} ($p< 0.0001$) respectively, while there were no significant differences in I_{max} value for the green and white stimuli. The same color ranking was observed for K_a, that is, subjects were most sensitive to blue followed by green, white and finally red. Post-hoc analyses revealed that every wavelength generated a K_a significantly different from each other ($p< 0.001$). This aspect of our results is best illustrated at the bottom of Figure 4 where the four luminance-response curves (for a- and b-waves) are superposed. The suggestion of an enhanced sensitivity to the 410 nm blue stimulus is further evidenced at the Figure 5 where the b-wave luminance-response curves are normalized relative to the V_{max} of each subject. The data are also re-centered in order to line up the V_{max} obtained with the different stimuli. One can note that the overall shapes of the four photopic hills are quite similar. Interestingly however, both the ascending and descending limbs of the 410 nm blue photopic hill are shifted to the left by approximately 0.3 log units. Finally, statistical analyses were not performed on K_d and

$K_{a=b}$, since not all wavelengths yielded measurable values for these parameters (see bottom of Table 1).

In all instances, an increase in the intensity of the flash resulted in faster a-waves (Figure 6). For a given intensity of stimulation, the peak time of the a-wave was always fastest in responses evoked to the blue stimulus. In comparison, with progressively brighter flashes, the timing of the b-wave first shortens gradually (until around -1.0 log cd.sec.m^{-2}) than lengthens (up to intensities of 1.0 log cd.sec.m^{-2}) and finally shortens again with the brightest stimuli. This pattern was similar for every wavelength, except for the green and red stimuli which did not show the final decrease in timing.

A similar difference in chromatic sensitivity was also observed for the post-b-wave component identified as i-wave. This is best illustrated with the data shown at Figure 7 where its amplitude increases, saturates and then decreases with progressively brighter flashes, and that, irrespective of wavelength. As shown at the bottom of Figure 7, where the four graphs are superposed, the V_{max} of the i-wave is highest in response to the white stimulus and lowest in response to the blue and red (see also Table 2). It is of interest to note that the flash intensity needed to reach the i-wave V_{max} varied according to wavelength being dimmest with the blue stimuli and brightest with the red stimuli, thus demonstrating a chromatic differentiation identical to that observed with the b-wave V_{max} (Table 2). Finally,

irrespective of the wavelength, the i-wave reached its maximal amplitude at a flash intensity approximately 0.2 log unit dimmer than that needed for the b-wave.

Also illustrated at Figure 3 are representative OPs obtained from a normal subject in response to white, blue, green and red flashes presented in photopic condition. Data on OP amplitude measurements obtained at the intensity of flash which produced the maximal OP amplitude (sum of all three major OPs, i.e. SOPs = $OP_2 + OP_3 + OP_4$) are presented at Table 2. For example, for the chromatic luminance-response functions illustrated at Figure 3, the flash intensities required to generate the maximal OP responses (i.e. where all three OPs are of maximal amplitude; Lachapelle, 1994) are: 0.64 log cd.sec.m^{-2} (white), 0.30 log cd.sec.m^{-2} (410 nm blue), 0.69 log cd.sec.m^{-2} (510 nm green) and 0.57 log cd.sec.m^{-2} (640 nm red). The resulting tracings are compared at Figure 8. The amplitude of OP_2 is significantly larger in response to the blue stimulus ($p < 0.0005$), while the other three stimuli produced almost equivalent OP_2 amplitudes (Table 2). In comparison, all wavelengths generated identical OP_3 ($p > 0.10$). The most pronounced chromatic effect on the OP response was observed with the red stimulus where we noticed a significant reduction ($p < 0.0001$) in the amplitude of OP_4 to a value 69% of that reached with the white stimulus. In comparison, the amplitudes of the white, blue and green OP_4 were not significantly different from each other. As a result of the above, the SOP amplitude is

highest to the blue ($p < 0.05$) followed by the green and white and smallest in responses evoked to the red stimuli ($p < 0.0001$).

Discussion

The purpose of this study was to explore, with chromatic stimuli, if the typical photopic hill shape of the cone ERG luminance-response function was a universal feature irrespective of the wavelength of the stimulus. It should be emphasized here that our goal was not to isolate the S-cone response from the M- and L-cone response such as it is performed in other laboratories (Sawusch, Pokorny, & Smith, 1987; Gouras & MacKay, 1990; Gouras, MacKay, & Yamamoto, 1993; Swanson, Birch, & Anderson, 1993; Simonsen & Rosenberg, 1996; Yamamoto, Nitta, & Kamiyama, 1997; Yamamoto, Hayashi, & Takeuchi, 1999; Gouras, 2003) but rather to investigate if the previously reported photopic hill shape of the cone ERG luminance-response function was wavelength-dependent.

In all instances, a gradual increase in the strength of the stimulus progressively brought the amplitude of the b-wave to a maximum value (V_{max}) before showing a decrease with brighter flash intensities, thus resulting in the typical photopic hill shape previously evidenced by others with a white light stimulus (Wali & Leguire, 1992, 1993; Kondo et al., 2000; Lachapelle, Rufiange, & Dembinska, 2001; Rufiange et al., 2002a, b, 2003). There

were however some differences, the most striking being that the amplitude of the red V_{max} was significantly smaller (20%; $p< 0.0001$) than those obtained with the white, blue and green stimuli. The latter finding is of utmost importance given that in previous studies (Rufiange et al., 2002b, 2003), we had shown that the amplitude of the b-wave V_{max} did not vary significantly despite an increase in background illumination from 18 to 525 $cd.m^{-2}$, which resulted in a significant rightward shift along the intensity axis. This finding led us to postulate that the value of V_{max} was an immutable feature of the cone ERG b-wave luminance-response function that probably reflected the maximal voltage that can be generated by the retina irrespective of the stimulation conditions. The results presented here would suggest that this is not always the case.

It is important to stress that the smaller V_{max} that we obtained with the red stimulus was not due to our inability to generate a flash bright enough since, in 24 of the 30 subjects tested, this V_{max} was followed by progressively smaller b-waves evoked in response to gradually brighter flashes. Thus, the maximal amplitude of the cone ERG b-wave that was reached with the red flashes did represent the maximal output (as measured with the cone ERG b-wave) that the retina could generate in response to this type of stimulus delivered in photopic condition. Some could argue that our finding of a significantly smaller cone b-wave V_{max} in response to a red stimulus could have resulted from a sampling error inherent to the protocol we used. In other words, we would have missed the "real" V_{max} because our

interval between two consecutive intensities was too wide. We do not believe that this was the case since the same intensity intervals were used for all stimuli and only the red stimulus showed such a marked attenuation in V_{max}. Furthermore, if a sampling error would have been at the origin of the smaller V_{max} to 640 nm red and given that retinal sensitivity was not exactly the same for all subjects, we would have expected that the "real" V_{max} (i.e. of an amplitude identical to that reached with the other stimuli) would have appeared at least in some subjects. In fact, for all the subjects tested, the V_{max} to red was always the smallest of all, a finding which further supports our claim that this is what characterizes photopic hills obtained to a long-wavelength stimulus.

In his study, Nagata (1963) was first to demonstrate that the b-wave of the short-flash ERG, especially the last segment (i.e. that responsible for the peak), most probably resulted from the summation of the ON- and OFF-components evoked to flashes of longer duration. Of particular interest are his findings (see his figure 8) that a photopic b-wave of maximal amplitude was reached with a stimulus of 5 msec in duration and that further increases in the duration of the stimulus yielded progressively smaller responses, thus resulting in a duration-response function reminiscent of the luminance-response function (i.e. photopic hill) described in the present study. The latter would suggest that the descent of the photopic hill results from an imbalance between the ON-ERG and the OFF-ERG components generated by the brief flashes used in this study. Either the ON- is too strong to

be overpowered (or limited) by a normal OFF-, or the OFF- too weak to limit a normal ON-response. Supportive of the latter claim are the findings reported by Kondo et al. (2000) who studied the cone ERG luminance-response function with flashes of light of a duration long enough to allow for the separation of the ERG ON- and OFF- components. Their results suggest that the decrease in the amplitude of the short-flash b-wave, which yields the down-slope of the photopic hill, most probably results from the gradual weakening of the ERG OFF-response (or ERG d-wave). It is of interest to tie the latter result with those of Evers & Gouras (1986) who showed, with long-flash ERGs in monkeys, that the ON-ERG was of longest duration and the OFF-ERG included less oscillations in responses evoked to red stimuli compared to shorter wavelengths. These observations are consistent with the ON- and OFF-components being somewhat different in response to red compared to any other wavelengths; a phenomenon that could explain the lower amplitude of the V_{max} observed in the present study. Consequently, from the above, we postulate that the red stimulus either evoked a weaker or delayed (due to the longer duration of the ON-response) OFF-response compared to that generated with the other stimuli used. Both effects would result in a weaker summation of the ON- and OFF-responses and consequently yield a smaller b-wave (and OP_4) and a faster descent of the photopic hill.

The morphology of the OP signal evoked in response to the red stimulus (see Figures 3 and 8) was also unique. There was a significant reduction in amplitude of OP_4 to a value

approximately 70% of that reached with the other wavelengths. Similarly the amplitude of OP_2 was also smallest with red flashes while that of OP_3 did not appear to be wavelength-dependent. As a result of the above, the sum of OPs (SOPs) generated with the red stimulus was approximately 20% smaller than that reached with the other wavelengths, a reduction in amplitude similar to that reached with b-wave V_{max} measurements. The latter is in agreement with previous observations that the genesis of the b-wave and the OPs are probably more intimately tied than originally thought (Lachapelle, 1987; Peachey et al., 1991; Rousseau & Lachapelle, 2000). Similarly, it is noteworthy to remember that a specific modulation in the amplitude of OP_4 was previously reported elsewhere. For example, OP_4 is the only OP to be specifically and significantly enhanced during the light adaptation effect which is evidenced following a prolonged period of dark adaptation: its amplitude nearly doubling (like that of the b-wave) within the first 10 minutes of exposure to the photopic background (Lachapelle, 1987; Peachey et al., 1991; Benoit & Lachapelle, 1995). OP_4 was also the ERG component most affected in another light adaptation phenomenon observed this time following exposure to a bright photopic background light (Rousseau & Lachapelle, 2000). Along the same line of thoughts, we showed (Lachapelle et al., 1998) that two complementary retinal disorders, namely congenital stationary night blindness (CSNB, an alleged retinal ON-pathway anomaly; Barnes, Alexander, & Fishman, 2002) and a familial form of cone dystrophy (alleged retinal OFF-pathway anomaly; Sieving, 1994), also had complementary photopic oscillatory potential anomalies. In CSNB, the first two OPs (OP_2 and OP_3), suggested to signal the activation of the retinal

ON-pathway, are specifically abolished, while in the family with cone dystrophy it is the later one (OP_4), suggested to signal the activation of the retinal OFF-pathway, which was abolished. It is noteworthy to remember that Kojima and Zrenner (1978) had previously suggested an OFF- origin for the long latency OP of the photopic response. These results thus suggest that ON- and OFF- retinal pathways can be separately monitored with the OPs and consequently, our demonstration of a significantly smaller OP_4 in response to the red stimulus would again attest to the fact that this wavelength is less efficient in triggering a retinal OFF-response.

Another interesting finding reported in the present study is the shift of the photopic hill along the intensity axis noted in response to blue and red relative to the green and white stimuli. According to our results, the human retina would be most sensitive to short-wavelengths and less to long-wavelengths. Similar ERG sensitivities have been reported by other laboratories (Evers & Gouras, 1986; Hood et al., 1995). The weaker sensitivity to the 640 nm red stimulus could be explained by the fact the three types of cones in humans, even the L-cones, are not very sensitive to wavelengths above 600 nm (Forrester et al., 2002). Thus, fewer photoreceptors could be responding to the red stimulus. The greater sensitivity to short-wavelength is harder to explain. One could easily ponder on a possible rod intrusion in cone ERGs evoked to 410 nm blue light, since rods are most sensitive to short-wavelengths (Forrester et al., 2002). However, our previous demonstration that the

amplitude of the cone b-wave V_{max} remains identical while the rod-desensitizing background light varies between 18 and 525 cd.m^{-2} (Rufiange et al., 2002b) suggests that rods most probably do not contribute to cone ERGs, irrespective of the wavelength of stimulus used. Furthermore, when the four photopic hills are normalized and re-centered around the V_{max} (Figure 5), the ascent of the blue photopic hill is markedly shifted to the left (dimmer intensities) compared to the others which are superposed, suggesting that the sensitivity change is mainly reflected by a translation of the entire photopic hill on the intensity axis rather than by a change in the shape of the photopic hill itself.

The hill shape of the luminance-response curve of the i-wave has been demonstrated elsewhere (Kondo et al., 2000; Rufiange et al., 2002b). Results presented in this study further confirm, as previously alluded to, that the fate of two waves are intimately tied since, irrespective of the wavelength of the stimulus, the i-wave V_{max} was reached at an intensity 0.2 log unit dimmer than that of the b-wave. Hence, the b-wave and the i-wave generators appear to be either the same or highly dependent upon each other. Also, the fact that the i-wave was previously alleged to originate through the activation of the OFF-pathway (Nagata, 1963), probably at the level of the retinal ganglion cells (Rousseau, McKerral, & Lachapelle, 1996) would again bring further support to the above mentioned claim that the descent of the photopic hill result from the gradual weakening of the OFF-ERG component caused by progressively brighter flashes (Kondo et al., 2000).

In summary, use of chromatic stimuli to generate the unique photopic ERG luminance-response function provided us with a new means to further distinguish the different components of the photopic ERG signal as well as hypothesize on the possible origin of the photopic hill. Further work on patients with color vision deficiencies would help better define the relative contribution of the S-, M- and L-cones to the making of the different ERG components and the resulting photopic hill.

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Figure legends

Figure 1: Normalized light transmittance of the three color filters used. λ_{max} = 410 (solid), 510 (dotted) and 640 nm (interrupted).

Figure 2: Schematic representation of a photopic ERG luminance-response function illustrating the major parameters that were measured for each subject. Refer to text for the definition of the parameters. Solid line: b-wave; dotted line: a-wave.

Figure 3: Representative ERG (left) and oscillatory potential (right) responses evoked to progressively brighter (from bottom to top) white, 410 nm blue, 510 nm green and 640 nm red flashes. All responses were obtained from the same subject. Flash intensities are indicated in log $cd.sec.m^{-2}$ on the left hand side of the ERG responses. For the ERG responses, the a-, b- and i-waves are indicated with the corresponding letter while for the oscillatory potential responses, OP_2, OP_3 and OP_4 are identified with the corresponding numerals except for OP_{4a} and OP_{4b}, which are identified with the corresponding letters. The vertical arrow represents the flash onset. Calibration: Vertical, 50 μV (ERG) and 25 μV (OPs); Horizontal, 25 msec.

Figure 4: Mean (± SD) a- and b-wave luminance-response functions to white, 410 nm blue, 510 nm green and 640 nm red flashes. In the top four graphs, the squares represent the a-wave and the circles, the b-wave. In the bottom two graphs, the four curves are superposed to ease comparison. The abscissa is in log cd.sec.m^{-2} and the ordinate is in μV.

Figure 5: Normalized (%) b-wave luminance-response curves obtained with the white, 410 nm blue, 510 nm green and 640 nm red stimuli. Flash intensities are relative to the V_{max} intensity, which is reported as log 0 along the abscissa.

Figure 6: A- and b-wave peak time luminance-response functions obtained to the four light stimuli used. The abscissa is in log cd.sec.m^{-2} and the ordinate is in msec.

Figure 7: Mean (± SD) i-wave luminance-response functions to broad-band white, 410 nm blue, 510 nm green and 640 nm red flashes. In the bottom graph, the four curves are superposed to ease comparison. The abscissa is in log cd.sec.m^{-2} and the ordinate is in μV.

Figure 8: Representative oscillatory potential responses evoked to the white, 410 nm blue, 510 nm green and 640 nm red stimuli. Responses were obtained at the intensity where the maximal amplitude of the three major OPs was reached (see text). OP_2, OP_3 and OP_4 are represented with the corresponding number. The flash intensity (in log cd.sec.m^{-2}) is indicated at the left of each tracing. Calibration: Vertical, 25 μV; Horizontal, 25 msec.

Table 1: ERG photopic hill parameters (± SD) for the 30 subjects. Refer to text for definition of the parameters and statistical analyses. V_{max} and a_{max} are expressed in µV whereas I_{max}, K_a, K_d and $K_{a=b}$ are in log cd.sec.m^{-2} and b/a_{max} represents a ratio.

color of flash	V_{max}	a_{max}	I_{max}	b/a_{max}	K_a	K_d	$K_{a=b}$
white	92.2 (21.3)	26.7 (5.5)	0.35 (0.21)[3]	3.49 (0.70)[3]	-0.47 (0.12)[1]	1.59 (0.48)	1.45 (0.22)
blue	95.1 (20.0)[2]	34.7 (8.8)[1]	0.05 (0.25)[1]	2.84 (0.72)[2]	-0.90 (0.17)[1]	1.00 (0.19)	0.99 (0.23)
green	90.6 (22.6)	28.0 (5.9)	0.30 (0.20)[3]	3.26 (0.62)	-0.57 (0.15)[1]	1.04 (0.11)	1.14 (0.00)
red	74.3 (20.2)[1]	24.5 (5.8)	0.63 (0.23)[1]	3.10 (0.84)	-0.32 (0.17)[1]	—	—
ANOVA	$F_{(3,87)}=98.3$ $p< 0.0001$	$F_{(3,87)}=31.9$ $p< 0.0001$	$F_{(3,87)}=49.8$ $p< 0.0001$	$F_{(3,87)}=12.4$ $p< 0.0001$	$F_{(3,87)}=204.9$ $p< 0.01$	—	—

K_d was obtained for 19 subjects with white, 9 with blue, 5 with green and 0 with red stimuli. $K_{a=b}$ was obtained for 24 subjects with white, 14 with blue, 7 with green and 0 with red stimuli. Post Hoc analyses: [1] = different than the 3 others, [2] = different than green, [3] = different than blue and red, $p< 0.005$.

Table 2: ERG i-wave and oscillatory potential (OP) parameters (± SD) for the 30 subjects. Amplitudes are given in μV and intensities in log cd.sec.m^{-2}.

color	i-wave V_{max}		OP amplitude			
of flash	amplitude	intensity	OP_2	OP_3	OP_4	Sum of OPs
white	29.3 (8.6)[1]	0.11 (0.33)[3]	14.7 (5.4)[4]	17.1 (7.2)	28.0 (9.2)	59.8 (17.9)[3]
blue	22.6 (7.4)[2]	-0.20 (0.52)[1]	17.9 (6.3)[1]	17.9 (7.3)	29.7 (10.1)	65.5 (20.5)[1]
green	25.5 (8.1)[1]	0.08 (0.25)[3]	14.4 (4.9)	18.9 (7.5)	26.4 (10.2)	59.7 (19.8)[3]
red	22.5 (8.8)[2]	0.44 (0.26)[1]	12.9 (4.3)	17.9 (7.3)	19.2 (7.5)[1]	49.7 (14.5)[1]
ANOVA	$F_{(3,87)}=19.4$	$F_{(3,87)}=17.3$	$F_{(3,84)}=19.7$	$F_{(3,84)}=1.9$	$F_{(3,84)}=19.1$	$F_{(3,84)}=21.6$
	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p > 0.10$	$p < 0.0001$	$p < 0.0001$

OP results with the red flashes include 29 subjects. Post Hoc analyses: [1] = different than the 3 others, [2] = different than white and green, [3] = different than blue and red, [4] = different than red, $p < 0.05$.

Figure 1

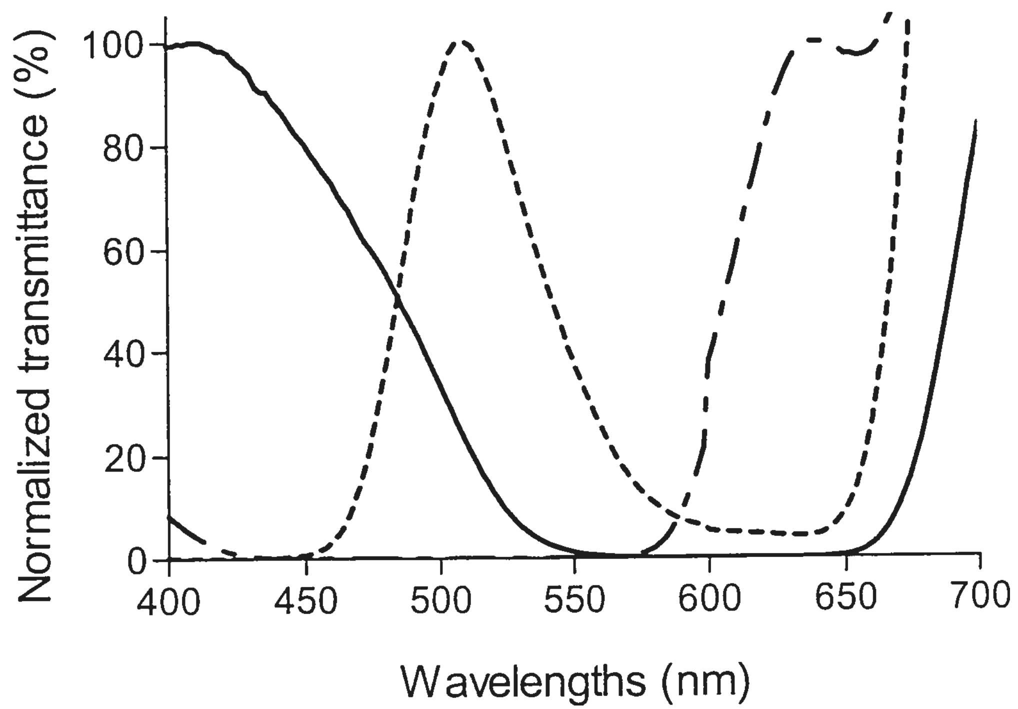


Figure 2

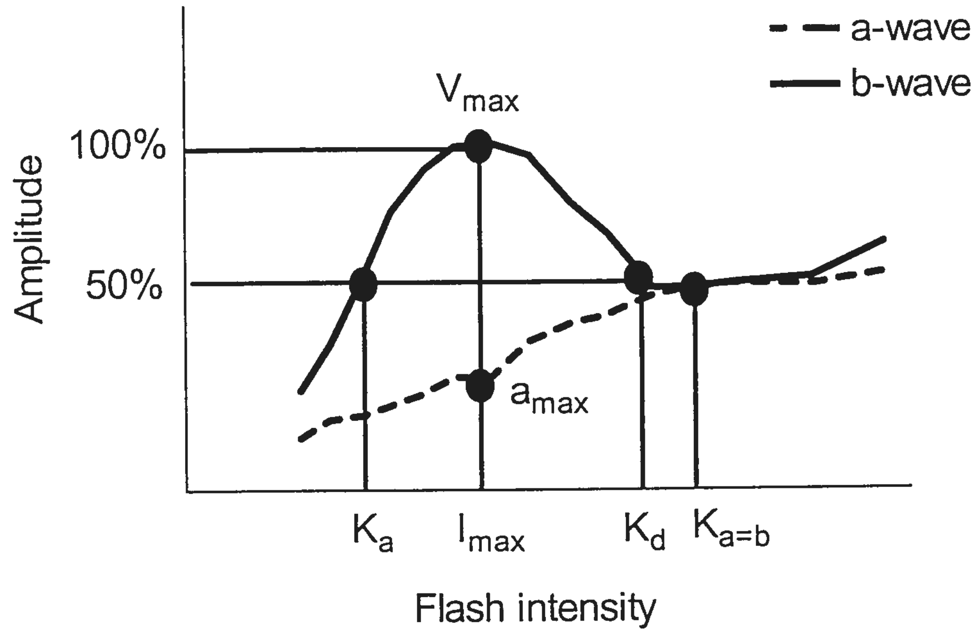


Figure 3

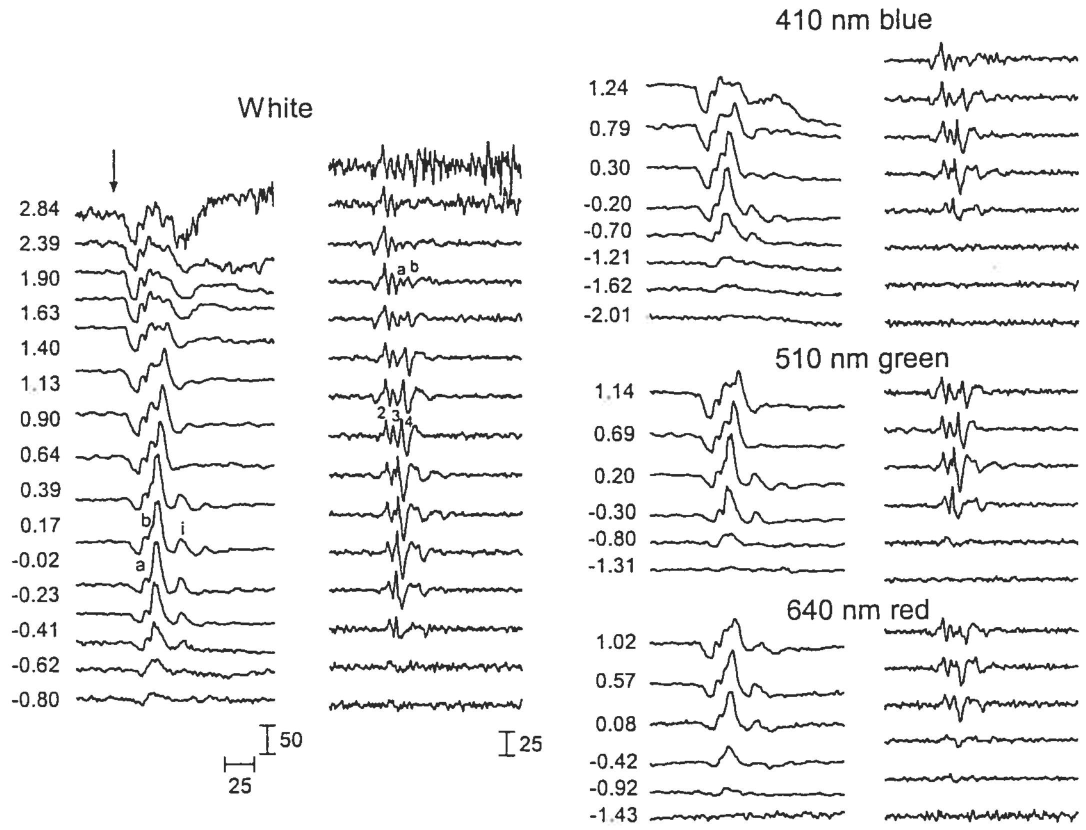


Figure 4

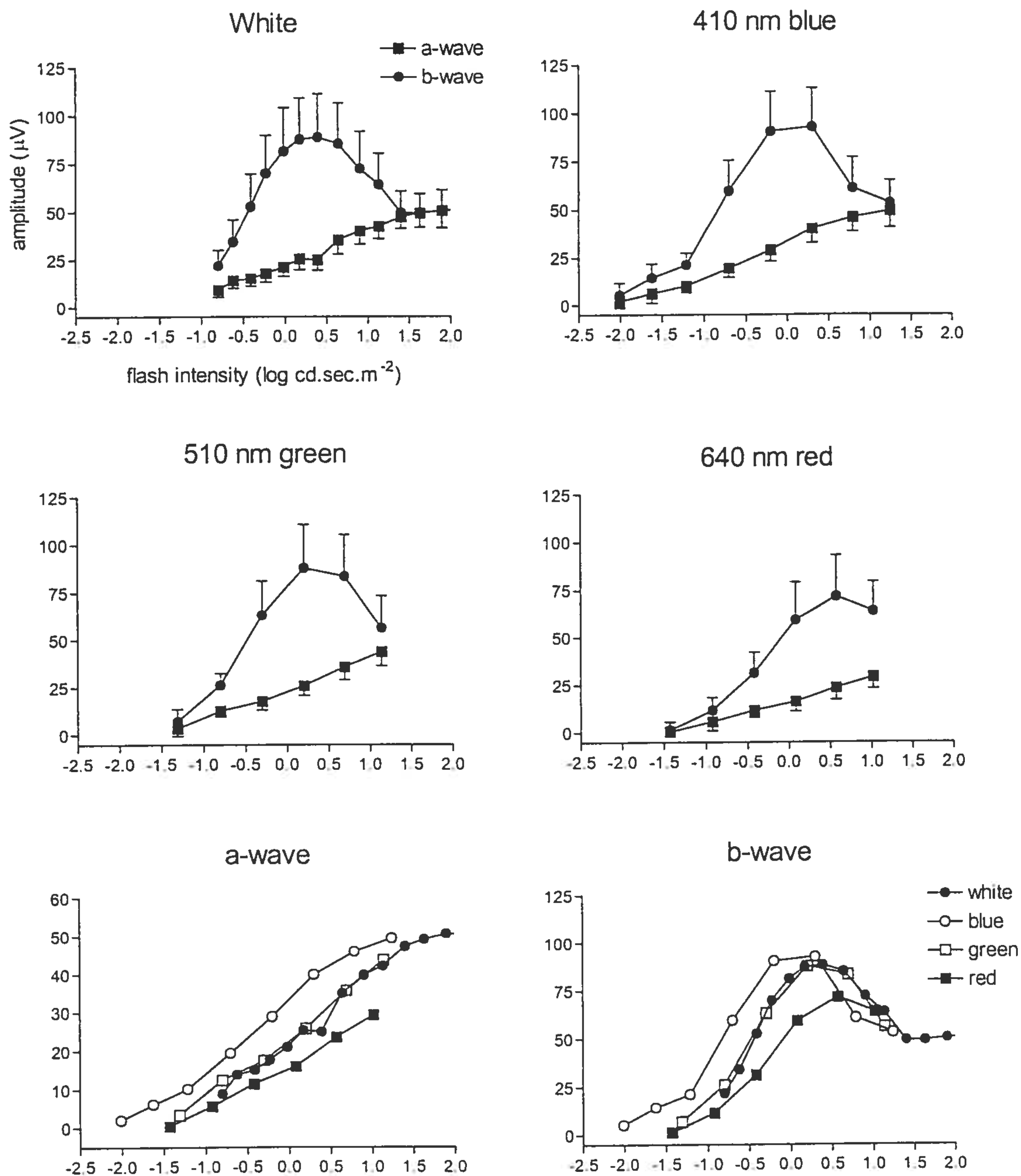


Figure 5

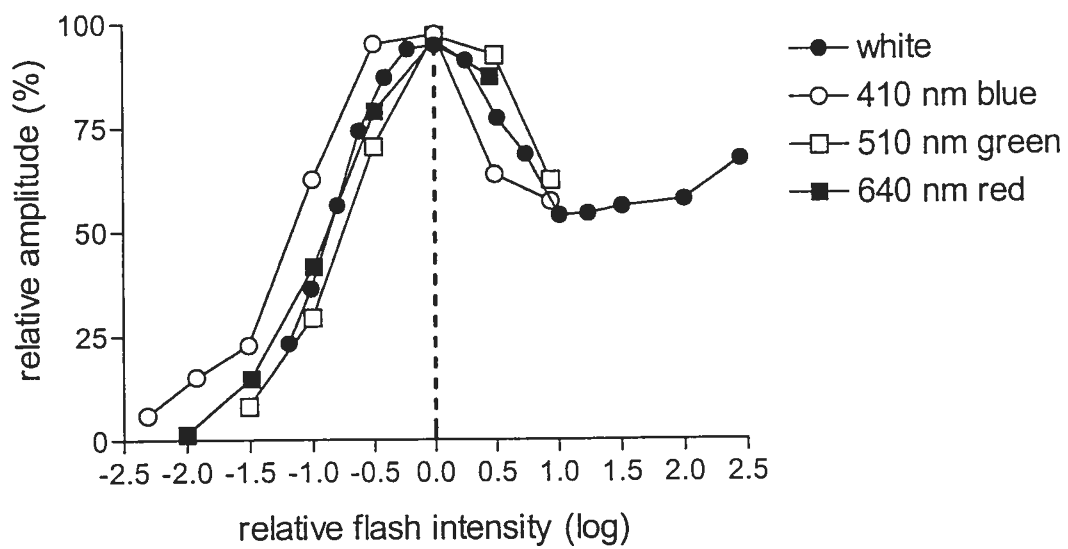


Figure 6

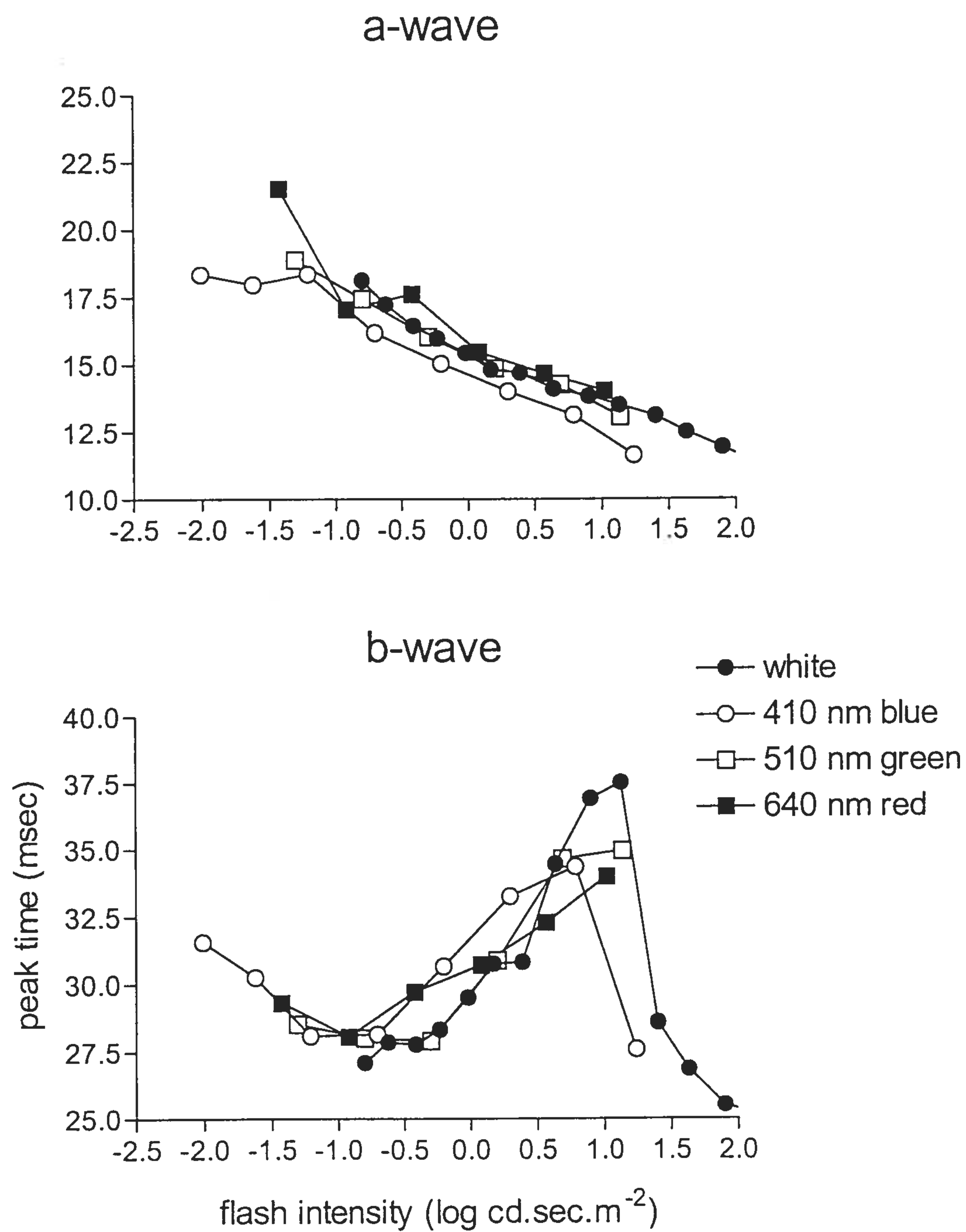


Figure 7

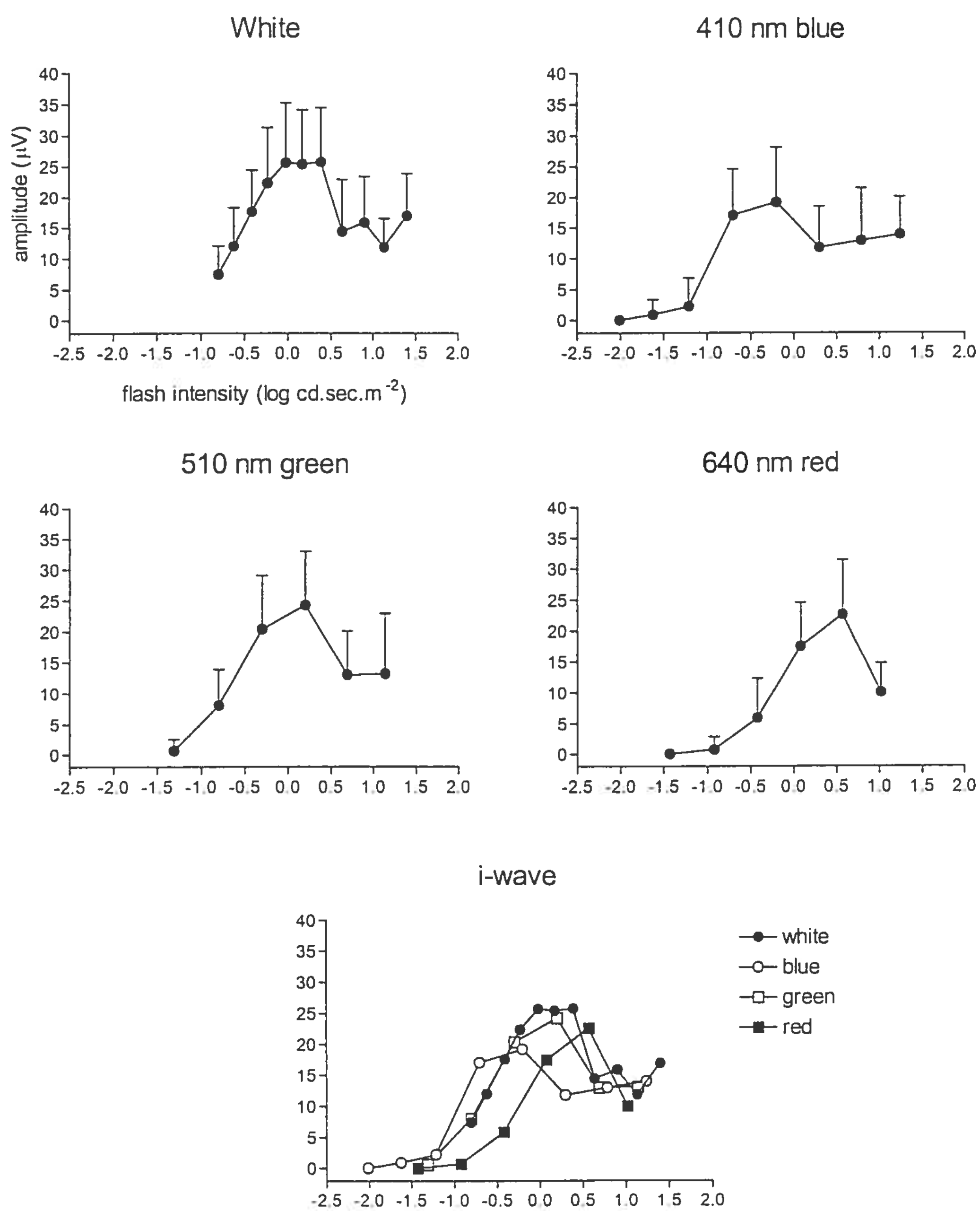
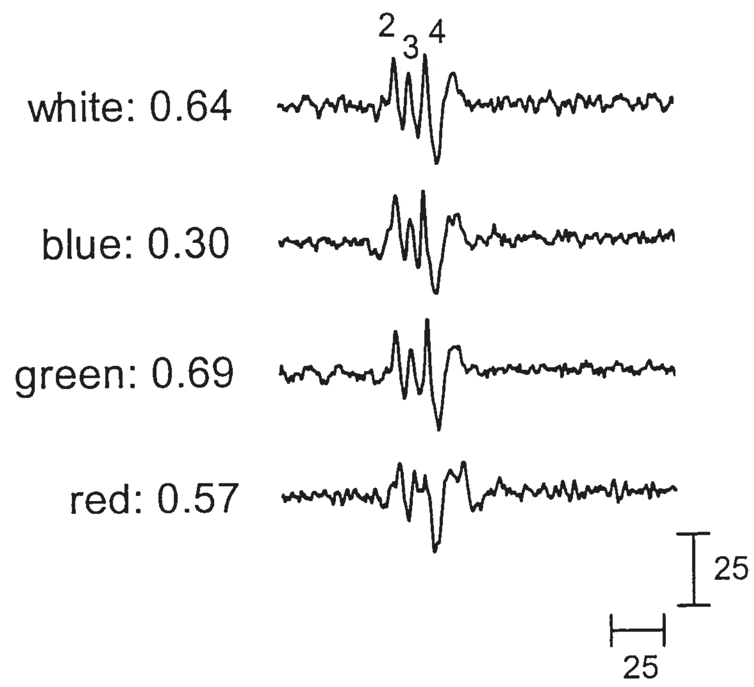


Figure 8



3.4. Quatrième article

Correlating retinal function with melatonin secretion in subjects with an early or late circadian phase.

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Correlating retinal function with melatonin secretion in subjects with an early or late circadian phase.

Marianne Rufiange, B.Sc.[1,2], Marie Dumont, Ph.D.[2], Pierre Lachapelle, Ph.D.[1]

[1] Department of Ophthalmology, McGill University, Montreal Children's Hospital Research Institute; [2] Department of Psychiatry, University of Montreal, Chronobiology Laboratory, Sacré-Coeur Hospital

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Corresponding author:

Pierre Lachapelle, PhD

Department of Ophthalmology (D-164)

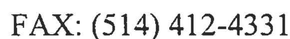
McGill University - Montreal Children's Hospital Research Institute

2300 Tupper Street

Montreal, Quebec, Canada, H3H 1P3

TEL: (514) 412-4400, ext 23890

FAX: (514) 412-4331



Abstract

PURPOSE: Evaluate the diurnal variation of retinal function, as measured with the electroretinogram (ERG), in subjects with an early (morning type: M-Type) or a late (evening type: E-Type) circadian phase. METHODS: Subjects (n=24) were recruited according to their scores on a Morningness-Eveningness Questionnaire assessing preferences in bedtime, waketime, timing of performance, etc. ERG testing was performed twice on each subject, at 22:30h and at 08:00h. Luminance-response functions were obtained in scotopic (blue flashes) and in photopic conditions (white, blue, green and red flashes). Salivary melatonin samples were taken every half-hour from 20:30h to 00:00h and from 06:30h to 09:30h. RESULTS: In scotopic conditions, both groups had lower ERG amplitudes and retinal sensitivity at 08:00h. In photopic conditions, the two groups showed an opposite pattern of diurnal variations. The E-Types demonstrated a significant reduction in ERG amplitudes at 08:00h whereas the M-Types showed an increase in amplitude at the same time. In addition, negative correlations were found between both the cone ERG and mixed rod-cone ERG and the concentration of salivary melatonin, indicating that the ERG amplitude is lowest when melatonin concentration is highest. CONCLUSIONS: The reduction in scotopic ERG responses at 08:00h seen in both groups might be due to the peak of rod disk shedding which takes place, in some mammals, at around light onset. The strong correlation between the cone ERG and salivary melatonin could be attributable to a

direct effect of retinal melatonin on the physiology of cones or of the circadian phase of the subjects.

Keywords: electroretinography, melatonin, circadian rhythm, diurnal variation, luminance-response function.

Introduction

Diurnal variations in human retinal functions as measured with the electroretinogram (ERG) have been previously reported. Nozaki et al.[1] observed a diurnal rhythm in the scotopic mixed (rod-cone) b-wave amplitude in more than 60% of their subjects where the minimal amplitude was observed early in the morning. Birch et al.[2-3] reported a 24-h rhythm for the rod b-wave amplitude in eyes entrained to a 14h-light/10h-dark cycle. Again, the lowest amplitude was observed at 09:30h, a time that the authors associated with the diurnal peak of rod outer segment disk shedding. More recently, Hankins et al.[4-5] reported an increase in the implicit time of the photopic b-wave at night compared to daytime measurements for subjects kept in natural lighting conditions, a finding which is also consistent with the presence of diurnal variations in the human ERG.

The above-mentioned variations in ERG parameters could either be the consequences of the light-dark cycle or that of the sleep-wake rhythm. However, several studies[6-10] have shown diurnal variations of the ERG in animals kept under constant environmental conditions. It is therefore possible that the diurnal variations in retinal functions are under the control of an endogenous circadian oscillator. Among the several hypotheses proposed to explain this diurnal rhythm, there is the role of dopamine and melatonin within the retina. Dubocovich[11] suggested that interactions between retinal dopamine and melatonin could control the retinal sensitivity to light. A circadian rhythm in the secretion of melatonin has been

demonstrated in isolated retinas of some mammals, including rats and mice, that were maintained in constant darkness[12-14]; showing that the rhythmic secretion of melatonin is endogenous to the retina itself. According to most studies, the rod and cone photoreceptors would be the most probable candidates for melatonin synthesis in the retina[15].

Retinal melatonin was shown to be involved in cone elongation[16], aggregation of melanin pigments in the pigmented epithelium[17-18], suppression of light-adaptive horizontal cell spinule formation[19], priming of the light-evoked rod outer segment disk shedding[20-21] and inhibition of dopamine release[22-24]. Melatonin is considered by many as the major signal of dark-adaptation or nighttime within the retina and dopamine as that of light adaptation or daytime[15,19,25-26]. It is therefore possible that the diurnal variations observed in retinal functions could in part be the consequences of the endogenous rhythm of retinal melatonin secretion.

In humans, it is impossible to measure directly the secretion of retinal melatonin. However, with the development of highly sensitive radioimmunological assays, it is now possible to determine repeatedly and non-invasively the concentration of circulating melatonin in the saliva. This measure accurately reflects the plasmatic concentration of pineal melatonin[27]. Although the phase relationship between retinal and pineal melatonin rhythms is still uncertain, both episodes of melatonin secretion are essentially nocturnal and are acutely

suppressed by exposing the retina to light. In addition, both circadian rhythms are similarly entrained by the light-dark cycle[15]. Therefore, it is reasonable to assume that the two rhythms of melatonin secretion will have the same circadian phase under a normal light-dark cycle.

Consequently, the aim of this study was to assess the relationship between the timing of melatonin secretion and the diurnal variations in retinal function as measured with the ERG. We also examined if the variation in the cone response could be influenced by the wavelength of the stimulus since a previous report of ours on the photopic luminance-response function showed that the maximal amplitude of the cone b-wave was wavelength-dependent[28], a finding which suggests that the cone function can be further characterize with this technique. Retinal function was evaluated at the same clock time in the late evening and in the early morning, in two groups of healthy volunteers kept under the same sleep/dark schedule but differing as to their timing of melatonin secretion.

Methods

Subjects:

A French version of the Horne and Ostberg[29] questionnaire was used to characterize Morningness-Eveningness orientation in 569 university students. This questionnaire is composed of 19 questions from which a score (M/E score) between 16 and 86 is obtained. A score higher than 58 identifies morning type individuals (M-Types) whereas an index lower than 42 identifies evening types (E-Types). It is expected that M-Types will have earlier habitual waketimes and bedtimes, and earlier onset and offset of melatonin secretion compared to E-Types[30]. Among the respondents, 108 were M-Types and 142 were E-Types. From the above, 12 M-Types (M/E scores: 60 to 74, mean = 66.6; age 19 to 25 years, mean = 21.1 years; 9 women, 3 men), and 12 E-Types (M/E scores: 22 to 38, mean = 27.8; age 20 to 25 years, mean = 21.3 years; 8 women, 4 men) were selected for the study. All subjects had a regular sleep-wake schedule without sleep or vigilance complaints. Subjects were not using medications known to affect sleep, vigilance or melatonin secretion (e.g.: NSAIDs, β-blockers, anxiolytics, hypnotics). No caffeine or tobacco products could be used during the experiment. A complete ophthalmologic examination was performed prior to the experiment in order to rule out any retinal disorders. All subjects had best corrected visual acuity of 20/20 or better. Finally, subjects were excluded if they had worked on night shifts during the past six months or had made a transmeridian flight in the past month. Each participant signed an informed consent form approved by the ethics

committee of the Montreal Children's Hospital and received a financial compensation. Furthermore, the research followed the tenets of the Declaration of Helsinki.

Procedures:

Schedule:

The experiment was performed from July 1999 to June 2000 and the subjects from the two groups were tested in random order. Ambulatory activity measures (Actiwatch®, Mini-Mitter Co, OR, USA) were obtained during two to seven days prior to testing. Each participant also completed a seven-day sleep-wake diary. On the day of testing, the subjects were admitted to the Visual Electrophysiology Laboratory (Department of Ophthalmology, Montreal Children's Hospital) at 19:30h, and stayed until 09:30h the following morning. Each subject participated to two ERG sessions: the first one from 22:30h to 23:40h and the second from 08:00h to 09:10h on the following morning. The subjects were kept under dim illumination (<10 lux) from 19:30h to 09:30h, except during the photopic part of the ERG, i.e. from 22:50h to 23:40h and from 08:20h to 09:10h. Subjects slept in complete darkness from 00:00h to 06:00h and had to wear dark goggles (5 log units of attenuation) to go to the bathroom.

Melatonin Measurements:

In order to measure the concentration of melatonin, saliva samples were collected (using Salivettes®, Sarstedt Inc, Newton, NC) every half-hour from 20:30h to 00:00h and from 06:30h to 09:30h. Melatonin concentrations were determined by radioimmunoassay with a ^{125}I-labelled tracer (Bühlmann Laboratories, Switzerland). With this method, the minimum detectable dose of melatonin (analytical sensitivity) is reported to be of 0.2 pg/ml whereas the functional least detectable dose is of 0.65 pg/ml[31]. All samples from a given subject were assayed in the same run. Each sample was divided in two for the extraction and sample duplicates with a coefficient of variation (CV) larger than 10% were rejected (3.2% of all samples). The intra-assay CVs for control samples of 1.9 and 14.7 pg/ml were 6.1 and 7.3%, respectively.

ERG Recordings:

Electrophysiological recordings were performed as previously reported[32-33] and in accordance with the ISCEV ERG standards[34]. The pupils were maximally (8-9 mm) dilated with Tropicamide 1% and the pupil size was measured at the beginning and at the end of the recording procedure. There were no pupil size differences noted either between the evening and morning sessions or between the beginning and end of the ERG procedure. DTL fiber electrodes (27/7 X-Static® silver coated nylon conductive yarn; Sauquoit Industries, Scranton, PA, USA) were positioned deep into the inferior conjunctival bag and

secured with double-sided adhesive tape at the external and internal canthi. Reference and ground electrodes (Grass gold cup electrode filled with Grass EC2 electrode cream) were pasted at the external canthi and forehead, respectively. ERGs (bandwidth: 0.3-500Hz; amplification: 10000X scotopic and 20000X photopic; attenuation: 6dB) and oscillatory potentials (OPs) (bandwidth: 75-500Hz; amplification: 10000X scotopic and 20000X photopic; attenuation: 6dB) were recorded simultaneously from both eyes with a LKC UTAS-E-3000 system which included a Ganzfeld of 30 centimeters in diameter.

Subjects were first dark-adapted for 30 minutes (from 22:00h to 22:30h and from 07:30h to 08:00h, respectively). Scotopic luminance-response functions were then obtained (from 22:30h to 22:50h and from 08:00h to 08:20h, respectively) with the use of 11 intensities of blue (GamColor filter 850, λ_{max}=410nm) flashes ranging from -5.01 to -0.96 log cd.m^{-2}.sec. Each flash had a duration of 20 µsec and the interstimulus interval was fixed at ten seconds. Five responses were recorded and averaged at each flash intensity. To avoid the light adaptation effect previously reported[35], the subjects were then light-adapted for 10 minutes (from 22:50h to 23:00h and from 08:20h to 08:30h, respectively) to a white light background of 17 cd.m^{-2} after which photopic ERGs were recorded against the same background light (from 23:00h to 23:40h and from 08:30h to 09:10h, respectively). The interstimulus interval was reduced to 2.3 seconds for the photopic ERG. Ten responses were recorded and averaged at each flash intensity. Luminance-response functions were

obtained with the use of 15 intensities of white (-0.8 to 2.84 log $cd.m^{-2}.sec$), 8 intensities of blue (GamColor filter 850, λ_{max}=410nm; -2.01 to 1.24 log $cd.m^{-2}.sec$), 6 intensities of green (GamColor filter 650, λ_{max}=510nm; -1.31 to 1.14 log $cd.m^{-2}.sec$) and 6 intensities of red (GamColor filter 250, λ_{max}=640nm; -1.43 to 1.02 log $cd.m^{-2}.sec$) light. Flash intensities and background luminance were measured with a research radiometer (International Light, IL 1700).

Data Analysis:

Of the 24 subjects, two had saliva samples with insufficient volume for accurate melatonin measurements. Therefore, melatonin results are available only for 11 M-Type and 11 E-Type subjects. Since there are large interindividual variations in melatonin concentration[36], all analyses were performed on data transformed into percent of the maximum concentration observed in each individual subject. The onset of melatonin secretion was defined as the clock time of the first evening saliva sample with a melatonin concentration greater than or equal to 33% of the maximum. Similarly, melatonin offset was defined as the clock time of the first morning sample with a melatonin concentration lower than or equal to 33% of maximum concentration. For comparisons with ERG parameters, relative melatonin concentrations were averaged among the samples collected during the evening ERG session (at 22:30h, 23:00h and 23:30h) and during the morning ERG session (at 08:00h, 08:30 and 09:00h). For each subject, habitual bedtime and waketime were

estimated by averaging the times reported in the sleep-wake diary for the 5 days prior to the experiment. These estimates were validated with the activity data recorded with the ambulatory monitor.

Figure 1 illustrates typical scotopic and photopic ERG recordings evoked to progressively brighter flashes (from bottom to top). The corresponding luminance-response functions (mean of both eyes) are graphically reported at the bottom part of the figure. In scotopic conditions, the amplitudes of the a-wave (black squares) and the b-wave (white squares) increase gradually with flash intensity. The maximal amplitude of the scotopic b-wave or rod V_{max} was determined by fitting (GraphPad Software, San Diego, CA, USA) the b-wave amplitude data - from the lowest intensity to the intensity at which an a-wave of twice the baseline noise was obtained (here, at -2.22 log cd.m-2.sec) - to a sigmoidal curve (solid line)[32,37]. The point of maximal amplitude on this curve was identified as the rod V_{max}. In addition, we also considered the ERG recorded at the maximal intensity (-0.96 log cd.m^{-2}.sec) used in scotopic conditions, which we identified as the scotopic mixed rod-cone response[34]. In comparison, the luminance-response curve of the photopic ERG also shows that the a-wave augments in amplitude with intensity, but the behavior of the b-wave differs significantly. With progressively brighter flashes, the amplitude of the b-wave first increases to reach a maximum (V_{max}) then gradually decreases with higher flash intensities

to finally form a plateau. This unique luminance-response function was previously described as the Photopic Hill[28,38-39].

The analysis of the ERG included peak time and amplitude measurements of the a-, b- and i-waves. The data from both eyes were averaged to yield a single data point. The amplitude of the a-wave was measured from baseline (which was recorded for 40 msec prior to flash onset) to trough and that of the b-wave from the trough of the a-wave to peak of the b-wave. The i-wave, which is the small positive peak after the b-wave[40], was measured from the trough following the peak of the b-wave to the peak of the i-wave. The amplitude of each of the three major photopic OPs was also calculated from the preceding trough to the peak except for OP_2 that was measured from the baseline to the peak. Peak times were measured from flash onset to the peak of each wave. The rod sensitivity (K_s) was determined as the flash intensity necessary to produce a b-wave 50% of the scotopic V_{max} while the cone sensitivity (K_p) was defined as the flash intensity needed to produce the photopic V_{max} (see Figure 1). It should be noted that one outlier (> ±2 SD) was rejected of the analyses for the amplitude of the scotopic mixed a-wave and three outliers who were also rejected for the peak time of the scotopic mixed b-wave.

Statistical comparisons between groups and times of testing were performed with 2x2 analyses of variance (ANOVAs), with the factor Group (M-Types and E-Types) and the factor Time-of-Day (22:30h and 08:00h, repeated measures). Contrast analyses (planned comparisons) were performed when interaction effects were found. Melatonin onset and offset, as well as habitual bedtime and waketime were compared between groups with Student t-tests for independent samples. Correlations between diurnal variations in melatonin concentration and ERG parameters were computed with the Pearson test on the ratio of the results obtained during the evening session over the results obtained during the morning session. Melatonin ratios were log-transformed in order to normalized their distribution for the statistical analyses.

Results

Melatonin Secretion:

As expected, M-Types had earlier habitual bedtimes and waketimes compared to E-Types, and also showed an earlier circadian phase, as estimated with the onset and offset of melatonin secretion (Table 1). Averaged relative melatonin concentrations measured during the ERG sessions showed a strong Group-by-Time interaction ($F_{1,20}$=14.18, p<.001). The relative melatonin concentration was significantly higher in the M-Type group compared to the E-Type group during the evening session ($F_{1,20}$=8.69, p<.01) whereas the reverse was

seen during the morning session ($F_{1,20}$=10.13, p<.01). Group differences in profiles of melatonin secretion in relation with the timing of the ERG recordings are illustrated at Figure 2. The episode of melatonin secretion occurred earlier in the M-Type group compared to the E-Type group as revealed by the earlier increase in concentration in the evening as well as the earlier decrease in the morning in former group. A drop in melatonin concentration was observed at 23:30h in the E-Type group, that is 40 minutes after the background light had been turned on. This decrease was seen in 8 out of the 11 E-Type subjects. No similar decreases were detected with the M-Types during the evening session or during the morning session for either group.

Retinal Function:

Figure 3 shows representative ERG responses (i.e. scotopic V_{max}, scotopic mixed response, photopic V_{max} and photopic OPs) obtained from one E-Type and one M-Type subject during the evening (22:30h, dash line) and the morning (08:00h, solid line) sessions. It can be observed that the overall morphology of the ERG responses remained the same irrespective of the recording session. However, there are suggestions of amplitude changes that are most prominent for the E-Type subject. This is best visualized at Table 2 where the mean (± 1 SD) of the scotopic and photopic results obtained from all 24 subjects are given along with their respective diurnal variations, where 08:00h ERG measurements are expressed as a percentage (%) of the 22:30h measurements.

In photopic conditions (Table 2), irrespective of the wavelength of the stimulus, the amplitude of the a- and b-waves at V_{max} intensity as well as the sum OPs amplitude showed an opposite pattern in diurnal variation between the two groups of subjects. The E-Type group demonstrated a 4 to 16% reduction in amplitude at 08:00h compared to 22:30h whereas the M-Type group showed a 3 to 13% increase in amplitude. Furthermore, all Group-by-Time interactions resulted from a significant reduction in the amplitude at 08:00h for the E-types. For the M-Types, none of the amplitude increases at 08:00h was significant. Finally, no diurnal changes were observed for either group in the cone sensitivity (K_p), in the i-wave maximal amplitude (see Table 2) or in the photopic a- and b-wave peak times at V_{max} intensity.

In scotopic conditions (Table 2), both groups showed a 08:00h decrease in rod sensitivity (K_s), in amplitude of the b-wave V_{max} as well as in the amplitude of the mixed a- and b-waves. The magnitude of these diurnal changes was however greater in the E-Type group. The amplitudes of the a- and b-waves in scotopic mixed ERGs both showed a Group-by-Time interaction. Compared to 22:30h, the amplitudes of the a- and b-waves of the E-Type group measured at 08:00h were significantly reduced by 24% and 17%, respectively, whereas the M-Type group showed no significant diurnal changes (less than 5%). In contrast, the two groups could not be differentiated with their diurnal variations in K_s and

b-wave V_{max} amplitude (Time effect, see Table 2) despite the fact that E-Types showed a slightly greater morning decrease (14% for V_{max} and 6% for K_s) compared to M-Types (7% for V_{max} and 2% for K_s). No diurnal variations in peak times were found for E-Types and M-Types except for the a- and b-waves of the scotopic mixed response (see Table 2).

Correlations between Melatonin Secretion and Retinal Function:

Figure 4 presents the evening session over morning session ratios of ERG amplitude results plotted against the corresponding log melatonin ratios obtained for each M-Type and E-Type subjects. In both scotopic and photopic conditions, the ERG amplitude ratios decreased as the ratios of melatonin concentration increased. The negative correlation was significant for the scotopic mixed a- and b-waves ($r= -0.52$, $p<.01$ and $r= -0.45$, $p<.05$, respectively) as well as for the photopic b-wave V_{max} obtained with the white flash ($r= -0.44$, $p<.05$, see Figure 4). However, significance was not reached for the scotopic b-wave V_{max} nor for the photopic a-wave at V_{max} intensity. Although not shown at Figure 4, the correlation was also significant for the photopic a- and b-waves at V_{max} obtained with the green ($r= -0.42$ and $r= -0.50$, $p<.05$, respectively) and red ($r= -0.49$ and $r= -0.43$, $p<.05$, respectively) stimuli. Furthermore, a higher peak time ratio (scotopic mixed a- and b-waves) was associated with a higher ratio of salivary melatonin ($r= +0.50$ and $r= +0.51$, $p<.05$, respectively). No correlations were found with the K_s or with photopic sum OPs.

Discussion

Our results provide support to the hypothesis that parameters of the human ERG change according to the time of day, and that melatonin (pineal and/or retinal) is associated with this diurnal variation. For E-Types, the amplitudes of the a- and b-waves in photopic conditions were significantly lower in the morning ERG compared to the evening. This decrease was accompanied by a higher concentration of salivary melatonin compared to the level obtained during the evening session. In contrast, in the M-Type group, ERG tended to increase as melatonin concentration decreased in the morning session relative to the evening session. Furthermore, the significant correlations obtained in photopic (cone response) and scotopic mixed (rod-cone response) conditions between the daily variation in amplitude and peak time of the a- and b-waves and that of the salivary melatonin concentration suggest a close relationship between the two phenomena. Two hypotheses arise from the above assertion: either melatonin has a direct effect on the visual physiology or the same circadian oscillator regulates both rhythms.

It appears from the results reported in this study that the presence of melatonin in the organism is associated with a decrease in the amplitude and an increase in the peak time of the cone response. Our findings thus confirm those of Emser et al.[41] who reported a decrease in the amplitude of the human ERG following oral administration of melatonin in the afternoon. Unfortunately, the authors did not distinguish the cone from the rod effect.

Similarly, Lu et al.[8] also showed that an intramuscular injection of melatonin during the day decreased the amplitude of the photopic b-wave of chickens. The above effects of exogenous melatonin on the ERG amplitude could be due to the fact that this hormone was shown to stimulate cone elongation[16] and suppress the light-adaptive horizontal cell spinule formation[19]. These two phenomena could contribute to the decrease in the cone response, the former by reducing the photon catch and the latter by diminishing the efficiency of synapses between the cones and the horizontal cells.

On the other hand, it has been suggested that a circadian oscillator could in part regulate retinomotor movements of the cone inner segments[42]. This hypothesis is supported with the observation that the initiation of the cone contraction precedes the actual light onset at dawn and that this rhythm persists even in constant darkness conditions[42]. Therefore, we might be looking here at the impact that the circadian phase of our subjects exerted on the photoreceptor physiology rather than at the direct effect of melatonin on the cone ERG. On the other hand, the retinal secretion of melatonin could be the mediator by which the circadian oscillator modulates the retinomotor movements.

In this study we also examined if the diurnal variation of the cone response could be wavelength dependent since a previous report of ours[28] showed that the color of the flash stimuli had an impact on the maximal amplitude of the cone b-wave as revealed with the

photopic luminance-response curve or Photopic Hill. Our results do not reveal wavelength dependent effects either in the pattern of diurnal variation or in the correlation with melatonin concentration. There is however a trend for the green and red stimuli to show a greater diurnal variation and a stronger correlation with melatonin compared to the white and blue stimuli.

For the scotopic response, both groups of subjects showed similar diurnal variations, that is a decrease in ERG amplitudes and retinal sensitivity at the morning session compared to the evening session. Furthermore, there was no correlation between melatonin and ERG parameters. A similar reduction in rod ERG at around the usual light onset was previously reported. Birch et al.[2-3] observed the lowest rod ERG amplitudes in humans an hour and a half after light onset. Similarly, a study conducted in albino rabbits showed that the scotopic b-wave demonstrated a 16% reduction in amplitude some 30 minutes after the onset of light[6]. This reduction correlated with an increase in the phagosome count within the pigmented epithelium that is a marker of the disk shedding activity. In pigmented rats, ERG analysis also revealed a decrease in rod sensitivity occurring some 1.5 hour after light onset, a finding also highly correlated with the phagosome count[43].

Photopigment renewal shows a circadian rhythm controlled intraocularly, the timing of the peak of disk shedding being determined by the light history of each eye[6]. The daily peak of disk shedding activity takes place between 30 minutes and 1.5 hours after the usual light onset. In our subjects, the mean light onset (waketime) during the 5 days prior to the ERG sessions was 09:04h for the E-Type group and 07:40h for the M-Type group (see Table 1). Consequently, we would have expected the disk shedding effect to be less important for the E-Type group since only two out of the twelve subjects were usually awake at the time of the morning ERG (08:00h). Instead, the decrease in ERG amplitude and retinal sensitivity measured in scotopic conditions tended to be larger in E-Types compared to M-Types. Interestingly, Grace et al.[44] recently reported evidence suggesting that the disk shedding process might actually begin before the expected light onset and consequently would extend over a relatively longer period of time than originally postulated. This could suggest that the reduction in amplitude and in sensitivity of the scotopic ERG that we report might in fact result from the rod disk shedding. This decreased response could be the consequence of a momentary shortening of the rod outer segments and/or a decrease in the amount of functional rhodopsin. However, although melatonin was previously suggested to be responsible for the circadian regulation of the disk shedding and phagocytosis activities[15], we believe that the difference in circadian phase between our two groups (about 1.2h, on average) may have been insufficient to precisely reveal the impact of melatonin secretion on those two phenomena.

The results obtained in scotopic mixed conditions, where both rods and cones contribute to the response, support those obtained in both pure rod (scotopic ERG) and cone (photopic ERG) conditions. The largest reduction in amplitude, which we observed in the morning measurements for the E-Type group (24% for a-wave and 17% for b-wave), could reflect the combined contribution of both the rod disk shedding process and the higher concentration of melatonin in the morning. In contrast, the opposite effects of rod disk shedding and low concentration of melatonin in the morning for the M-Types resulted in a smaller decrease in amplitude (5% for b-wave). A similar explanation could also be offered to elucidate the larger diurnal variation observed in the E-Type group compared to the M-Type group in photopic conditions (see Table 2, contrast analyses). The rod disk shedding could not only contribute to the morning decrease seen in the rod ERG but also to that of the cone ERG. The used disks and the phagosomes might diminish the rebound of light on the pigmented epithelium thus reducing both the rod and the cone responses.

In conclusion, our study brings new evidence supportive of a diurnal variation in the human ERG. Our results further suggest that the circadian oscillator and/or the hormone melatonin would play a key role in this rhythm. This is consistent with several animal studies showing the circadian nature of the diurnal variation of the ERG[6-10]. In our study, melatonin was measured in saliva and thus reflects systemic pineal melatonin. It is impossible to state at

this point if the level of retinal melatonin in humans follows the plasmatic one. However, based on immunocytochemical results, it was recently suggested that retinal and pineal melatonin would cooperatively regulate the retinal ganglion cell activity[45], thus supporting our claim that melatonin (retinal and/or pineal) would be necessary to the normal processing of the visual information.

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Figure legends

Figure 1: Representative example of individual scotopic (left) and photopic (right) ERG recordings (top) and their corresponding luminance-response functions (bottom). Each tracing represents an average of 5 responses for the scotopic and 10 for the photopic. Arrows indicate flash onset and letters a, b and i identify the a-, b- and i-waves, respectively. Scotopic and photopic V_{max} and K measurements are indicated on the luminance-response curves. Black squares indicate a-wave and white squares indicate b-wave. The curve fitting line (solid line) in the scotopic graph was obtained with the Naka-Rushton equation.

Figure 2: Mean (± SEM) salivary melatonin concentration expressed as % of the maximum for each subject. Black dots represent E-Types and white dots represent M-Types. The black rectangles on the X-axis represent the timing of scotopic ERGs and the white rectangles, the timing of photopic ERGs.

Figure 3: Typical ERG responses (scotopic V_{max}, scotopic mixed response, photopic V_{max} and photopic OPs) of a E-Type (top) and M-Type (bottom) subject obtained during the evening (dash line) and morning (full line) sessions. The Y-axis is in μV and the X-axis in msec where 0 represents flash onset. Scotopic V_{max} and mixed response were evoked to -

2.22 log cd.m^{-2}.sec and -0.96 log cd.m^{-2}.sec respectively. Photopic V_{max} for the E-Types and M-Types were evoked to 0.39 log cd.m^{-2}.sec and 0.17 log cd.m^{-2}.sec, respectively. Photopic maximal OPs for the E-Types and M-Types were evoked to 0.64 log cd.m^{-2}.sec and 0.39 log cd.m^{-2}.sec, respectively. The OP_2, OP_3 and OP_4 are identified on the upper right recording.

Figure 4: Correlations between the evening session over morning session ratios [(22:30h/08:00h) X100] of ERG amplitude results and log melatonin concentrations obtained from E-Type (filled symbols) and M-Type (open symbols) subjects. Numbers higher than 100 on the Y-axis and than 0 on the X-axis identify measurements which were higher during the evening session compared to the morning one. See text for statistical description.

Table 1:

Mean (±1 SD) clock times for habitual sleep episode and onset/offset of melatonin secretion in evening-type (E-Types) and morning-type (M-Types) subjects. N=12 in each group for bedtime and waketime; N=11 for melatonin onset/offset.

Variables	E-Types	M-Types	t-Tests
Habitual Bedtime	01:36 (01:05)	23:23 (00:29)	$p<.001$
Habitual Waketime	09:04 (01:25)	07:40 (00:37)	$p<.01$
Melatonin Onset	23:25 (01:12)	22:14 (00:56)	$p<.05$
Melatonin Offset	08:35 (01:01)	07:25 (00:38)	$p<.01$

Table 2:

Mean (±1 SD) scotopic and photopic ERG results obtained for both groups of subjects with statistical analyses (ANOVAs and Contrast analyses on the factor Time-of-Day). The M/E ratios represent the diurnal changes and are equivalent to the 08:00h ERG measurements expressed as a percentage (% ±1 SD) of the 22:30h measurements. Scotopic results include the amplitude (in µV) of b-wave V_{max}, the rod sensitivity (K_s, in log $cd.m^{-2}.sec$) and the amplitudes and peak times (in msec) of a- and b-waves in scotopic mixed conditions. Photopic results obtained with the 4 wavelengths of flash include the amplitude of b-wave V_{max}, the amplitude of a-wave obtained at V_{max} intensity, the sum amplitude of maximal OPs and the maximal amplitude of the i-wave (white only).

Variables		E-Types			M-Types			ANOVAs	Contrast analyses
		22:30h	08:00h	M/E (%)	22:30h	08:00h	M/E (%)		
Scotopic	V_{max}	192.4 (44.0)	160.2 (34.0)	86 (20)	177.2 (37.5)	164.2 (36.9)	93 (15)	time effect: $F_{1,22}$=10.77, p<.005	
	K_s	-3.72 (0.31)	-3.49 (0.38)	94 (11)	-3.75 (0.20)	-3.65 (0.28)	98 (10)	time effect: $F_{1,22}$=4.34, p<.05	
Scotopic mixed	b-wave ampl	235.8 (69.2)	194.8 (61.4)	83 (13)	224.3 (51.9)	212.3 (48.4)	95 (12)	interaction: $F_{1,22}$=5.14, p<.05	E-Types: $F_{1,22}$=15.76, p<.001
	a-wave ampl	112.6 (27.1)	86.4 (35.1)	76 (25)	86.3 (23.9)	84.1 (19.9)	102 (25)	interaction: $F_{1,22}$=6.58, p<.05	E-Types: $F_{1,22}$=20.43, p<.000
	b-wave time	46.1 (1.3)	48.0 (3.1)	104 (6)	48.5 (2.6)	47.9 (3.1)	99 (5)	interaction: $F_{1,22}$=5.12, p<.05	E-Types: $F_{1,22}$=4.89, p<.05
	a-wave time	23.9 (0.8)	25.1 (1.6)	104 (6)	24.3 (1.2)	23.3 (1.5)	96 (6)	interaction: $F_{1,22}$=12.48, p<.005	E-Types: $F_{1,22}$=7.23, p<.01 M-Types: $F_{1,22}$=5.32, p<.05
Photopic V_{max}	white	92.8 (23.0)	85.9 (21.6)	93 (8)	85.3 (20.7)	89.9 (18.3)	108 (22)	interaction: $F_{1,22}$=7.41, p<.01	E-Types: $F_{1,22}$=5.28, p<.05
	blue	94.0 (22.4)	86.1 (21.5)	91 (10)	90.8 (19.3)	95.2 (20.2)	105 (11)	interaction: $F_{1,22}$=9.94, p<.005	E-Types: $F_{1,22}$=8.19, p<.01
	green	92.1 (24.9)	84.1 (22.8)	92 (14)	84.3 (21.4)	91.8 (18.1)	113 (30)	interaction: $F_{1,22}$=6.95, p<.01	E-Types: $F_{1,22}$=3.66, p<.10
	red	71.8 (20.6)	63.5 (18.6)	89 (8)	70.3 (19.3)	74.9 (19.1)	110 (29)	interaction: $F_{1,22}$=7.70, p<.01	E-Types: $F_{1,22}$=6.41, p<.05
Photopic a-wave	white	25.9 (4.3)	24.7 (6.6)	96 (23)	25.1 (5.1)	25.8 (5.4)	104 (19)	n.s.	
	blue	34.9 (8.0)	32.3 (10.5)	92 (19)	33.3 (10.7)	33.4 (9.0)	103 (21)	n.s.	
	green	27.6 (5.9)	24.3 (6.0)	89 (19)	26.6 (5.1)	28.7 (6.6)	110 (25)	interaction: $F_{1,22}$=5.45, p<.05	E-Types: $F_{1,22}$=4.05, p<.05
	red	24.5 (5.2)	20.3 (6.1)	86 (31)	22.9 (6.5)	23.4 (6.6)	105 (29)	interaction: $F_{1,22}$=4.28, p<.05	E-Types: $F_{1,22}$=6.86, p<.01
Photopic sum OPs	white	59.9 (18.4)	58.0 (20.0)	96 (9)	52.3 (16.4)	54.8 (16.6)	111 (37)	n.s.	
	blue	65.8 (22.3)	55.5 (21.5)	84 (12)	57.5 (18.6)	58.8 (18.2)	104 (15)	interaction: $F_{1,22}$=12.57, p<.005	E-Types: $F_{1,22}$=19.72, p<.000
	green	62.2 (21.4)	55.8 (20.1)	90 (17)	54.0 (19.9)	53.6 (19.3)	103 (28)	n.s.	
	red	52.6 (16.0)	48.7 (16.2)	93 (13)	44.3 (14.0)	46.7 (15.8)	109 (31)	n.s.	
i-wave	white	30.5 (8.4)	27.1 (8.4)	90 (23)	25.9 (8.8)	24.8 (6.9)	103 (40)	n.s.	

Figure 1

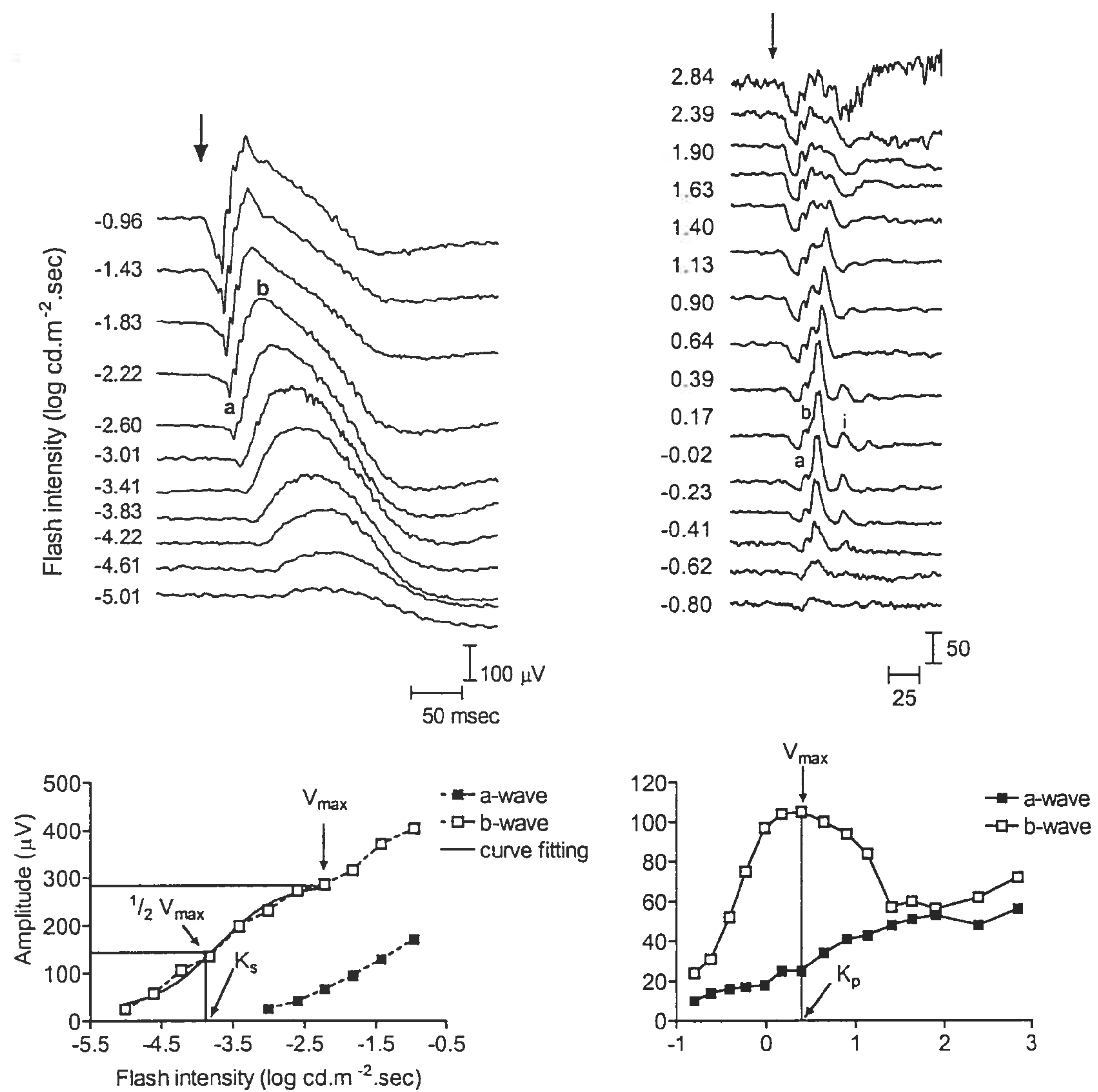


Figure 2

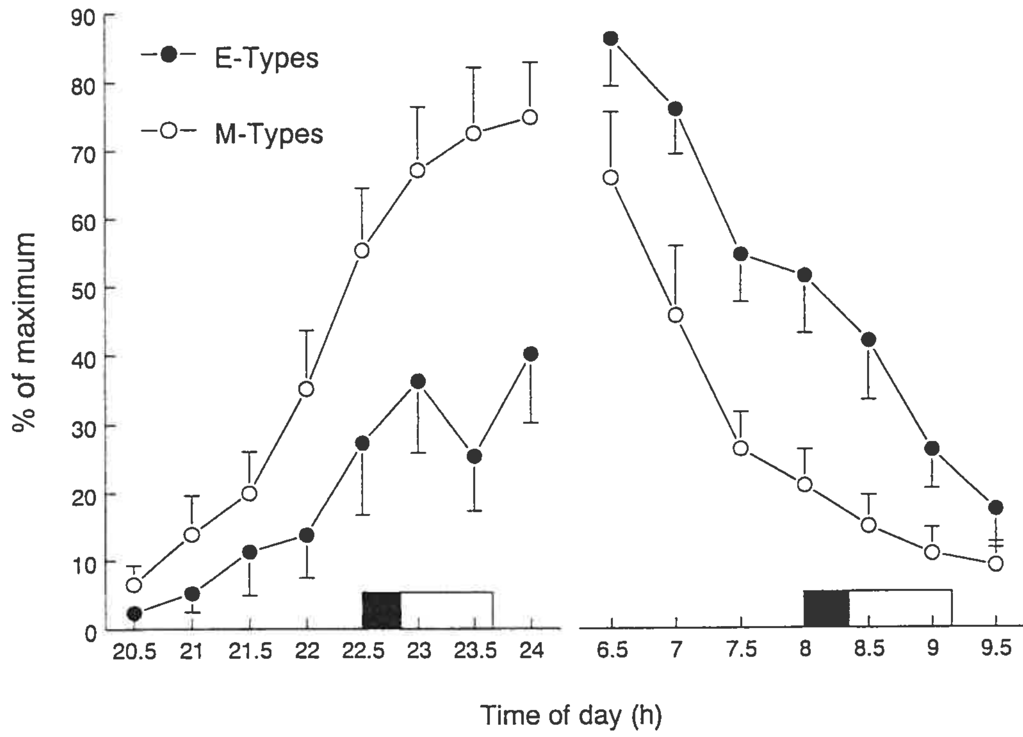


Figure 3

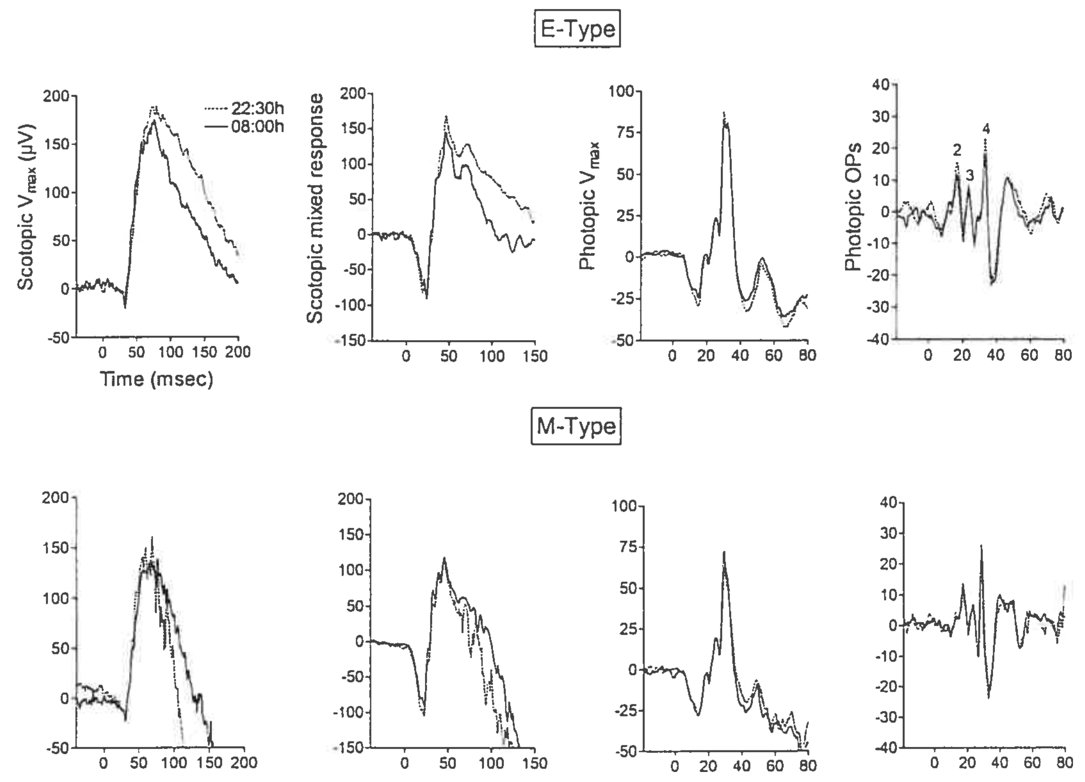
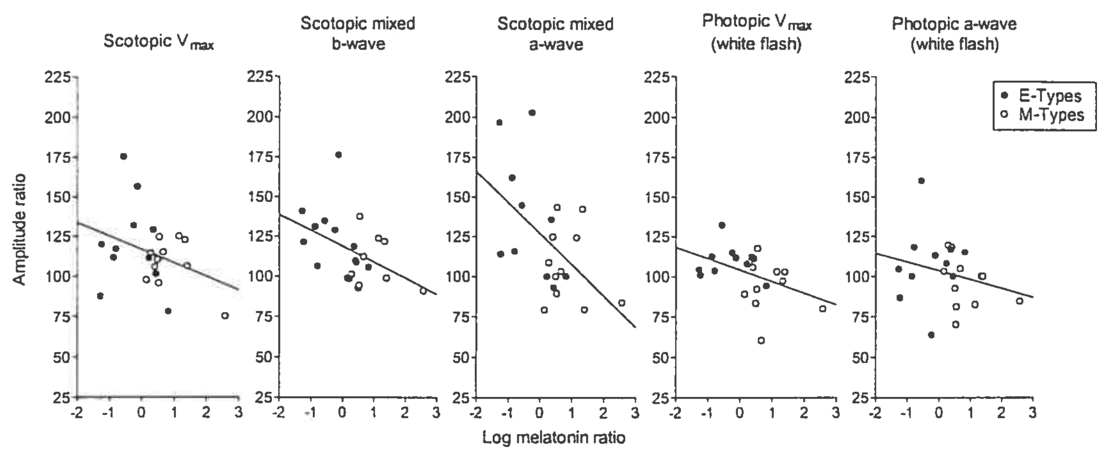


Figure 4



3.5. Cinquième article

Circadian and retinal sensitivity to light in association wih light exposure in the work environment.

Article en préparation

pour soumission dans « Journal of Neuroscience »

Circadian and retinal sensitivity to light in association with light exposure in the work environment.

Marianne Rufiange, M.Sc.[1,2], Pierre Lachapelle, Ph.D.[1], Marie Dumont, Ph.D.[2]

[1] Department of Ophthalmology, McGill University and Montreal Children's Hospital Research Institute; [2] Department of Psychiatry, University of Montreal and Chronobiology Laboratory, Sacre-Coeur Hospital of Montreal

In preparation for submission in *Journal of Neuroscience*

Corresponding author:

Marie Dumont, Ph.D.

Chronobiology Laboratory

Sacré-Cœur Hospital

5400 blvd Gouin West

Montreal (Quebec)

Canada, H4J 1C5

Tel: (514) 338-2222 ext. 2246

Fax: (514) 338-2531

Abstract

Both animal and human studies have suggested that the sensitivity to light of the circadian system could have the ability of adapting to the light regimen on a long-term basis. Previous light history has also been associated with changes in retinal physiology that permit a constant daily photon catch, a phenomenon called photostasis. In the present study, light exposure was continuously recorded with ambulatory monitors (Actiwatch) in two populations of full-time workers: one group working indoor, in a dim environment without access to natural light, and one group working mainly outdoors. Circadian light sensitivity, measured by the test of melatonin suppression, and retinal sensitivity, assessed with an electroretinogram (ERG), were compared between these two groups and correlated with levels of daily light exposure. The circadian phase was also assessed with the dim-light melatonin onset (DLMO). Indoor workers received less light than outdoor workers, the difference being noticeable only during work hours. Dim light history was associated with a greater circadian sensitivity, a later circadian phase, a faster dark-adaptation, a greater retinal sensitivity in scotopic conditions and a lower retinal sensitivity in photopic conditions compared to bright light history. Results are consistent with previous reports suggesting that the circadian system seems to adapt its sensitivity to light according to previous light history. This long-term adaptation could originate from a modulation of the light sensitivity at the level of the retina.

Keywords: work, light history, circadian system, melatonin, retina, ERG

Introduction

The light-dark cycle is the main environmental cue used by the circadian pacemaker to synchronize its endogenous rhythmicity with the external 24-h day. Recent studies have suggested that the light sensitivity of the circadian system could be modified by variations in the previous light regimen or light history. Circadian light sensitivity is often measured by the amount of suppression of melatonin secretion in response to a light stimulus (Lewy et al., 1981, 1985). The nocturnal secretion of this pineal hormone is regulated by the circadian pacemaker and can be directly suppressed by an ocular exposure to light in a dose-response manner, i.e. brighter light triggering more suppression (McIntyre et al., 1989). The circadian system compares the relative intensities of the light-dark cycle to distinguish night from day. In animals, it has been shown that the exposure to a given light intensity is interpreted as diurnal or nocturnal depending on the intensity of the light during the other part of the light-dark cycle (Lynch et al., 1981, 1985). Therefore, that dim light intensity suppressed melatonin secretion in animals reared in a dark/dim-light cycle but not in those reared in a dim-light/bright-light cycle. This observation suggests that the sensitivity to light of the circadian system was increased in animals reared in dimmer light levels compared to those reared in brighter environments. Similarly, in another study, the circadian system of animals living in the wild has been found to be less sensitive to light than that of their counterparts, which were raised in the darker environment of the laboratory (Reiter et al., 1983).

The effect of light history on circadian light sensitivity can be related to a change in the sensitivity of the circadian pacemaker itself and/or can originate from a change in light sensitivity at the level of the retina. Some long-term light adaptation have been observed in the retina of rodents. In rats, the structure and composition of photoreceptors are modified with changes in the lighting environment. The term photostasis refers to these retinal changes, which allow for a constant daily photon catch (Boulos & Terman, 1998). The renewal of the disks of rod outer segments is believed to be the process from which originates photostasis (Schremser & Williams, 1995a, b). Hence, disks that are produced while the animal is in dim light contain more rhodopsin (rod photopigment) than when produced in bright light. In addition, the length of the rod outer segments also adjusts according to the light history, becoming longer as the light regimen becomes dimmer (Battelle & LaVail, 1978; Penn & Williams, 1986). Those changes are noticeable two weeks after the implementation of the new light regimen. Until now, photostasis has only been demonstrated in rods although this process could also be present in cones, with possibly a different rate of adjustment to the new lighting environment (Boulos & Terman, 1998).

Few studies have looked into the effects of light history in humans. Some preliminary observations reported by Lewy in 1983 suggested that circadian sensitivity to a 500-lux

stimulus is lower when the subjects are exposed to bright natural light during the preceding day compared to when exposed to lower daytime light intensities. In a recent study, most subjects exposed successively to one week of dim light and one week of bright light showed a greater circadian sensitivity to light following the dim-light week compared to the bright-light one (Hebert et al., 2002b). However, there was a large inter-individual variability possibly resulting from the combined effect of the short duration of light exposure and the absence of a time interval between the two lighting regimens. Finally, another study reported an increased circadian light sensitivity in the winter compared to summer in four subjects living in Antarctica where huge seasonal differences in lighting can be observed (Owen & Arendt, 1992). However, other seasonal studies performed in more equatorial locations failed to demonstrate a variation in melatonin suppression with seasons (Thompson et al., 1990; Nathan et al., 1999). None of those studies directly measured light exposure of the subjects. Comparisons could therefore have been affected by unknown changes in the living habits of the subjects according to the seasons.

On the other hand, to our knowledge the light sensitivity of retinal functions has never been tested in humans in relation with light history. Retinal function can be assessed with an electroretinogram (ERG), which is a biopotential recorded at the level of the cornea that represents the electrical activity generated by the retina in response to light stimuli. There are two types of ERGs: the scotopic ERG is recorded in dark-adapted conditions and

measures the rod response whereas the photopic ERG is recorded in light-adapted conditions and measures the cone response. Previous studies have demonstrated that the ERG recorded in humans is a useful tool to measure variations in retinal function with, for example, the time of day (Rufiange et al., 2002a) and different pathologies (Hebert et al., 2002a; Rufiange et al., 2003). Furthermore, a recent study has shown that albino rats transfered in 400-lux-dark cycles demonstrated a gradual decrease in the outer nuclear layer (ONL, i.e. photoreceptors layer) thickness and in the scotopic ERG amplitude compared to animals kept in 5-lux-dark regimen (Li et al., 2003). Those decreases were noticeable after only one day spent in the new bright light cycle and became significantly different from the dim light condition after three (ONL thickness) and six (ERG) days, respectively.

In the working population, most of the daily light exposure depends on light exposure at work (Heil & Mathis, 2002). Therefore, the comparison of workers exposed to different lighting conditions can be used as a model to evaluate the effect of chronic exposure to a dimmer or a brighter environment on light sensitivity. In the present study, light exposure was continuously measured with ambulatory monitors in two populations of full-time workers: one group working indoor, in a relatively dim environment without access to natural light, and one group working mainly outdoors in natural light. Circadian light sensitivity as well as retinal sensitivity in scotopic and photopic conditions were compared between these two groups and correlated with levels of daily light exposure. Since

differences in light exposure are expected to be associated with differences in circadian entrainment to the 24-h day, the circadian phase was also estimated in the two groups using the timing of the onset of melatonin secretion.

Materials and Methods

Subjects:

Twenty-five subjects were recruited according to the level of light exposure in their work environment. Thirteen (5F, 8M; 29.2 ± 6.2 y.o.) subjects worked indoors, in an environment without access to natural light, and twelve (5F, 7M; 30.1 ± 6.4 y.o.) worked mostly outdoors. At the time of testing, all subjects had been working full time (at least 4 days or 32 hours weekly) on a daytime schedule and in the same environment for at least 6 consecutive weeks. The work duration prior to testing, without interruption for vacations or other reasons, was the same in both groups (indoor: 3.5 ± 2.0 months and outdoor: 3.8 ± 2.4 months). Participants had not been working on a night shift during the last six months nor had they gone to another time zone in the last month prior to the study. All participants had a regular sleep-wake schedule and did not report sleep or vigilance disorders. Subjects were not using medications known to affect vision, sleep, vigilance or melatonin secretion (e.g., NSAIDs, β-blockers, anxiolytics or hypnotics). No caffeine could be used during and five hours prior to the measurements and all subjects were nonsmokers except for one light

smoker who accepted to stop smoking five hours before each visit to the laboratory. A complete ophthalmological examination was performed prior to the study to rule out any retinal disorders. All subjects had best corrected visual acuity of 20/20 or better and refraction errors of less than ± 5 Diopters. One male subject was included in the indoor group even though his myopia was higher than 5 Diopters but he was excluded from the ERG measurements. This research study followed the tenets of the Declaration of Helsinki and each participant signed an informed consent form approved by the ethics committees of the Montreal Children's and Sacre-Cœur Hospitals and received a financial compensation.

Procedures:

Schedule:

The study was conducted from August 2000 to November 2002 and the subjects from the two groups were tested in random order all year-round. On average, daylight duration at the time of testing was the same in the two groups (indoor workers: 12.2 ± 1.7 hours and outdoor workers: 12.2 ± 2.1 hours). For each subject, the study included three visits. Those visits took place at the end of three consecutive working weeks, each including a minimum of four days of work. At the end of week 1, the ERG recording was performed at the Visual Electrophysiology Laboratory (Department of Ophthalmology, Montreal Children's Hospital) early in the evening (mean start time: 19:22 ± 52 min) in order to avoid the circadian bias previously observed (Rufiange et al., 2002a). During this first visit, the

subjects also received an ambulatory monitor (Actiwatch-L, Mini-Mitter Co, OR, USA), which they had to wear during the next two consecutive weeks in order to record both light exposure and activity levels. They were also given a 14-day diary to log daily information concerning hours of work and sleep, time spent outside, and periods when the monitor was not worn. During the second and third visits, which took place at the Chronobiology Laboratory (Sacre-Cœur Hospital of Montreal) at the end of week 2 and week 3, circadian light sensitivity and circadian phase were assessed.

Ambulatory Light Recordings:

Subjects were instructed to wear the ambulatory monitor continuously, on their non-dominant wrist, and to remove it only during incompatible activities (e.g., shower, contact sports, etc). Light exposure (in lux) and activity (activity counts) were recorded every minute. Data recorded when the monitor was not worn was excluded from the analyses. These data included those identified as such in the subject's diary and data recorded while there was no activity for periods of 30 consecutive minutes or more outside of the sleep episodes. One indoor female and one outdoor male did not wear their monitor properly. Therefore, light results include only 12 indoor and 11 outdoor subjects. For each subject, bedtime and wake time, as well as the beginning and end of the work days, were estimated by averaging the hours reported in the 14-day diaries, validated by the activity and light data recorded with the ambulatory monitor (when available).

For each subject, light data were averaged over 12 days (i.e., the 6 days preceding each of the two visits to the Chronobiology Laboratory) after being submitted to the following analyses. The first analysis was performed to establish the proportion of waking episodes spent under predetermined ranges of illumination. The ranges of light intensities were chosen according to Hebert et al. (1998), namely: less than 100 lux, 100 to 1000 lux and over 1000 lux. A 100 lux represent a low light level found exclusively indoors, in rooms without access to natural light and 1000 lux is an outdoor illuminance, typical of a cloudy day. For this analysis, light exposure recorded during sleep was excluded. Only days with less than 10% of missing data (i.e., monitor not worn) and less than 15% of time spent between 0 and 0.1 lux (i.e., monitor covered with a piece of clothing) were included in the analyses. The second analysis was performed to estimate the daily pattern of light exposure. Data were log transformed before statistical analyses to reduce the distortion caused by the inclusion of very high light intensities during exposure to sunlight (> 10,000 lux) and to normalize the distributions. Light exposure was averaged over each hour that included a minimum of 30 valid data.

Circadian Measures:

Circadian light sensitivity was evaluated with a test of suppression of melatonin secretion (Gaddy et al., 1993) performed during the two visits at the Chronobiology Laboratory. Procedures were the same during the two visits, except that the subjects were exposed to a 500-lux light stimulus during the last 90 min of their second visit. Subjects were admitted five hours before their habitual bedtime (HB) and were asked to remain awake in seated position for a total of eight hours. In order to measure the concentration of melatonin, saliva samples were collected every half-hour using Salivettes devices (Sarstedt Inc, Newton, NC, USA). Sampling started 4.5 hours before HB and finished 3 hours after HB, for a total of 16 samples per visit. Subjects were kept in dim light (< 15 lux), except for the duration of the test of melatonin suppression during the second visit. During that test, subjects were exposed to 500 lux of white light delivered with a ceiling lighting system made of 196 fluorescent (type 32T8, 32 watts) lamps. Light intensity was precisely controlled (Lutron Graphic Eye Liaison program) and monitored in the angle of gaze every 15 minutes with a research photometer (International Light, IL 1400).

Salivary melatonin concentrations were determined by radioimmunoassay with a ^{125}I-labelled tracer (Bühlmann Laboratories, Switzerland). The reported functional least detectable dose is 0.65 pg/ml (Weber et al., 1997). All samples from a given subject were analyzed in the same run. Samples were assayed in duplicate and those with a coefficient of

variation (CV) larger than 10% were rejected (4.8% of all samples). Intra-assay CVs for control samples of 1.9 and 14.7 pg/ml were 3.6 and 2.3%, respectively.

The amount of suppression of melatonin secretion was determined with the control-adjusted method reported by Gaddy et al. (1993). First, a change ratio was computed for each of the two visits, as the percent change over the 90-min testing period [100 x (melatonin concentration after 30, 60 or 90 min of light – concentration at beginning of testing period) / concentration at beginning of testing period]. Second, control-adjusted suppression was computed by subtracting the change ratio calculated during light exposure (visit 2) from the change ratio calculated during the control condition (visit 1). The control-adjusted method has the advantage of including in the calculation of melatonin suppression the variation in melatonin secretion observed in dim light at this time of night for each individual.

Circadian phase was estimated with the timing of the onset of melatonin secretion (Dim Light Melatonin Onset or DLMO, Lewy & Sack, 1989), measured in dim light during the first laboratory visit. DLMO was defined as the clock time of the first saliva sample with a melatonin concentration greater than or equal to twice the detection limit of the method (i.e., 1.3 pg/ml) that was followed by two other samples also above this value (Deacon & Arendt, 1994). Of the 25 subjects, three (1 indoor and 2 outdoors) showed large

discrepancies in their melatonin pattern between the two visits and a fourth subject (an indoor female) withdrew from the study. Therefore, melatonin suppression results include 11 indoor and 10 outdoor subjects, while the DLMO results include 12 subjects in each group.

Retinal Measures:

Electrophysiological recordings were performed as previously reported (Hebert et al., 1996, 1999; Rufiange et al., 2002a, b, 2003) and in accordance with the ISCEV ERG standards (Marmor & Zrenner, 1998). The pupils were maximally (8-9 mm) dilated with Tropicamide 1% and the pupil size was measured at the beginning and at the end of the recording procedure. DTL fiber electrodes (27/7 X-Static® silver coated nylon conductive yarn; Sauquoit Industries, Scranton, PA, USA) were positioned deep into the inferior conjunctival bag and secured with double-sided adhesive tape at the external and internal canthi. Reference and ground electrodes (Grass gold cup electrode filled with Grass EC2 electrode cream) were pasted at the external canthi and forehead, respectively. ERGs (bandwidth: 0.3-500Hz; amplification: 10000X scotopic and 20000X photopic; attenuation: 6dB) were recorded simultaneously from both eyes with a LKC UTAS-E-3000 system that included a Ganzfeld of 30 centimeters in diameter.

Subjects were first light adapted for 10 minutes to a background light of approximately 480 cd.m^{-2} (range: 410-573) in order to desensitize the rods. During the following dark adaptation period of 30 minutes, series of five blue (GamColor filter 850, λ_{max}= 410 nm) flashes of –3.41 log cd.sec.m^{-2} (an intensity that produce in normal subjects a b-wave amplitude equal to half the V_{max}, Rufiange et al., 2002a, b, 2003) were delivered every three minutes to measure the rate of adaptation of the rods. Scotopic luminance-response functions were then obtained with the use of 11 intensities of blue flashes ranging from -5.01 to -0.96 log cd.sec.m^{-2}. Each flash had a duration of 20 μsec and each recording included a pre-stimulus baseline of 40 msec. For the scotopic ERGs, the inter-stimulus interval was fixed at ten seconds and five responses were averaged for each flash intensity. To avoid the light adaptation effect previously reported (Lachapelle, 1987), the subjects were then light adapted for 10 minutes to a white light background of 30 cd.m^{-2} following which photopic ERGs were recorded against the same background light. Photopic luminance-response functions were obtained with the use of 15 intensities of white flashes ranging from -0.8 to 2.84 log cd.sec.m^{-2}. Ten responses were recorded and averaged at each flash intensity. The inter-stimulus interval was reduced to 2.3 seconds for the photopic ERGs.

Flash intensities and background luminances were measured with a research photometer (International Light, IL 1700). Data from both eyes were averaged to yield a single data

point. The analysis of the ERG included peak time and amplitude measurements of the a- and b-waves. The amplitude of the a-wave was measured from baseline to trough and that of the b-wave from the trough of the a-wave to peak of the b-wave. Peak times were measured from flash onset to the peak of each wave. Unless mentioned otherwise, ERG results reported in this paper include 12 subjects in each group (5F, 7M).

Statistical Analysis:

Statistical comparisons between the two groups were performed with Student t-tests for independent samples. The daily pattern of light exposure was analyzed with a 2x24 analysis of variance (ANOVA), with the factor Group (Indoor and Outdoor) and the factor Time-of-Day (repeated measures). Simple effect analyses were performed when an interaction effect was found. Correlations between light exposure and ERG and melatonin results were computed with the Pearson test when the variables were normally distributed. If this were not the case, the non-parametric Spearman test was used. For the comparisons of ranges of light intensities between the two groups, the Bonferroni correction was applied and significance was set to $p < 0.01$. In all other analyses, statistical significance was set to $p < 0.05$.

Results

Sleep and Work Schedule:

On average, both groups woke up and went to bed at about the same time (Table 1). On average, they also started to work at the same time but the indoor workers finished approximately one hour later than the outdoor workers ($p < 0.05$). Nevertheless, the total duration of the workday did not vary significantly between both groups, the average being between 7 and 8 hours of work. This could be due to the large inter-subject variability that can be noticed in Table 1.

Light Exposure:

Figure 1 shows two pie graphs that depict the daily (excluding the sleep episode) percentage of time spent in various ranges of light intensity in the two groups, namely between 0.1-100, 100-1000, and over 1000 lux. The amount of time spent between 0 and 0.1 lux (monitor covered by clothing) as well as when the monitor was not worn (i.e. missing data) are also shown. The total length of the daily waking period was similar in both groups, that is 15.6 h for the indoor group and 16.0 h for the outdoor one ($p= 0.37$). Only the amount of time spent at light intensities greater than 1000 lux was found to be significantly different between the two groups ($p < 0.001$), being less than 6% (52 min) for indoor workers and 21% (3.3 h) for their counterparts.

Since there was no differences between the two weeks of recording in any of the groups (week effect: $p > 0.19$ in both groups), group comparisons were computed on data averaged over both weeks. Figure 2 thus presents the mean 24-h pattern of light exposure for both groups of subjects over those 12 days. The ANOVA revealed a significant Group-by-Time interaction ($p < 0.0001$). Light exposure was significantly higher in the outdoor group compared to the indoor group between 09:00 and 16:00 ($p < 0.05$), a period during which both groups of subjects were at work (Table 1).

Circadian Measures:

The amount of melatonin suppression, determined with the control-adjusted method, is illustrated in Figure 3, after 30, 60 and 90 minutes of 500-lux light testing. Melatonin tended to be more suppressed in the indoor group than in the outdoor group although the difference did not reach significance ($p > 0.25$ at all time points). As shown in Table 2, the inter-group difference was approximately 15% for all three measurements but the variability was very high (SDs around 30%). A Pearson correlation was computed on all subjects between the amount of light exposure during work time (mean of light data from 09:00 to 16:00, see Figure 2) and the amount of melatonin suppression. As illustrated in Figure 4A, brighter light exposure during the day was associated with less melatonin suppression at 90 min ($r = -0.50$, $p = 0.03$). Although not illustrated, the same association

was noted with the suppression after 30 min ($r= -0.45$, $p= 0.04$) and almost reached the significance level for suppression at 60 min ($r= -0.43$, $p= 0.07$).

DLMO was on average 30 minutes later in indoor subjects compared to outdoor workers (Table 2), but the difference was not significant ($p= 0.18$). As shown in Figure 4B, brighter light exposure during work time was associated with an earlier circadian phase, although this last correlation only approached significance ($r= -0.40$, $p= 0.06$).

Retinal Measures:

The rate of dark adaptation was obtained by plotting the amplitude of the b-wave recorded at regular time interval following the onset of dark adaptation. Figure 5 shows typical ERGs recorded in response to flashes of -3.41 log cd.sec.m^{-2} along with the mean amplitude gain calculated in both groups. The results are expressed in percentage (%) of the maximal amplitude reached by each subject. The amplitude of the b-wave increases progressively with the time spent in darkness and reaches a plateau at around 20 minutes. Inspection of the graph suggest that the indoor subjects seem to adapt faster than the outdoor ones, particularly in the last part of the process. The amplitude values were fitted to a sigmoidal curve (GraphPad Software, San Diego, CA, USA) and the time required to reach the maximal b-wave amplitude was identified as the first entire minute during which

the amplitude stayed stable according to the fitting. The variables measured were the time required to reach the maximal amplitude and that of half that amplitude (see Table 3). The 50% amplitude was reached at the same time in both groups whereas the maximal amplitude was obtained faster in indoor subjects ($p < 0.05$), the difference being of about three minutes.

Typical scotopic (left) and photopic (right) ERG recordings evoked to progressively brighter flashes (from top to bottom, intensity of flash in log cd.sec.m^{-2} at left of tracings) for an indoor and an outdoor subject are illustrated at Figure 6. The mean luminance-response functions of each group of subjects are graphically reported at the bottom part of the figure. The purpose of recording luminance-response functions is to detect changes in the retinal sensitivity. A shift toward lower values along the intensity axis reveals increased retinal sensitivity. The variables identified on the bottom graphs are measured to quantify those changes in the ERG (see Table 3) and have been described extensively in previous reports (Rufiange et al., 2002a, b, 2003).

In scotopic conditions, the amplitudes of the a-wave (squares) and the b-wave (circles) increase gradually with flash intensity. The maximal amplitude of the scotopic b-wave (rod V_{max}) was determined by fitting to a sigmoidal curve (GraphPad Software, San Diego, CA, USA) the b-wave amplitude data, from the lowest intensity to the intensity at which an a-

wave of twice the baseline noise was obtained. The point of maximal amplitude on that curve was identified as the rod V_{max}. The K_s measurement was determined as the flash intensity necessary to produce a b-wave 50% of the rod V_{max}. In comparison, the luminance-response curve of the photopic ERG also shows that the a-wave (squares) augments in amplitude with intensity, but the behavior of the photopic b-wave (circles) differs significantly. With progressively brighter flashes, the amplitude of the b-wave first increases to reach a maximum (V_{max}) then gradually decreases with higher flash intensities to finally form a plateau. This distinctive luminance-response function was previously described as the Photopic Hill (Wali & Leguire, 1992, 1993; Rufiange et al., 2002a, b, 2003). The K_a and K_d measurements are defined as the flash intensity needed to produce a b-wave 50% of the photopic V_{max} on the ascending (K_a) and the descending (K_d) phases of the photopic hill. The intensities at which the scotopic and photopic V_{max} are reached are indicated as I_{max}. The amplitude of the a-wave at the photopic V_{max} intensity, identified as a_{max}, was also considered.

A general inspection of the shape of the luminance-response curves of each group (Figure 6) reveals that the indoor subjects reach their maximal b-wave amplitude at a lower intensity compared to outdoor subjects in scotopic conditions. However, the reverse is true in the photopic conditions, with the most pronounced difference between the two groups being during the descending phase of the photopic hill. In scotopic conditions, the V_{max}

amplitude and the K_s are similar in both groups (see Table 3). The main result for the rod response is the intensity at which the V_{max} was obtained (I_{max} in the graph of Figure 6). The indoor workers reached their rod V_{max} at a significantly lower intensity than the outdoor subjects ($p < 0.01$). In photopic conditions, the amplitude and the peak time of the a-wave at the V_{max} intensity was the same in both groups. At that intensity, the amplitude of the b-wave was also similar in the two groups but its peak time was longer in indoor workers ($p < 0.05$). Here again, the main result is the intensity at which the V_{max} was obtained, as well as the K measurements on the ascending and descending phases of the photopic hill. All three variables were shifted to higher intensities in indoor compared to outdoor subjects ($p < 0.05$). The shift being more pronounced for the K_d than for the K_a, the resulting photopic hill is larger in the indoor than in the outdoor group (see Figure 6). Moreover, this leads to b-wave amplitudes for intensities beyond V_{max} being larger in indoor subjects than in their counterparts. Finally, the photopic I_{max} and the b-wave peak time at V_{max} were correlated with the light exposure during the work day, i.e. between 09:00 and 16:00 (Spearman correlations: $R = -0.44$, $p = 0.04$ and $R = -0.48$, $p = 0.02$, respectively). Hence, higher light exposure during the work day was correlated with a more sensitive and faster cone response. No other significant correlations were noted for the ERG results.

Discussion

As expected, indoor workers were exposed to less bright light (> 1000 lux) during the day compared to outdoor workers. The lower exposure to bright lights was associated with a greater circadian sensitivity, a later circadian phase, a faster dark adaptation, a greater retinal sensitivity in scotopic conditions and a lower retinal sensitivity in photopic conditions. Light exposure in the two groups differed only during work time. This is in accordance with a recent study showing that light exposure within the work environment is the major determinant for total light exposure (Heil & Mathis, 2002). This result also suggest that indoor and outdoor workers spend their free time in similar luminous environments. The data obtained for the time spent in different ranges of light corresponds to what was obtained in the same city (Montreal, Canada) in the experiment of Hebert et al. (1998). In that study, the light exposure in winter was compared with that recorded in summer. Surprisingly, the distribution observed for the indoor group resembles that obtained in winter and that of the outdoor group corresponds to that of summer. The time spent above 1000 lux was also the sole significant difference between the two groups, being 3% of time awake or 26 min per day in winter and 18% or 2.6 h per day in summer. It is important to stress that the two groups of workers were tested all-year round, meaning that working in low-light environments all-year round mimics the "general" winter lighting distribution and working outdoors all-year round mimics the light distribution observed in summer in the general population. This means that the present report did not study any "extreme" lighting conditions but rather the two ends of a normal lighting continuum. On

the other hand, it should be mentioned that even if the light exposure was measured from the wrist, and thus not exactly represent the light received at the level of the eyes, one can assume that the exposure to light above 1000 lux surely denotes time spent outdoor.

A negative correlation between melatonin suppression and the light exposure during work hours was found. These results are in agreement with that of Lynch et al. (1981, 1985) and Reiter et al. (1983) in animals and that of Owen & Arendt (1992) and Hebert et al. (2002b) in humans. All five studies concluded that a low-level light history was associated with an increased circadian sensitivity compared to high-level light history. The fact that the level of melatonin suppression in the two groups did not differ significantly in spite of the above correlation could be due to a high inter-subject variability in the measure. Previous studies have also reported such high variability (Laakso et al., 1991; Hebert et al., 2002b). Furthermore, even if on average the indoor and outdoor groups were exposed to significantly different light levels, Figure 4 shows that the light exposure of the two groups is not well differentiated and forms a continuum rather than two separated lighting conditions. This means that the selection of subjects could have been more restricted on the basis of the light exposure at work in order to reach more "extreme" lighting conditions. Nevertheless, the difference in suppression between the indoor and outdoor group was very stable throughout the light testing (i.e. at time points 30, 60 and 90 minutes) at around 15%. This suggest that the group difference really exists although the measure is highly variable.

One could suggest that this variability is due to false evaluations of the habitual bedtime in certain subjects that would have caused the light testing to occur at various circadian phases. This is not the case because the circadian time of the test of melatonin suppression was the same in both groups, i.e. 3.7h after DLMO in the indoor group and 3.9h in the outdoor group ($p > 0.65$), with low variability (SD of 1.1h in indoor group and 0.8h in outdoor group).

Our hypothesis pertaining an adjustment of the entrained phase that would be related to the change in light sensitivity of the circadian system was partly verified in the present study. Here again, the two groups did not significantly differ as per their circadian phase (DLMO) but the correlation between this measure and the light exposure at work was nearly significant ($p = 0.06$). Low light exposure tended to be associated with a later onset of melatonin secretion. It appears that the phase of circadian entrainment would be affected by the force of the zeitgeber (here, light) and by the amplitude of the light intensity difference between night and day (Roenneberg et al., 2003). For most people, the endogenous circadian period is longer than 24h and thus needs to be advanced on a daily basis to maintain a stable phase relationship with the 24h light-dark cycle. For those individuals, a weak zeitgeber, such as in the work environment of the indoor group, would diminish this phase advance.

Furthermore, the dark adaptation process as well as the scotopic luminance-response function showed different results for the two groups. Looking at Figures 5 and 6 (left graph), one can note that both curves present the same differential effect between the indoor and the outdoor group. The first part of the curves was similar in both groups whereas the second part occurred faster (for the adaptation) and at lower intensities (for the scotopic ERG) in the indoor group compared to the outdoor one. This can be assessed in Table 3 by the time for 50% of maximal amplitude and K_s, which are both the same in the two groups as well as the time for maximal amplitude and V_{max} intensity (I_{max}), which significantly differ between the indoor and the outdoor group. The fact at the first part of the regeneration process was the same in both groups (until 9 minutes in darkness) could be related to cone physiology rather that of rods. Indeed, the typical dark adaptation curve that measures the lowering of the light detection threshold with time spent in darkness shows what is called a cone-rod break, meaning that the first part of the process is mediated by regeneration of cone opsins and the second by that of rhodopsin (Reeves, 2004). On the other hand, Penn & Williams (1986) showed that the regeneration rate of rhodopsin varies with light history in rats. The present findings on dark adaptation seem to agree with their observation. Here, the rhodopsin seem to regenerate faster after low-level light history compared to high-level, particularly in the second portion of the process.

The results obtained with the scotopic luminance-response function appear to be in agreement with the photostasis phenomenon in animals, which postulates that in order to catch a constant amount of photons per day, the rods adjust, over-time, the length of their outer segments (Batelle & LaVail, 1978; Penn & Williams, 1986) and their concentration in rhodopsin (Schremser & Williams, 1995a, b). Here, one could suggest that in order to compensate for the chronic exposure to low light levels, the rods become more sensitive to light. The reverse could also be true for the subjects exposed to high light levels. Another study that looked into seasonal variations of the scotopic ERG found no changes in normal subjects (Hebert et al., 2002b) but did not measure the I_{max}, which is the variable showing an inter-group difference in the present study. In albino rats, an important decrease in scotopic b-wave amplitude has been noted after only 6 days spent in 400 lux-dark regimen compared to the 5 lux-dark condition (Li et al., 2003). Here again, the intensity at which the V_{max} was obtained was not reported. In the present study, no significant differences in the amplitude of the scotopic b-wave were noted although outdoor workers tended to show lower amplitudes than indoor workers (see Figure 6). Thus, the main observations in scotopic conditions were probably due to a modulation of the efficiency of photon catching by rods, which produced sensitivity and dark adaptation changes, rather than of the total amount of rhodopsin that would have impacted on the amplitude of the ERG.

To our knowledge this is the first study that explore the effect of the light history on the cone response in humans. The effect noted in photopic condition was the reverse of what seen in scotopic. The photopic luminance-response curve (photopic hill) of the outdoor group was shifted to the dimmer intensities compared to the indoor group. The three sensitivity measurements, i.e. K_a, I_{max} and K_d, were all significantly lower in the outdoor group relative to the indoor one. One could suggest a possible "long-term potentiation" of the cones, or other retinal elements in the cone pathway, in the outdoor group because of high level light exposure. This alleged potentiation could be similar to what can be observed in other neurons of the central nervous system, where a given stimulus triggers a higher response after such potentiation (Kandel, 2001).

Regarding the more important difference between the two groups noted in the descending part of the photopic hill compared to the ascending portion, one could suggest some kind of protection against the harmful effects of sunlight in the outdoor group. This protection would lead to an "earlier" decrease in amplitude of the cone response (the b-wave) with increasing intensities of light. Since this decrease happened sooner along the photopic hill, the peak time of the b-wave was also significantly shorter at the V_{max} of the outdoor group compared to the indoor one. Such a protection phenomenon has been noticed by Li et al. (2001, 2003) when the retina of albino rats raised in bright light conditions was found to be protected against light damage compared to that of animals kept in dim light cycles. Indeed,

they observed a better preservation of the ONL thickness and of the scotopic ERG b-wave amplitude with increasing time spent in bright light conditions. Those changes were observable after only one day in bright light regimen and became significantly different from dim light raised animals after 2-3 days.

Finally, assuming that the changes in retinal and circadian sensitivities observed in this study do result from the difference in light history in the two groups of workers, what comes first? The retinal and circadian changes could occur independently or the circadian effect could be the consequence of the retinal adaptation to a new lighting environment. Recent studies suggest that the main circadian receptors would be the intrinsically photosensitive retinal ganglion cells (ipRGC), which represent one to three percent of all RGCs (Hattar et al., 2002). The photopigment of those cells is called melanopsin and axons of the ipRGCs seem to form the retino-hypothalamic tract (RHT). Nevertheless, even if melanopsin is now considered by many as the main circadian photopigment, conventional photoreceptors that convey visual information to the brain, namely the rods and cones, should not be totally discarded from the circadian neuronal network (Hattar et al., 2003; Berson, 2003; Van Gelder, 2003). Indeed, mice lacking rods and cones show some disturbances in circadian entrainment and phase shifts (Yoshimura et al., 1994; Mrosovsky, 2003) whereas melanopsin-knockout mice retain in part those two abilities (Panda et al., 2002; Ruby et al., 2002). Moreover, it was recently shown that mice lacking rod, cone, and

melanopsin functions all together lost each of the circadian responses to light (Hattar et al., 2003; Panda et al., 2003). The rod and cone pathway and that of melanopsin thus seem sufficient and essential for a normal response of the circadian system to light (Van Gelder, 2003). In addition, there are some evidences that rods and cones drive neurons of the suprachiasmatic nucleus (SCN) (Aggelopoulos & Meissl, 2000) and that ipRGCs receive direct inputs from bipolar and amacrine cells (Belenky et al., 2003), and thus are linked to the "conventional" visual pathway. Finally, as mentioned by Belenky et al. (2003), as many as 20% of RGCs forming the RHT do not express melanopsin and thus could solely receive information from visual photoreceptors. Keeping the latter in mind, it could be that, in the present study, a modulation of the light sensitivity of the rod and cone pathways by the lighting environment would have an impact on the sensitivity of the ipRGCs and thus on that of the circadian system.

In conclusion, the human organism could be able of adjusting on a long-term basis to a given lighting environment. The sensitivity to light of the retina and circadian system seem to adapt according to the light history. Further studies that would compare the retinal and circadian sensitivity to light in the same individuals after they been exposed to different lighting environments would help us defining the changes observed in the present study.

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Figure legends

Figure 1: Daily period (expressed in percentage (%) of the time awake) spent in different ranges of illumination, i.e. 0.1-100 (dark gray), 100-1000 (light gray), over 1000 lux (hatched) as well as missing data (white = monitor not worn and black = monitor covered, i.e. between 0 and 0.1 lux). * $p < 0.01$. Indoor group: N=12; Outdoor group: N=11.

Figure 2: Daily pattern of light exposure (expressed in log lux ± SD). Time point 0 indicates light received between 00:00 and 01:00. Gray box indicates time points significantly different between both groups, $p < 0.005$ except for 09:00, $p < 0.05$. Refer to text for analysis details. Indoor group: N=12; Outdoor group: N=11.

Figure 3: Mean (± SD) suppression (expressed in %) of salivary melatonin by a 90-minute period of light testing in indoor (close symbols) and outdoor (open symbols) groups. Refer to Table 2 for exact values.

Figure 4: Correlations of melatonin suppression (after 90 minutes of light testing, panel A) and DLMO (dim-light melatonin onset, panel B) with light exposure between 09:00 and

16:00 (log lux). Indoor: close symbols. Outdoor: open symbols. $r = -0.50$, $p = 0.03$ and $r = -0.40$, $p = 0.06$, respectively.

Figure 5: Representative ERG recordings during the 30-minute period of dark adaptation along with the mean gain of b-wave amplitude (expressed in % of maximal amplitude of each subject) for both groups of subjects. Arrow indicates flash onset. Legend: vertical: µV; horizontal: msec. ERG variables are indicated (for the indoor group) on the graph. Refer to text for definition of the variables. Indoor: close symbols. Outdoor: open symbols.

Figure 6: Typical scotopic (left) and photopic (right) ERG recordings (top) of an indoor and an outdoor worker along with the mean luminance-response curves (bottom) of both groups of subjects. Flash intensities are indicated in log $cd.sec.m^{-2}$ at the left of tracings. Arrows indicate flash onset and letter a and b identify the a- and b-wave, respectively. Legend: vertical: µV; horizontal: msec. ERG variables are indicated (for the indoor group) on the luminance-response curves. Refer to text for definition of the variables. Indoor: close symbols. Outdoor: open symbols. Squares: a-wave. Circles: b-wave.

Table 1: Mean (± SD, and range on second line) sleep and work schedules for both groups of subjects.

Variables	Indoor group	Outdoor group	t-tests
wake time	07:46 (00:39) 06:33 – 08:59	07:22 (01:01) 06:26 – 09:32	n.s.
bedtime	23:31 (01:01) 21:32 – 00:52	23:13 (00:52) 22:15 – 00:47	n.s.
beginning of work day	08:58 (00:48) 08:00 – 10:05	08:52 (01:34) 06:45 – 11:35	n.s.
end of work day	17:01 (00:46) 15:06 – 18:04	16:08 (01:08) 14:11 – 18:02	$p< 0.05$
duration of work day	8.0 (0.8) 7.0 – 9.4	7.3 (1.7) 4.4 – 9.5	n.s.

Results include 12 subjects in each group and are expressed in clock time (except duration in hours). n.s. $p> 0.05$

Table 2: Melatonin suppression after 30, 60 and 90 minutes of light testing at 500 lux and dim-light melatonin onset (DLMO) (± SD) for both groups of subjects.

Variables	Indoor group	Outdoor group	t-tests
30 min	52.2 (42.1)	37.3 (32.0)	n.s.
60 min	63.4 (33.4)	49.8 (26.2)	n.s.
90 min	74.7 (34.0)	58.4 (32.4)	n.s.
DLMO	20:50 (00:59)	20:20 (00:47)	n.s.

Suppressions are expressed in percentage (%) and DLMO are in clock time. Both groups include 12 subjects except for melatonin suppression where the indoor group comprise of 11 subjects and the outdoor group of 10. n.s. $p > 0.05$

Table 3: Scotopic and photopic ERG results (± SD) for both groups of subjects.

Condition	Variables	Indoor group	Outdoor group	t-tests
Dark adaptation	time for 50% of maximal amplitude	11.3 (2.1)	11.8 (1.5)	n.s.
	time for maximal amplitude	20.1 (3.5)	23.2 (3.1)	$p < 0.05$
Scotopic	V_{max} amplitude	192.2 (47.2)	183.5 (28.2)	n.s.
	V_{max} intensity	-2.06 (0.20)	-1.83 (0.17)	$p < 0.01$
	intensity for 50% of V_{max} (K_s)	-3.61 (0.13)	-3.61 (0.25)	n.s.
Photopic	V_{max} amplitude	97.7 (23.1)	91.6 (19.9)	n.s.
	V_{max} intensity	0.54 (0.19)	0.37 (0.12)	$p < 0.05$
	intensity for 50% of V_{max} – ascent (K_a)	-0.15 (0.09)	-0.23 (0.10)	$p < 0.05$
	intensity for 50% of V_{max} – descent (K_d)	1.58 (0.25)	1.38 (0.13)	$p < 0.05$
	a-wave amplitude at V_{max} intensity	26.9 (7.1)	22.2 (8.3)	n.s.
	a-wave peak time at V_{max} intensity	14.2 (1.1)	14.6 (1.3)	n.s.
	b-wave peak time at V_{max} intensity	30.5 (2.3)	28.3 (2.5)	$p < 0.05$

Adaptation times are expressed in minutes, amplitudes in μV, intensities (including K measurements) in log $cd.sec.m^{-2}$ and peak times in msec.

Both groups include 12 subjects except for dark adaptation where the indoor group comprise of 10 subjects. n.s. $p > 0.05$

Figure 1

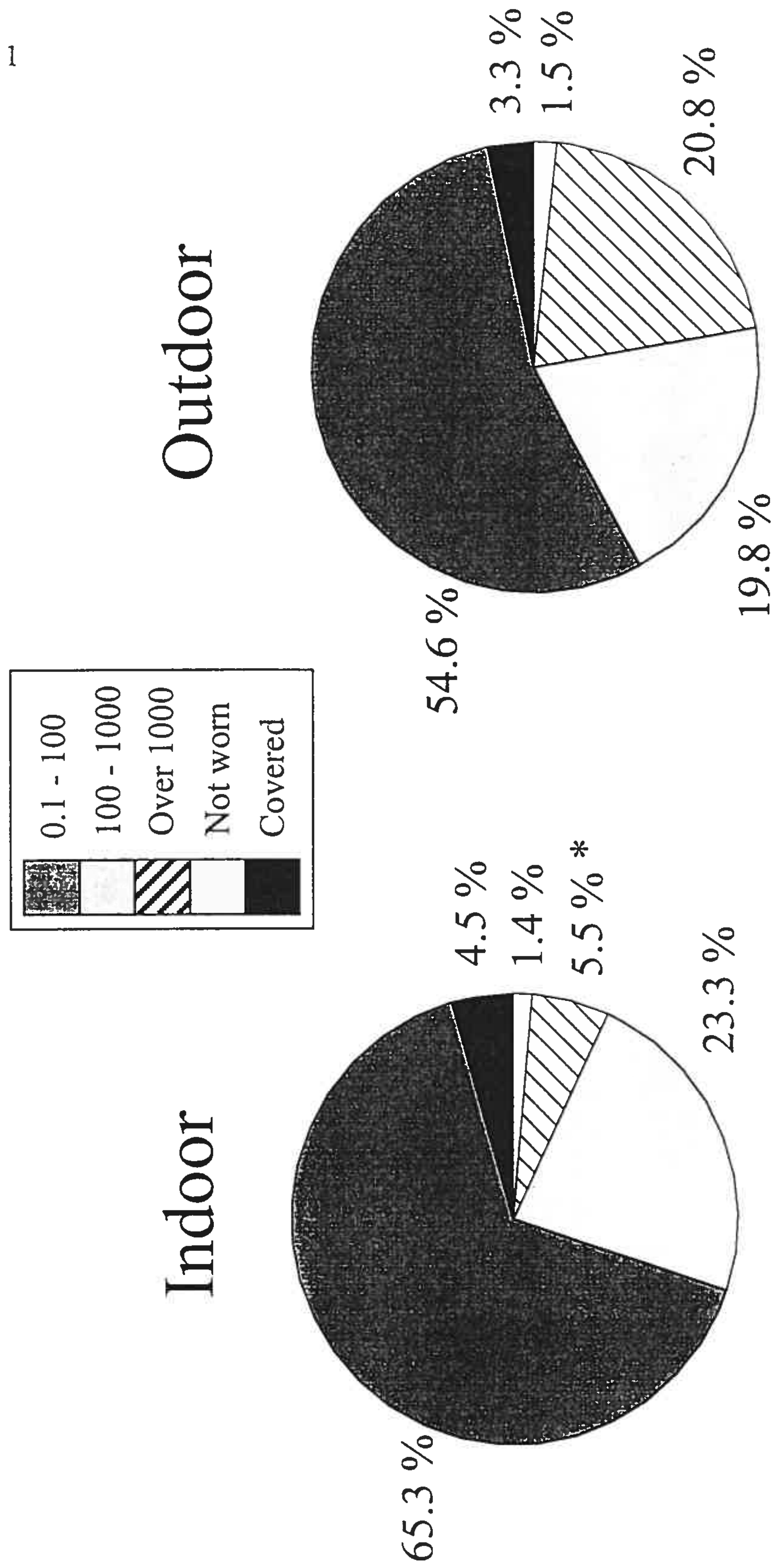


Figure 2

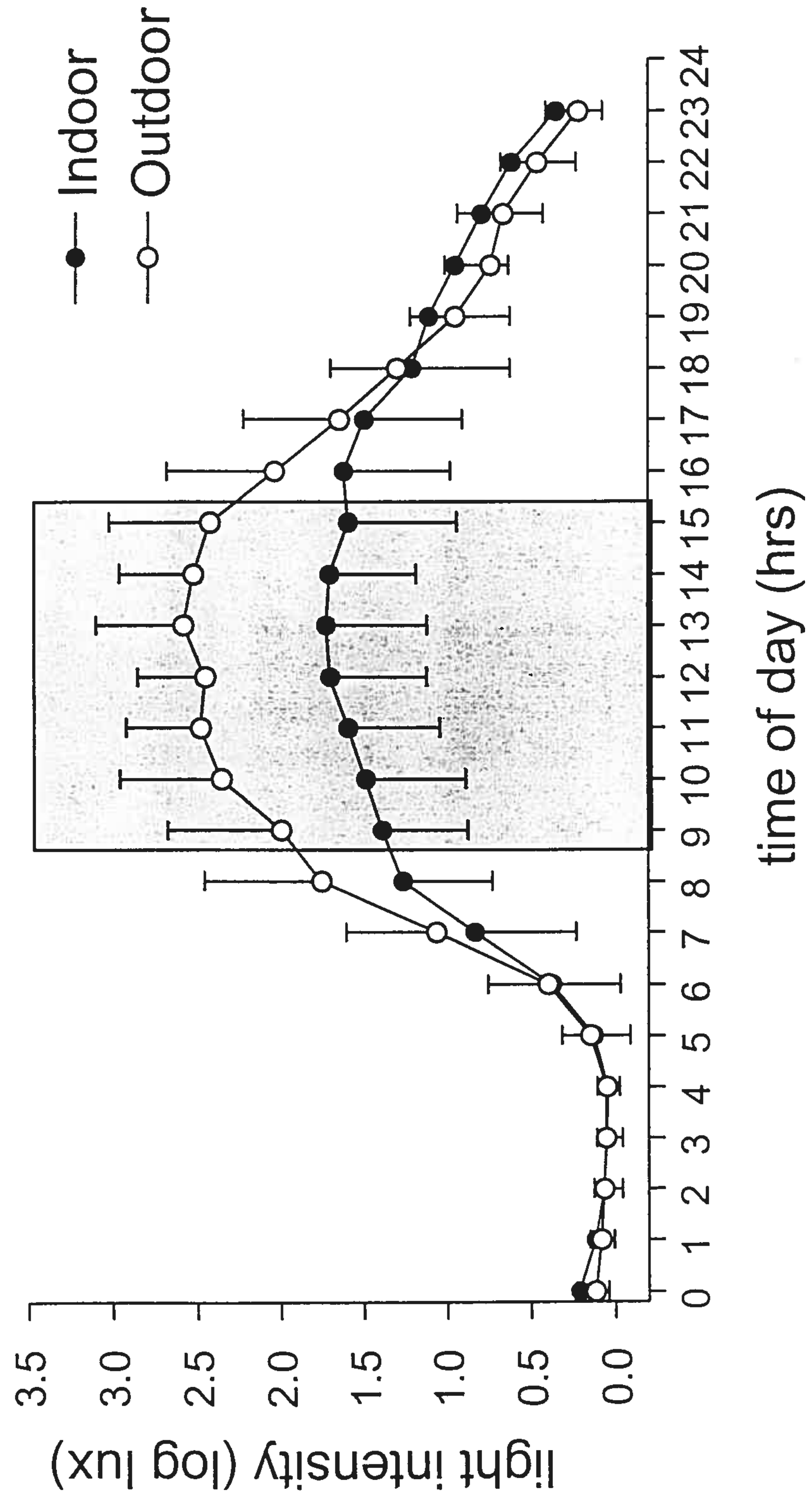


Figure 3

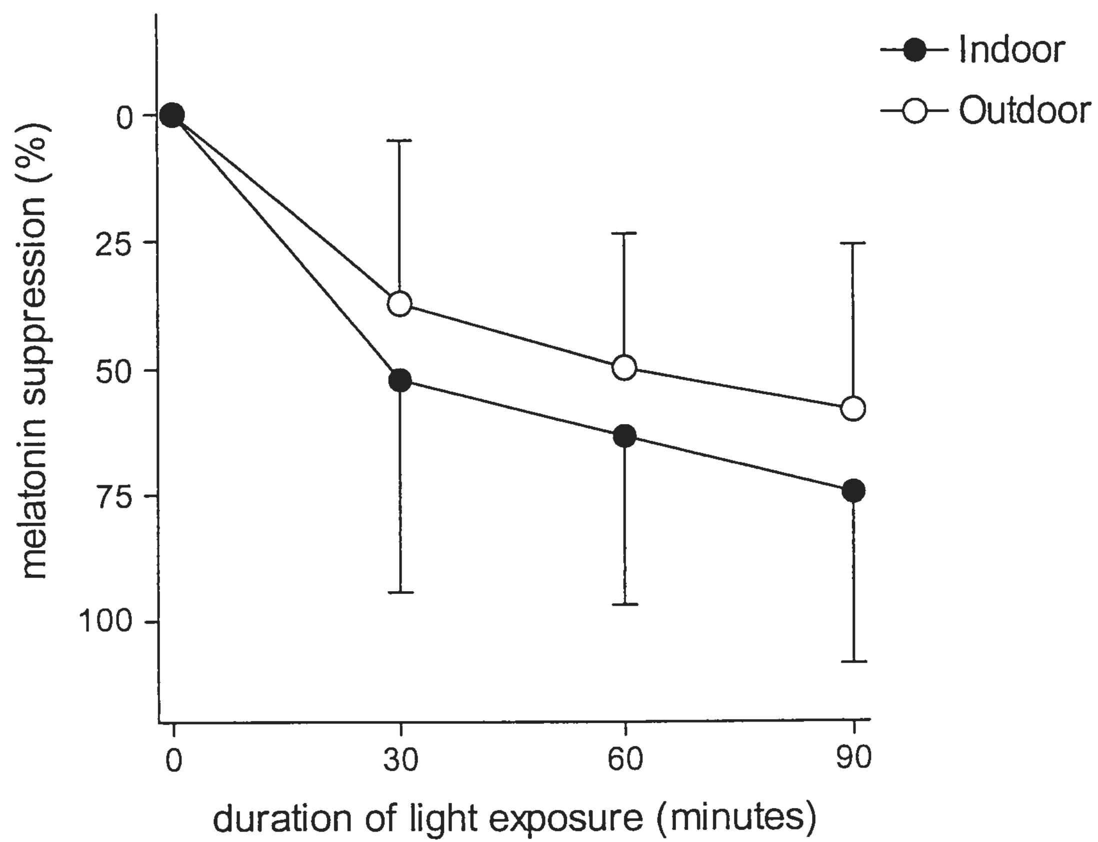
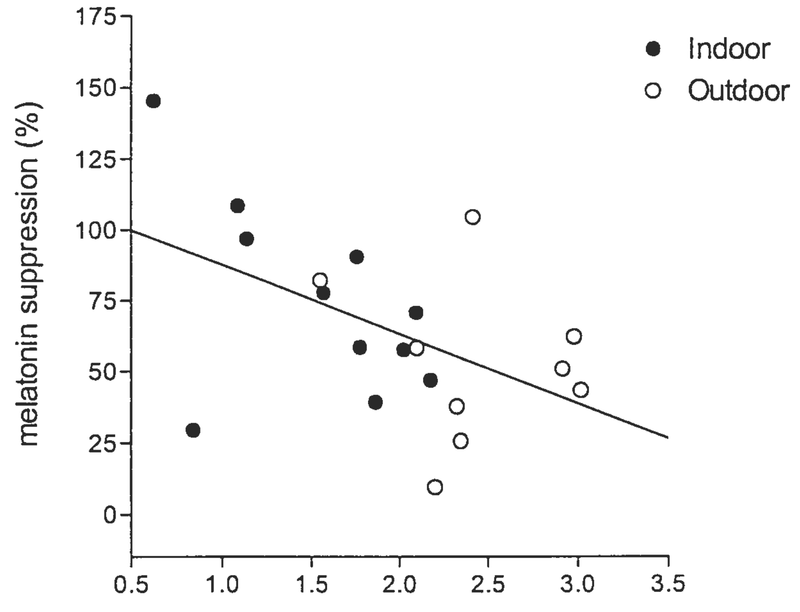


Figure 4



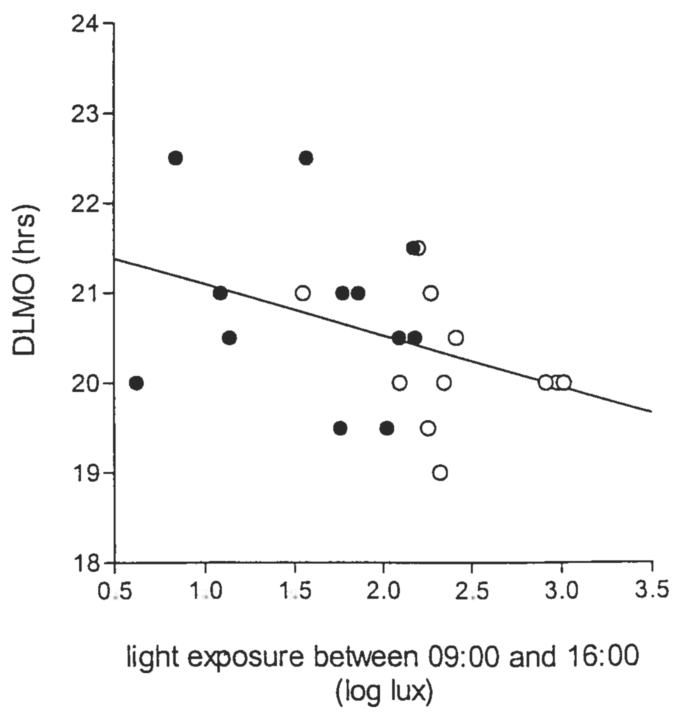


Figure 5

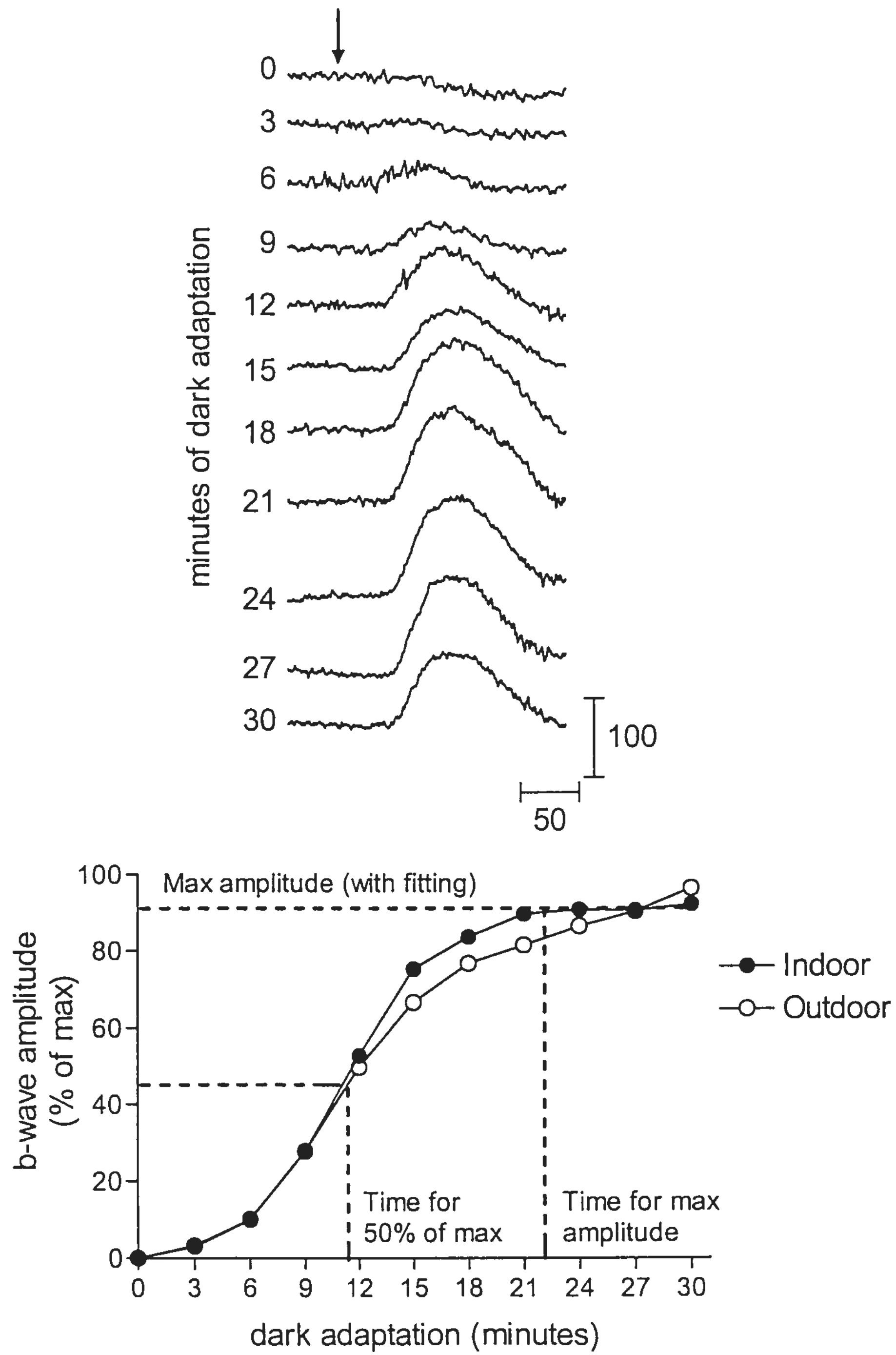
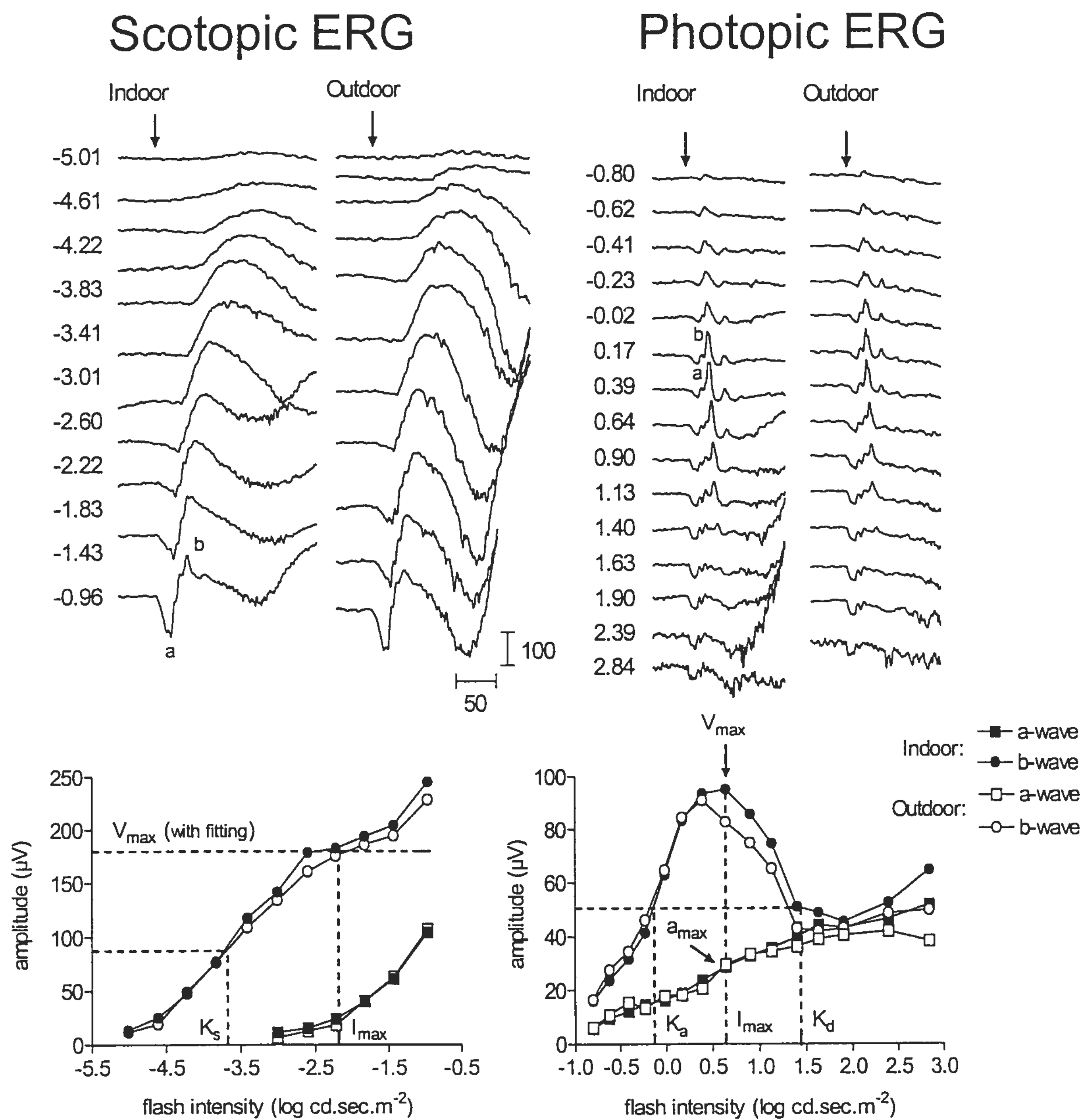


Figure 6



4. Discussion

4.1. Modulation de la fonction rétinienne en condition photopique

Dans le cadre d'études sur les modalités de la sensibilité rétinienne, il est primordial d'utiliser une mesure précise qui est basée sur un enregistrement électrophysiologique à diverses intensités de stimulation et qui permet une comparaison efficace des résultats obtenus d'un sujet à l'autre et/ou d'une condition à l'autre. Un tel outil existait déjà pour l'ERG scotopique; il s'agit de la courbe luminance-réponse modélisée à l'aide de l'équation de Naka-Rushton et couramment utilisée par les électrophysiologistes de la vision (Naka & Rushton, 1966; Peachey et al., 1989; Evans et al., 1993; Hébert et al., 1996). Jusqu'à ce jour, très peu de chercheurs ont examiné la courbe luminance-réponse en condition photopique. Cette fonction possède une forme particulière qui lui vaut son nom de « photopic hill » ou colline photopique et qui rend sa modélisation difficile (Wali & Leguire, 1992, 1993). En effet, l'amplitude de l'onde-b photopique augmente graduellement avec l'intensité du stimulus lumineux jusqu'à l'obtention d'un ERG d'une amplitude maximale, que l'on nomme V_{max}. Puis, en réponse à des intensités lumineuses encore plus fortes, l'amplitude de l'onde-b s'affaisse pour finalement atteindre un plateau, où l'onde-a et l'onde-b sont, à toutes fins pratiques, d'égale amplitude.

Les causes physiologiques de cette baisse d'amplitude à forte intensité ne sont pas encore connues. Une origine plausible pourrait être un phénomène d'habituation dû à une adaptation à la lumière ou un phénomène de fatigue dû à une sur-stimulation. Pourtant, il a

été démontré par Wali et Leguire (1992) que la forme du « photopic hill » n'est affectée ni par un changement dans l'intervalle entre chacun des stimuli, ni par l'ordre dans lequel les stimuli de différentes intensités sont présentés, c'est-à-dire en ordre croissant ou décroissant. L'hypothèse alors retenue par ces chercheurs était que la baisse d'amplitude de l'onde-b soit due à la sommation des potentiels électriques de l'onde-a et de l'onde-b. A forte intensité lumineuse, le potentiel hyperpolarisant de l'onde-a interférerait avec celui dépolarisant de l'onde-b.

L'objectif du premier volet de ce programme de recherche était donc d'approfondir les connaissances sur le « photopic hill » en le modulant à l'aide de diverses conditions d'enregistrement. Nous avons également testé la pertinence de l'enregistrement du « photopic hill » dans un cadre clinique. Dans la première étude, nous avons examiné l'impact de l'éclairage ambiant sur le « photopic hill ». Tous les niveaux d'éclairage (de 18 à 150 $cd.m^{-2}$) ont engendré un « photopic hill ». L'effet principal d'un éclairage ambiant plus intense sur l'enregistrement des ERG a été de déplacer la courbe luminance-réponse vers les intensités plus fortes, ce qui signifie une baisse de sensibilité. Ce résultat semblait prévisible si l'on considère le stimulus qui est perçu par la rétine comme étant constitué de la soustraction de l'intensité du stimulus envoyé par l'appareil par l'intensité de l'éclairage ambiant. De plus, il est intéressant de noter que l'amplitude du V_{max} était stable peu importe l'intensité de la lumière de fond. Nous avons relevé que les courbes luminance-réponse de l'onde-i et du potentiel oscillatoire OP_4 présentaient également une forme ressemblant au

« photopic hill », c'est-à-dire que leur amplitude baissait en réponse aux plus fortes intensités de stimulus.

Cette stabilité de l'amplitude maximale de la réponse des cônes laisse croire qu'il y aurait une limite intrinsèque du voltage intra-rétinien. Il semblerait que cette limite s'applique seulement au système des cônes car une onde-b d'une amplitude supérieure à celle du V_{max} photopique s'observe en condition scotopique en raison de la grande contribution des bâtonnets à la réponse. Par ailleurs, une rétine totalement adaptée à la noirceur (30 minutes en obscurité) ne présente normalement pas de « scotopic hill », l'amplitude de l'onde-b augmentant graduellement avec l'intensité du stimulus lumineux. Toutefois, nous avons démontré qu'une courbe luminance-réponse ressemblant au « photopic hill » peut s'observer au tout début de l'adaptation à la noirceur, une condition dans laquelle répondent les deux types de photorécepteurs (Rousseau & Lachapelle, 1999). Le V_{max} est alors d'une amplitude équivalente à 150% de celle du V_{max} photopique.

Dans le cadre de la deuxième étude, nous avons établi sept paramètres facilement identifiables et reproductibles pour permettre une analyse approfondie et objective du « photopic hill » de rétines normales et pathologiques. En effet, il ne serait pas approprié de modéliser la courbe luminance-réponse photopique à l'aide d'une équation de type sigmoïdal comme celle de Naka-Rushton, utilisée pour l'analyse de l'ERG scotopique, car aucune information sur la portion descendante ne serait incluse. Cette partie pouvant, tout

comme la portion ascendante, être soumise à des variations physiologiques particulières, il apparaît important de l'analyser également. Les sept paramètres sont les suivants : 1) V_{max} : l'amplitude maximale de l'onde-b; 2) a_{max} : l'amplitude de l'onde-a à l'intensité du V_{max}; 3) I_{max} : intensité du V_{max}; 4) b/a_{max} : ratio de l'amplitude de l'onde-b sur celle de l'onde-a à l'intensité du V_{max}; 5) K_a : intensité nécessaire pour obtenir une onde-b d'amplitude équivalente à 50% du V_{max} sur la portion ascendante du « photopic hill »; 6) K_d : intensité nécessaire pour obtenir une onde-b d'amplitude équivalente à 50% du V_{max} sur la portion descendante du « photopic hill »; 7) $K_{a=b}$: intensité évoquant une réponse où l'onde-a et l'onde-b sont d'égale amplitude. Ces variables se sont avérées adéquates pour la comparaison des « photopic hills » obtenus avec une lumière de fond de 17 et de 30 $cd.m^{-2}$. La seule variable significativement différente entre les deux conditions d'éclairage ambiant était le K_a. Cela signifie qu'il existe une différence de sensibilité entre les deux conditions qui s'observe principalement en réponse aux stimuli de faible intensité lumineuse.

Dans cette étude, nous avons également testé la pertinence clinique de ce protocole d'ERG photopique avec quatre patients atteints d'une héméralopie congénitale, d'une anomalie de cônes, d'une rétinopathie pigmentaire et d'une micro-ophtalmie unilatérale. Les quatre cas ont démontré un « photopic hill ». L'atteinte majeure observée avec l'héméralopie congénitale est une onde-b anormalement petite dans la portion descendante du « photopic hill ». Pour le patient présentant une anomalie de cônes, c'est dans la partie ascendante que l'on observe une faible amplitude d'onde-b, ceci repousse également le V_{max} qui survient à

une intensité plus élevée que chez les sujets normaux. La rétinopathie pigmentaire amène, quant à elle, une onde-b d'amplitude anormale tout au long du « photopic hill », avec un V_{max} et une portion descendante situés à des intensités plus élevées que la normale.

L'exemple le plus probant de l'utilité clinique de l'enregistrement du « photopic hill » vient du patient affecté d'une micro-ophtalmie unilatérale. Une comparaison des réponses des deux yeux révèle des « photopic hills » décalés l'un par rapport à l'autre. Ainsi, on observe une onde-b de plus grande amplitude dans l'œil sain que dans l'œil pathologique, mais seulement pour les intensités relativement faibles. On constate l'effet inverse à la fin du « photopic hill ». Ce phénomène fait ressortir l'importance d'utiliser des stimuli de différentes intensités car un seul test à forte intensité aurait mené à un faux constat, c'est-à-dire une meilleure réponse dans l'œil pathologique que dans l'œil normal.

Dans la dernière étude de ce volet portant sur la modulation de la fonction rétinienne en condition photopique, nous avons examiné les variations des différents paramètres utilisés dans la précédente étude en réponse à des stimuli de différentes longueurs d'onde, soient blanc (large spectre), bleu (410 nm), vert (510 nm) et rouge (640 nm). Le principal effet observé est un abaissement marqué de l'amplitude du V_{max} en réponse aux stimuli de couleur rouge par rapport aux trois autres couleurs. Cette diminution du V_{max} était accompagnée d'une baisse importante de l'amplitude d'OP_4. Ainsi, contrairement à nos observations antérieures à l'effet que l'amplitude du V_{max} soit stable peu importe le niveau

d'éclairage ambiant, ce paramètre peut démontrer des variations dans certaines conditions d'enregistrement. Cette notion modifie l'hypothèse voulant que le « photopic hill » soit causé par une limite intrinsèque du voltage intra-rétinien. Cette limite varierait selon le type de stimulus lumineux, du moins selon sa longueur d'onde. Nous avons également montré une translation sur l'axe des intensités des différents « photopic hills », ceux de l'onde-b comme ceux de l'onde-i, selon la longueur d'onde. Nous interprétons ce résultat comme une différence de sensibilité. Ainsi, il semble que l'on soit plus sensible au bleu, suivi du blanc et du vert qui sont équivalents. La plus faible sensibilité est observée en réponse au stimuli de couleur rouge.

Ces trois études combinées nous ont amené à proposer un nouveau modèle pour expliquer la génèse du « photopic hill ». Ce modèle est une extension du modèle avancé par Sieving et coll. (1994) pour décrire la génèse de l'onde-b elle-même. Ce concept appelé « Push-Pull » avance que la phase ascendante de l'onde-b, jusqu'au pic, est contrôlée par les cellules bipolaires ON-dépolarisantes, ceci constitue la poussée ou « Push ». La force de traction ou « Pull » permet, quant à elle, la re-descente de l'onde-b jusqu'à la ligne de base et est sous le contrôle des cellules bipolaires OFF-hyperpolarisantes. Nous croyons que ce concept pourrait aussi être modulé par l'intensité du stimulus et ainsi expliquer la descente du « photopic hill ». En effet, les OP_2 et OP_3, qui sont les potentiels oscillatoires associés à la voie ON (Lachapelle et al., 1998), ne présentent pas de détérioration en réponse aux stimuli de forte intensité. Par contre, l'OP_4 et onde-i démontrent un affaissement

d'amplitude dans la phase descendante du « photopic hill ». Or, ces deux constituants de l'ERG sont associés à la voie OFF (Nagata, 1963; Kojima & Zrenner, 1978; Lachapelle et al., 1998). Une étude de Kondo et coll. (2000) vient d'ailleurs appuyer l'hypothèse selon laquelle la réponse « OFF » diminue avec l'augmentation de l'intensité du stimulus. Ainsi, cette équipe a observé, à l'aide de stimuli d'une durée suffisamment prolongée pour séparer les composantes « ON » et « OFF » dans le temps, une diminution dans l'amplitude de l'élément « OFF » en réponse à des stimuli de forte intensité.

À ce concept s'ajoute le constat de Nagata, datant des années 60 (1963). Ce dernier a démontré que l'onde-b photopique obtenu avec un stimulus court représente la sommation des éléments « ON » et « OFF » évoqués avec des stimuli longs. Il a observé que l'onde-b d'amplitude maximale était obtenu avec un stimulus de 5 msec et qu'une augmentation de la durée du stimulus provoquait une séparation des éléments « ON » et « OFF » entraînant ainsi des ondes-b progressivement plus basses en amplitude et plus étendues dans le temps. En se basant sur la morphologie des ondes obtenues, ceci résultait en une fonction durée-réponse semblable en tout point à la courbe luminance-réponse qui forme le « photopic hill ». Ainsi, un stimulus court d'intensité forte, tel qu'observé en fin de « photopic hill », semble évoquer une réponse semblable à un stimulus long. Un stimulus fort pourrait donc retarder l'élément « OFF » dans le temps, plutôt que le diminuer en amplitude comme mentionné plus haut. Cette théorie semble correspondre à ce qui est perçu subjectivement par le sujet. En effet, un stimulus lumineux de forte intensité peut paraître plus long à celui

qui le reçoit qu'un stimulus de faible intensité. Ceci s'apparente au phénomène d'éblouissement causé par un éclair d'appareil photo. Au niveau physiologique, ceci pourrait s'expliquer par le fait qu'un stimulus de forte intensité amène la capture d'une grande quantité de photons par les photorécepteurs. Les nombreux signaux qui parviennent ensuite à la rétine interne (post-photorécepteurs) et qui engendrent l'onde-b seraient donc moins synchronisés, dispersant ainsi le signal ERG dans le temps et affaissant son amplitude par le fait même.

Cette hypothèse vient de recevoir un nouvel appui avec la parution très récente d'un article d'Ueno et coll. (2004). En effet, cette équipe a observé chez le singe, à l'aide de deux agents pharmacologiques qui inhibent la réponse des cellules bipolaires « ON » et celle des « OFF », que la baisse d'amplitude de l'onde-b à hautes intensités semble être due à une baisse d'amplitude de l'élément « ON » et à un délai dans le temps de l'élément « OFF ». Ces auteurs ont également fait un parallèle avec la morphologie des réponses obtenues avec des stimuli de longue durée. Une des explications avancées était que le temps requis pour la récupération des cônes serait plus long lorsque le stimulus est fort. Ils ont d'ailleurs isolé cette réponse des cônes avec l'addition des deux mêmes agents pharmacologiques et ont observé une extension de l'hyperpolarisation des cônes dans le temps en réponse aux stimuli de forte intensité.

4.2. Variation diurne de l'ERG et association avec la mélatonine

Dans le deuxième volet de ce programme de recherche, nous avons évalué la fonction rétinienne aux mêmes heures, en fin de soirée et en début de matinée, dans deux groupes de sujets maintenus sur le même horaire éveil-sommeil mais dont le moment de sécrétion de mélatonine différait. Ce protocole nous a permis de discriminer l'effet potentiel de la mélatonine sur l'ERG de l'effet du moment de la journée. Les deux groupes étaient formés d'individus classés « types du soir » ou « types du matin » selon le questionnaire de Horne et Ostberg (1976). Comme prévu, le groupe des types du soir s'est distingué de celui des types du matin par une heure habituelle de coucher et de lever plus tardive et par un épisode de sécrétion de mélatonine également plus tardif. Ainsi, comme les ERG étaient effectués à la même heure pour tous, la concentration relative de mélatonine chez les types du matin était significativement plus élevée pendant la session d'ERG en soirée que chez les types du soir, et inversement durant la session matinale.

À l'ERG scotopique, les deux groupes ont démontré la même variation diurne, c'est-à-dire une baisse d'amplitude du V_{max} et une sensibilité diminuée (K plus grand) le matin par rapport au soir. Nous avons attribué cette détérioration de la réponse scotopique au pic diurne du renouvellement des disques des bâtonnets, qui se produit tôt le matin. Cet effet matinal avait déjà été observé chez l'humain (Birch et al., 1984, 1986), le lapin (White & Hock, 1992) et le rat (Sandberg et al., 1986). Dans ces deux dernières études, une

association avec la quantité de phagosomes dans l'épithélium pigmentaire, un indicateur de l'activité de renouvellement des disques, avait été rapportée. La baisse d'amplitude et de sensibilité observée ici pourrait être due soit à un raccourcissement momentané des segments externes et donc à une diminution de la quantité de rhodopsine disponible, soit à un encombrement de l'espace entre l'épithélium pigmentaire et les photorécepteurs par des débris de phagocytose ce qui nuirait à la pénétration de la lumière dans la rétine. Finalement, même si la mélatonine a déjà été impliquée dans la régulation circadienne du renouvellement des disques des bâtonnets et de la phagocytose (Tosini, 2000), nous croyons que la différence de phase circadienne entre nos deux groupes (1.2 h) n'était pas suffisante pour apprécier l'effet de la sécrétion de mélatonine sur ces phénomènes étant donné qu'ils sont relativement étendus dans le temps.

Pour l'ERG photopique, nous avons utilisé les mêmes longueurs d'onde que pour l'étude précédente, c'est-à-dire blanc (large spectre), bleu (410 nm), vert (510 nm) et rouge (640 nm). Les variations diurnes ont été, à toutes fins pratiques, identiques peu importe la couleur des stimuli. L'amplitude de l'onde-a et de l'onde-b au V_{max} ainsi que la somme des potentiels oscillatoires (SOP) ont démontré un patron de variation inverse dans les deux groupes. Ces amplitudes étaient plus élevées le soir pour les types du soir et le matin pour les types du matin, c'est-à-dire aux moments où la concentration de mélatonine était relativement faible. D'ailleurs, des corrélations négatives avec la concentration de mélatonine ont été mesurées pour l'onde-a au vert et au rouge et pour l'onde-b au blanc, au

vert et au rouge. Des corrélations semblables ont également été observées pour l'amplitude de l'onde-a et de l'onde-b en condition scotopique mixte (bâtonnet et cône), ainsi que pour leur temps de culmination, une plus faible concentration de mélatonine étant associée à une réponse plus rapide.

Il semble donc que la présence d'une forte concentration de mélatonine soit associée à une diminution d'amplitude et à un ralentissement de la réponse des cônes. Une association semblable avait déjà été rapportée chez l'humain et chez le poulet. Suite à l'administration de mélatonine en après-midi chez des sujets sains, une baisse d'amplitude d'ERG avait été constatée (Emser et al., 1993). Cette étude ne dissociait malheureusement pas la réponse des cônes de celle des bâtonnets. Chez le poulet, une injection intra-musculaire de mélatonine pendant la journée avait généré une baisse d'amplitude de l'ERG photopique (Lu et al., 1995). Il est possible que ces effets de la mélatonine exogène sur l'amplitude de l'ERG de cônes soient dûs au fait que cette hormone stimule l'élongation des cônes (Pierce & Besharse, 1985) et abolisse la formation des spinules des cellules horizontales lors de l'adaptation à la lumière (Behrens et al., 2000). Ces deux phénomènes pourraient contribuer à la réduction d'amplitude de la réponse à l'ERG, d'abord en réduisant la capture de photons par les cônes, puis en diminuant l'efficacité des synapses entre les cônes et les cellules horizontales.

Par ailleurs, il a été suggéré par le passé que les mouvements rétino-moteurs des segments internes des cônes (élongation-contraction) puissent être sous un contrôle circadien. Cette hypothèse est supportée, entre autres, par l'observation que la contraction des cônes s'initie avant même le début de la période de lumière et que ce rythme persiste en obscurité constante (McCormack & Burnside, 1992). Ainsi, les effets observés dans la présente étude sont peut-être dûs à la différence de phase circadienne entre les deux groupes de sujets au moment de l'expérimentation plutôt qu'à la mélatonine elle-même.

Les résultats obtenus en condition scotopique mixte, où les deux types de photorécepteurs contribuent à la réponse, sont en accord avec les résultats obtenus à l'ERG de bâtonnets seulement (scotopique) et de cônes seulement (photopique). En effet, nous avons observé une diminution plus importante de l'amplitude de la réponse cône-bâtonnet le matin chez les types du soir que chez les types du matin. Chez les types du soir, ceci pourrait être dû à l'effet combiné du renouvellement des disques des bâtonnets et de la concentration élevée de mélatonine. Parallèlement, chez les types du matin, on noterait les effets opposés du renouvellement des disques et de la faible concentration de mélatonine.

Par ailleurs, il est intéressant de noter la présence d'une baisse de la concentration de mélatonine 40 minutes après l'ouverture de la lumière de fond pour l'enregistrement de l'ERG photopique en soirée, et ce, uniquement dans le groupe des types du soir. Cette baisse a été notée chez plus de 70% des types du soir, alors qu'aucun type du matin n'a

démontré une telle diminution. Cette observation est en désaccord avec l'hypothèse voulant que la sensibilité du système circadien à la lumière augmente au cours de la nuit (Terman & Terman, 1985; McIntyre et al., 1989). En effet, lors de la session d'ERG du soir, les types du soir étaient moins avancés dans leur « nuit habituelle » que les types du matin. Leur système circadien aurait donc dû, par le fait même, être moins sensible à la lumière, ce qui ne semble pas être le cas. Par contre, l'hypothèse voulant que le système circadien des types du soir soit plus sensible à la lumière en fin de soirée que celui des types du matin a déjà été avancée par Aoki et coll. (2001). Il faut toutefois préciser que cette dernière étude avait été menée sur des patients atteints du syndrome de délai de phase, ce qui constitue l'extrême limite de la population des types du soir. Ainsi, ils avaient mesuré une plus grande suppression de la sécrétion de mélatonine suite à une exposition lumineuse à la même phase circadienne (pendant les 2 heures précédant le pic de mélatonine) chez ces patients que chez les sujets contrôles. Notre étude semble abonder dans le même sens, c'est-à-dire que les types du soir (même relativement modérés) présentent une plus grande sensibilité circadienne à la lumière en fin de soirée que les types du matin. Il est d'ailleurs possible d'envisager que cette dernière caractéristique puisse être une cause de leur délai de phase.

4.3. Effet de l'histoire lumineuse sur la sensibilité rétinienne et circadienne

Dans le dernier volet de ce programme de recherche, nous avons examiné, chez deux groupes de travailleurs, l'effet à long terme de l'environnement lumineux sur la sensibilité à la lumière. Ces deux groupes étaient formés de sujets travaillant à temps plein, soit dans un milieu sombre sans accès à la lumière naturelle, soit principalement à l'extérieur. L'exposition lumineuse a été enregistrée 24 h sur 24 pendant deux semaines consécutives à l'aide de moniteurs ambulatoires. Nous avons utilisé la mesure de suppression de sécrétion de mélatonine pour évaluer la sensibilité du système circadien à la lumière. Afin de déterminer si l'adaptation du système circadien pouvait être due à une modification de la sensibilité à la lumière au niveau de la rétine même, nous avons également pratiqué un protocole complet d'ERG en condition scotopique et photopique ainsi qu'une évaluation de la vitesse d'adaptation à la noirceur. De plus, la phase circadienne des sujets a été déterminée par le début de la période de sécrétion de mélatonine (DLMO).

Comme prévu, les travailleurs en milieu sombre ont été exposés à moins de lumière vive (>1000 lux) pendant la journée que les travailleurs en milieu éclairé. Cette exposition moindre à la lumière vive était associée à une plus grande sensibilité circadienne, une phase circadienne tardive, une adaptation rapide à la noirceur, une sensibilité rétinienne augmentée en condition scotopique et diminuée en condition photopique. L'exposition lumineuse des deux groupes différait seulement pendant les heures de travail. Ceci

concorde avec une étude récente démontrant que l'exposition lumineuse dans l'environnement de travail est la composante principale de l'exposition lumineuse totale (Heil & Mathis, 2002). Notre observation suggère également que les travailleurs en milieu sombre et en milieu éclairé passent leur temps libre dans des environnements lumineux semblables.

Nous avons obtenu une corrélation négative entre la suppression de mélatonine et l'exposition lumineuse pendant les heures de travail. Ces résultats corroborent ceux de Lynch et coll. (1981, 1985) et Reiter et coll. (1983) chez l'animal et de Owen et Arendt (1992) et Hébert et coll. (2002) chez l'humain. Ces cinq études s'accordent toutes à dire qu'une histoire lumineuse de faible intensité est associée à une augmentation de la sensibilité circadienne à la lumière par rapport à une histoire lumineuse de plus forte intensité. Malgré cette corrélation, le degré de suppression de mélatonine n'était pas significativement différent dans les deux groupes. Ceci pourrait être dû à la grande variabilité inter-individuelle de la mesure, qui a déjà été rapportée ailleurs (Laasko et al., 1991; Hébert et al., 2002). Une cause possible de cette variation pourrait être une mauvaise évaluation de la phase circadienne, par l'heure habituelle du coucher, chez certains sujets, le test de suppression de mélatonine étant alors administré à des phases circadiennes diverses. Ce n'est toutefois probablement pas le cas car le test fut administré, en moyenne, à la même heure circadienne dans les deux groupes, avec relativement peu de variabilité (écart type de 1.1 h pour milieu sombre et 0.8 h pour milieu éclairé). De plus, même si les

deux groupes de travailleurs étaient exposés, en moyenne, à des niveaux d'éclairage différents, notre représentation graphique de la corrélation mentionnée plus haut démontre clairement que l'exposition lumineuse des sujets forme un continuum plutôt que deux conditions différentes. Ceci signifie que la sélection des sujets aurait pu être plus stricte quant à l'exposition lumineuse au travail de façon à obtenir des conditions d'éclairage plus distinctes l'une de l'autre et plus homogènes dans chacun des groupes.

L'hypothèse voulant qu'un changement dans l'environnement lumineux (et dans la sensibilité du système circadien à la lumière) puisse entraîner une modification de la phase circadienne a été en partie vérifiée dans cette étude. Dans ce cas également, les deux groupes ne se sont pas différenciés significativement quant à leur phase circadienne (DLMO). Toutefois, la corrélation entre cette mesure et l'exposition lumineuse au travail a presque atteint le niveau de signification ($p=0.06$). Ainsi, une sensibilité circadienne accrue, combinée avec une exposition lumineuse de faible intensité pendant la journée de travail, pourrait engendrer un délai de phase circadienne. Il semble en effet que la phase d'entraînement circadien soit affectée par la force du synchroniseur (ici la lumière) et par l'ampleur de la différence d'intensité lumineuse entre le jour et la nuit (Roenneberg et al., 2003). Pour la majorité des individus, la période circadienne endogène est supérieure à 24 heures et a donc besoin d'être avancé de façon quotidienne pour se maintenir dans une relation de phase stable par rapport au cycle environnemental de 24 heures. Pour ces gens,

un synchroniseur faible, comme dans le cas des travailleurs en milieu sombre, aurait donc pour effet d'amoindrir cette avance de phase.

Le processus d'adaptation à la noirceur et la fonction luminance-réponse en condition scotopique ont varié entre les deux groupes. Les deux courbes (« temps-réponse » et luminance-réponse) ont montré la même différence inter-groupe, c'est-à-dire que la première partie était identique dans les deux groupes alors que la dernière portion s'est produite plus rapidement (pour l'adaptation) et à de plus faibles intensités (pour l'ERG scotopique) chez les travailleurs en milieu sombre que chez les travailleurs en milieu éclairé. Les travailleurs en milieu sombre s'adaptent donc plus rapidement à la noirceur et sont plus sensibles en condition scotopique que les travailleurs en milieu éclairé. La méthode traditionnelle d'évaluation de l'adaptation à la noirceur consiste à mesurer l'abaissement du seuil de détection de lumière en fonction du temps passé en obscurité (Reeves, 2004). Notre propre évaluation de l'adaptation à la noirceur, mesurée par le retour de l'onde-b à son amplitude maximale, suit la dynamique de la méthode traditionnelle; le temps requis pour obtenir l'asymptote (i.e. le maximum) étant d'une vingtaine de minutes. Par ailleurs, une étude de Penn et Williams (1986) a démontré une variation de la vitesse de régénération de la rhodopsine selon l'histoire lumineuse. Cette association était toutefois mal définie, la vitesse étant plus élevée dans les deux conditions extrêmes (la plus sombre et la plus éclairée) et plus basse dans les deux conditions intermédiaires. Ici, la rhodopsine semble se régénérer plus rapidement suite à une histoire lumineuse de faible intensité

qu'après une exposition prolongée à des intensités plus fortes. Ce mécanisme pourrait faire partie des modifications rétiniennes permettant une meilleure capture de photons suite à une exposition prolongée à un milieu sombre.

Les résultats obtenus avec la fonction luminance-réponse en condition scotopique semblent corroborer l'hypothèse d'un phénomème s'apparentant à la photostasie chez l'humain. Chez l'animal, on a démontré un ajustement à long terme des bâtonnets afin de capter une quantité fixe de photons par jour. Ainsi, la longueur des segments externes (Batelle & LaVail, 1978; Penn & Williams, 1986) et la quantité totale de rhodopsine (Schremser & Williams, 1995a, b) sont modulées en fonction de l'environnement lumineux. Dans notre étude, il semble que les bâtonnets deviennent plus sensibles à la lumière suite à une exposition chronique à un milieu de travail sombre. L'inverse pourrait également être le cas chez les travailleurs en milieu éclairé. Une étude sur les variations saisonnières de l'ERG n'a pas démontré de changements chez les sujets normaux avec la longueur de la photopériode (Hébert et al., 2002). Toutefois, ces chercheurs n'avaient pas rapporté le I_{max} (intensité du V_{max}), ce qui constitue la variable montrant une différence inter-groupe dans notre étude. À notre connaissance, une seule autre étude a examiné l'effet de l'histoire lumineuse sur l'ERG. Cette recherche effectuée sur des rats albinos a démontré une baisse de l'amplitude de l'onde-b scotopique, six jours seulement après un transfert d'un régime lumineux de 5 lux / obscurité vers 400 lux / obscurité (Li et al., 2003). Ici encore, le I_{max} n'était pas rapporté. Dans la présente étude, aucun changement d'amplitude important n'a

été noté même si, en moyenne, les ERG du groupe de travailleurs en milieu sombre étaient d'une amplitude supérieure à celle de l'autre groupe. Il semble donc que les résultats que nous avons obtenus en condition scotopique soient dûs à une modulation de l'efficacité de capture des photons par les bâtonnets et non de la quantité totale de rhodopsine disponible. Ceci expliquerait que les changements aient été observés dans la sensibilité et la dynamique d'adaptation à la noirceur plutôt que dans l'amplitude de l'ERG.

Notre étude est la première à examiner l'effet de l'histoire lumineuse sur la réponse des cônes chez l'humain. La différence inter-groupe est inverse à celle observée pour l'ERG scotopique. Ici, le groupe de travailleurs en milieu éclairé a démontré une sensibilité supérieure (telle que démontrée par les mesures K_a, K_d et I_{max}) à celle du groupe en milieu sombre. Nous postulons que cet effet soit dû à une « potentialisation à long terme » des cônes ou d'autres éléments rétiniens dans la voie des cônes, chez les travailleurs en milieu éclairé. Cette « potentialisation » pourrait ressembler à ce qui est observé dans d'autres neurones du système nerveux central, où un stimulus d'une intensité donnée déclenche une réponse plus forte après une telle « potentialisation » (Kandel, 2001). Ainsi, la voie des cônes pourrait devenir plus efficace dans le traitement de la lumière suite à une exposition fréquente et prolongée à des intensités lumineuses intenses.

En ce qui a trait à la différence plus prononcée entre les deux groupes dans la portion descendante du « photopic hill » comparativement à la partie ascendante, on pourrait

suggérer une certaine forme de protection contre les effets néfastes de la lumière du soleil. Cette protection chez les travailleurs en milieu éclairé amènerait une descente plus « hâtive » de l'amplitude de la réponse des cônes (onde-b) avec l'augmentation des intensités de stimulus. Une protection semblable contre les effets néfastes d'une lumière très vive a été notée chez des rats albinos élevés en milieu éclairé par rapport aux animaux gardés en milieu sombre (Li et al., 2001, 2003). En effet, cette étude révélait une meilleure sauvegarde de l'épaisseur de la couche des photorécepteurs et de l'amplitude de l'ERG scotopique avec une augmentation du temps passé en conditions d'éclairage de forte intensité. Ces changements étaient mesurables après une seule journée en milieu éclairé et devenaient significatifs après 2 à 3 jours. Malheureusement, aucun ERG photopique n'était rapporté dans cette étude.

Finalement, en supposant que les différences de sensibilité rétinienne et circadienne à la lumière soient causées par l'histoire lumineuse de nos deux groupes, on peut se demander si les deux processus sont indépendants ou si l'un découle de l'autre. En effet, l'adaptation à long terme du système circadien au régime lumineux pourrait être la conséquence d'une adaptation au niveau de la porte d'entrée de l'entraînement circadien, c'est-à-dire dans la rétine même. Les études récentes suggèrent que les récepteurs circadiens seraient des cellules ganglionnaires dotées de photosensibilité (Hattar et al., 2002). Le photopigment de ces cellules est la mélanopsine et leurs axones semblent formés la voie rétino-hypothalamique. La majorité des chercheurs s'entendent pour dire que, même si la

mélanopsine est considérée comme étant le photopigment circadien principal, les cônes et les bâtonnets ne devraient pas être éliminés complètement du réseau neuronal circadien (Hattar et al., 2003; Berson, 2003; Van Gelder, 2003). En effet, plusieurs études ont montré que l'unique absence de mélanopsine ou des photorécepteurs conventionnels n'est pas suffisante pour interrompre toute réponse circadienne à la lumière (Yoshimura et al., 1994; Panda et al., 2002; Ruby et al., 2002; Mrosovsky, 2003). L'absence combinée de mélanopsine, de cônes et de bâtonnets est nécessaire pour abolir l'entraînement et la suppression de la synthèse de mélatonine (Hattar et al., 2003; Panda et al., 2003). De plus, même si les cellules ganglionnaires photosensibles sont, par définition, indépendantes du reste du système visuel quant à la capture de photons, elles semblent posséder des liens synaptiques avec des cellules bipolaires et amacrines (Belenky et al., 2003). Ces cellules bipolaires seraient reliées à la voie des cônes plutôt qu'à celle des bâtonnets.

Ainsi, une interaction entre les voies visuelles classiques et la voie des cellules ganglionnaires photosensibles et de la mélanopsine paraît nécessaire à une réponse normale du système circadien à la lumière (Van Gelder, 2003). Il est donc plausible que les changements que nous avons observés dans l'ERG scotopique et photopique ainsi que dans l'adaptation à la noirceur soient non seulement dûs à l'histoire lumineuse mais soient également responsables de l'adaptation à long terme du système circadien à l'environnement lumineux.

5. Conclusion

Ce programme de recherche nous a permis d'approfondir les liens entre la sensibilité rétinienne et le système circadien central. D'une part, différents aspects de la physiologie rétinienne, tels que le renouvellement des disques des bâtonnets et les mouvements rétino-moteurs des photorécepteurs, semblent être sous un contrôle circadien. Nous croyons que ces rythmes rétiniens engendrent également des variations de la fonction rétinienne telle que mesurée par l'ERG. La sensibilité rétinienne semble donc être synchronisée au cycle lumière-obscurité par une horloge circadienne, qui pourrait être centrale (NSC) et/ou locale (rétinienne).

D'autre part, on sait que la sensibilité du système circadien à la lumière varie, elle aussi, avec la phase circadienne. De plus, les deux types de sensibilité à la lumière, rétinienne et circadienne, semblent modulés à plus long terme par l'environnement lumineux. Ainsi, il paraît plausible d'envisager une relation bi-directionnelle entre la rétine et les NSC avec une modulation à court et à long terme. Le système circadien central (et/ou local) entraînerait les rythmes rétiniens et, parallèlement, le rythme de la sensibilité rétinienne modulerait certains paramètres du système circadien, tels que la sensibilité circadienne à la lumière et la phase d'entraînement.

Plusieurs futurs projets de recherche pourraient complémenter les études présentées dans cette thèse. Il pourrait être pertinent d'examiner certaines fonctions du système circadien chez des patients souffrant de diverses atteintes du système visuel; et inversement, d'étudier

les fonctions rétiniennes de personnes présentant des anomalies circadiennes, telles que les syndromes de délai et d'avance de phase. L'étude de ces types de population permettrait d'examiner si les liens entre les deux systèmes sont maintenus lorsque l'un d'entre eux est perturbé ou anormal.

En ce qui a trait à l'étude de l'impact de l'environnement lumineux, il serait intéressant d'examiner les changements de sensibilité rétinienne et circadienne chez des individus dont l'exposition lumineuse est contrôlée de façon précise en laboratoire. Une série de mesures effectuées avant et après l'exposition à une luminosité donnée permettrait, d'une part, l'établissement d'une relation de cause à effet entre l'histoire lumineuse et la sensibilité circadienne, et, d'autre part, une corrélation entre les modulations de la sensibilité rétinienne et de la sensibilité circadienne.

Il serait également pertinent de reprendre l'étude des variations circadiennes et diurnes de la sensibilité rétinienne mais en incluant une évaluation de la sensibilité circadienne à la lumière à différentes phases circadiennes et à différents moments de la journée. Ceci permettrait d'établir une corrélation entre la sensibilité à la lumière des deux systèmes qui, cette fois, serait modulée sur une base quotidienne plutôt que sur plusieurs mois. ... En espérant que l'avenir saura répondre à toutes les questions qui restent en suspens !

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7. Annexes

7.1. Accord des coauteurs et permission des éditeurs

X

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7.2. Description de l'apport de chacun des coauteurs

Article 1 :

Rufiange M, Rousseau S, Dembinska O, Lachapelle P. Cone-dominated ERG luminance-response function : The *Photopic Hill* revisited. *Documenta Ophthalmologica*, 104 : 231-248, 2002.

L'expérimentation de cette étude a été réalisée par Sophie Rousseau, une étudiante à la maîtrise dirigée par Pierre Lachapelle. Mon apport personnel a consisté à colliger les résultats, faire les analyses statistiques et rédiger le manuscrit, Mme Rousseau ayant terminé ses études à ce moment. Olga Dembinska, alors étudiante au doctorat dans le laboratoire de Dr Lachapelle, a révisé le manuscrit et y a apporté quelques modifications. Pierre Lachapelle a agit comme chercheur principal de ce programme de recherche.

Article 2 :

Rufiange M, Dassa J, Dembinska O, Koenekoop RK, Little JM, Polomeno RC, Dumont M, Chemtob S, Lachapelle P. The photopic ERG luminance-response function (Photopic Hill) : Method of analysis and clinical application. *Vision Research*, 43 : 1405-1412, 2003.

Mon rôle dans cette étude a été à la fois de formuler les hypothèses de départ, d'élaborer le protocole expérimental, de procéder à l'expérimentation, d'analyser les résultats et d'écrire le manuscrit. Justine Dassa et Olga Dembinska ont participé à l'expérimentation ainsi qu'à l'interprétation des résultats. Les Drs Koenekoop, Little et Polomeno ont permis le recrutement des patients à titre d'ophtalmologistes à l'Hôpital de Montréal pour enfants. Les Drs Dumont et Chemtob ont révisé le manuscrit et y ont apporté quelques modifications. Pierre Lachapelle a agit comme chercheur principal de ce programme de recherche.

Article 3 :

Rufiange M, Dumont M, Lachapelle P. Modulation of the human photopic ERG luminance-response function with the use of chromatic stimuli. Soumis à *Vision Research*, janvier 2004.

Mon rôle dans cette étude a été à la fois de formuler les hypothèses de départ, d'élaborer le protocole expérimental, de procéder à l'expérimentation, d'analyser les résultats et d'écrire le manuscrit. Marie Dumont a révisé le manuscrit et y a apporté quelques modifications. Pierre Lachapelle a agit comme chercheur principal de ce programme de recherche. Cet article a été soumis à la revue *Vision Research* le 6 janvier 2004.

Article 4 :

Rufiange M, Dumont M, Lachapelle P. Correlating retinal function with melatonin secretion in subjects with an early or late circadian phase. *Investigative Ophthalmology & Visual Science*, 43 : 2491-2499, 2002.

Mon rôle dans cette étude a été à la fois de formuler les hypothèses de départ, d'élaborer le protocole expérimental, de procéder à l'expérimentation, d'analyser les résultats et d'écrire le manuscrit. Marie Dumont et Pierre Lachapelle ont agit comme directeurs de ce programme de recherche.

Article 5 :

Rufiange M, Lachapelle P, Dumont M. Differences in retinal and circadian sensitivity to light associated with light exposure in the work environment. Article en préparation pour soumission à *Journal of Neuroscience*.

Mon rôle dans cette étude a été à la fois de formuler les hypothèses de départ, d'élaborer le protocole expérimental, de procéder à l'expérimentation, d'analyser les résultats et d'écrire le manuscrit. Marie Dumont et Pierre Lachapelle agissent comme directeurs de ce programme de recherche. Cet article devrait être soumis dans les mois à venir à la revue *Journal of Neuroscience*.