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NONVISUAL BRAIN RESPONSES TO LIGHT EXPOSURE IN HUMAN AS ASSESSED BY FUNCTIONAL MAGNETIC RESONANCE IMAGING

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Résumé

La lumière influence profondément la physiologie humaine, en plus de permettre la vision. Elle constitue le synchronisateur principal des rythmes circadiens et induit des effets physiologiques immédiats. Ces effets concernent des fonctions non-visuelles telles que la régulation du rythme veille/sommeil, de la température corporelle, de fonctions endocrinologiques, de l'éveil et des performances. Plusieurs études de ces effets réalisées chez l'animal et chez l'homme ont montré l'implication d'un système de photoréception non-visuel sensible surtout aux courtes longueurs d'onde (~470nm ; bleu). Ce système utilise les photorécepteurs classiques (cônes et bâtonnets), en plus de cellules ganglionnaires rétiniennes (CGR) intrinsèquement photosensibles, et exprimant la mélanopsine. Ces CGR se connectent à de nombreux noyaux sous-corticaux et corticaux, ce qui suggère un rôle du système non-visuel dans de nombreuses fonctions cérébrales. Cependant, au delà de ces projections rétiniennes directes, les autres régions du cerveau impliquées sont très peu connues. Une étude en tomographie par émission de positons (TEP), réalisée à l'Université de Liège, a démontré que l'effet éveillant d'une lumière nocturne intense (>8000lux) pouvait moduler l'activité cérébrale liée à une tâche attentionnelle. Cette étude, ainsi que quelques données d'EEG, résume notre connaissance des mécanismes cérébraux impliqués dans le système non-visuel chez l'homme. De plus, la majorité des études sur ces effets ont été entreprises la nuit.

Nous avons réalisé trois études en imagerie fonctionnelle par résonance magnétique (IRMf) utilisant des expositions lumineuses diurnes pour mieux caractériser le système cérébral non-visuel chez l'homme. L'IRMf bénéficie d'une meilleure résolution spatiale et temporelle que la TEP et permet la caractérisation d'activités cérébrales liées à un processus cognitif précis.

La première étude met en évidence des réponses cérébrales liées à une tâche attentionnelle avant et après une exposition lumineuse intense (>7000lux) de 21min. L'amélioration de l'éveil subjectif induite par la lumière est liée à une augmentation de l'activité thalamique. De plus, la lumière augmente l'activité d'un réseau de régions corticales impliquées dans la tâche, prévenant les diminutions d'activités observées en obscurité continue. Ces augmentations déclinent en quelques minutes après l'arrêt de la lumière, en suivant des dynamiques diverses spécifiques à chaque région. Ces premiers résultats suggèrent que, via une modulation de l'activité de structures sous-corticales

régulant l'éveil, la lumière peut promouvoir dynamiquement l'activité corticale de réseaux impliqués dans un processus cognitif non-visuel.

La deuxième étude montre que de courtes expositions (18min) à des lumières monochromatiques ($3x10^{13}ph/cm^2/s$) bleues (470nm) ou vertes (550nm) affectent différemment les réponses cérébrales liées à une tâche de mémoire de travail. La lumière bleue augmente les réponses cérébrales ou, du moins, prévient les diminutions observées sous lumière verte dans des cortex pariétaux et frontaux impliqués dans la mémoire de travail, ainsi que dans le thalamus. Ces résultats montrent qu'une lumière monochromatique peut rapidement influencer les fonctions cognitives et suggèrent que ces effets sont induits via un système de photoréception qui utilise la mélanopsine.

La dernière étude répétait, au cours d'une même session, plusieurs courtes (50s) expositions lumineuses ($10^{13}ph/cm^2/s$) violettes (430nm), bleues (473nm), ou vertes (527nm) pendant la réalisation d'une tâche de mémoire de travail. Les réponses cérébrales, enregistrées à l'allumage et pendant la tâche, suggèrent que dès les premières secondes de l'illumination, les CRG exprimant la mélanopsine contribuent de manière prépondérante à la modulation des réponses cérébrales de régions impliquées dans la régulation de l'éveil et dans la tâche. Les résultats suggèrent un rôle du tronc cérébral ainsi que du thalamus dans l'établissement des réponses non-visuelles à la lumière.

Ces résultats démontrent qu'une exposition lumineuse diurne peut moduler l'activité cérébrale non-visuelle liée à deux fonctions cognitives complexes. La lumière agit rapidement en fonction de la région cérébrale et de la longueur d'onde considérées. Les sensibilités aux différentes longueurs d'ondes suggèrent l'implication d'un système de photoréception utilisant la mélanopsine. Quelques secondes de lumière sont suffisantes pour induire des changements dans des régions sous-corticales probablement impliquées dans l'initiation des réponses non-visuelles détectées à d'autres niveaux (physiologiques ou comportementaux). Les résultats suggèrent également une implication étendue de la lumière dans la régulation des fonctions cérébrales chez l'homme et soutiennent son utilisation pour contrecarrer la somnolence diurne et traiter des désordres circadiens et psychiatriques. Les résultats préliminaires de 2 autres études suggèrent une influence de la lumière sur la régulation des émotions et soulignent l'importance de facteurs génétiques dans les réponses non-visuelles à la lumière.

Summary

Light profoundly affects human physiology, in addition to allowing vision. Exposure to light is the primary synchronizer of circadian rhythms, but light also induces acute physiological responses. These responses involve functions not directly related to vision and include the modulation of the sleep/wake cycle, thermoregulation, endocrine functions, alertness, and performance. Animal and human studies demonstrated that a *nonvisual* photoreception system most sensitive to shorter wavelength light (~470nm; blue light) mediates these effects. This system recruits the classical retinal photoreceptors (rods and cones) and intrinsically photosensitive retinal ganglion cells (RGC) expressing melanopsin. These RGC project to numerous nuclei of the brainstem, hypothalamus, thalamus, and to cortical structures, an anatomical connectivity which suggests that the nonvisual system can influence many brain functions. However, beyond the direct melanopsin expressing RGC projections, little is known on the other brain structures involved. In addition these projections were mainly demonstrated in rodents. A positron emission tomography (PET) study carried out at the University of Liège established that night time bright white light exposure modulates the brain activity related to an attentional task. This study and sparse electroencephalogram data constitute the little knowledge of the human brain mechanisms of the nonvisual system. Furthermore, most investigations of the nonvisual effects of light took place at night.

We carried out three functional magnetic resonance imaging (fMRI) investigations to further unravel the brain system involved in nonvisual effects of light in healthy human subjects, using daytime exposures. The fMRI technique benefits from a much better spatial and temporal resolution than PET and allows the characterization of brain activities related to precise cognitive challenges.

The first experiment assessed the brain responses to an auditory attentional task before and after exposure to a 21min bright white light (>7000lux). Light-induced improvement in subjective alertness was linearly related to an increased responsiveness in the thalamus. In addition, light enhanced responses in a set of cortical areas involved in the task, preventing decreases of activity otherwise observed during continuous darkness. Importantly, the increases in responses declined within minutes after the end of the light stimulus, following various regionally-specific dynamics. These first findings suggest that light can modulate the activity of subcortical structures involved in

alertness, thereby dynamically promoting cortical activity in networks involved in ongoing nonvisual cognitive processes.

The second investigation showed that while participants perform an auditory working memory task, a short (18min) exposure to blue (470nm) or green (550nm) monochromatic light ($3x10^{13}$ph/cm^2/s) differentially modulates regional brain responses. Blue light typically enhanced brain responses or, at least, prevented the decline otherwise observed during green light exposure in frontal and parietal cortices implicated in working memory, and in the thalamus. These results imply that monochromatic light can affect cognitive functions almost instantaneously and suggest that these effects are mediated by a melanopsin-based photoreceptor system.

In the third experimentation, subjects were exposed to repeated very short (50s) monochromatic violet (430nm), blue (473nm), and green (527nm) lights of equal photon flux (10^{13}ph/cm^2/s) while they were performing an auditory working memory task. Brain responses were characterized at light onsets and during the task. Results support a prominent contribution of melanopsin RGC to nonvisual brain responses within the very first seconds of a light exposure in brain areas involved in arousal regulation and in the task. Results suggest the implication of the brainstem and of the thalamus in establishing nonvisual responses to light.

Overall, results show that daytime light exposure is effective in modulating nonvisual brain activity related to two complex cognitive functions. Light act swiftly in a region specific and wavelength specific manner. Wavelength sensitivities demonstrate that non-classical photoreception using melanopsin is involved. A few seconds of light are sufficient to induce significant changes in subcortical structures probably mediating the establishment of the nonvisual response observed at other physiological levels and in behavior. These results and multiplicity of brain areas involved speak for a broad involvement of light in the regulation of human brain function. These findings support the use of light exposure as a countermeasure to daytime sleepiness and in treating several circadian and psychiatric disorders. Light intensity and its spectral quality should however be taken into account when designing lighting environments of buildings or light therapy treatments. Finally, preliminary results of two other investigations suggest a role of light in emotion regulation and reveal the importance of genetic factors in mediating the nonvisual effects of light exposure.

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Abbreviations used

λ	wavelength
a.u.	arbitrary units
ANOVA	analysis of variance
ASPS	advanced sleep phase syndrome
BMI	body mass index
BOLD	blood oxygen level dependent
CBT	core body temperature
DLPFC	dorso-lateral prefrontal cortex
DMH	dorsomedial hypothalamus
DR	dorsal raphe
DSPS	delayed sleep phase syndrome
EEG	electroencephalogram
EPI	echo planar imaging
FOV	field of view
fMRI	functional magnetic resonance imaging
FWHM	full width at half maximum
GLM	general linear model
IGL	intergeniculate leaflet
IPS	intraparietal sulcus
IT	inferior temporal cortex
KSS	Karolinska Sleepiness Scale
LC	locus coeruleus
LDT	laterodorsal tegmental nuclei (of the brainstem)
LHA	lateral hypothalamus area
lm	lumen
LGN	lateral geniculate nuclei
MNI	Montreal Neuroscience Institute
MOP	medial preoptic
MFG	middle frontal gyrus

MRI	magnetic resonance imaging
MT	middle temporal area
NIF	non-image forming
OPN	olivary pretectal nuclei
PET	positron emission tomography
ph	photon
PPM	posterior probability maps
PPT	pedunculopontine tegmental nuclei (of the brainstem)
PVH	paraventricular nucleus of the hypothalamus
PVT	paraventricular nucleus of the thalamus
rCBF	regional cerebral blood flow
RF	radio frequence
RGC	retinal ganglion cells
RHT	retino-hypothalamic tract
SAD	seasonal affective disorder
SC	superior colliculus
SCN	suprachiasmatic nuclei
SD	standard deviation
SEM	standard error around the mean
SMG	supramarginal gyrus
SPM	statistical parametric mapping
SPZ	subparaventricular zone (of the hypothalamus)
T	Tesla
V1-4	visual areas 1 to 4
VLPO	ventrolateral preoptic area (of the hypothalamus)
VMH	ventromedial nucleus (of the hypothalamus)
W	Watt

1. Broad impact of light and circadian rhythms in humans

Definitions

Light

Light is an electromagnetic wave composed of energy quanta called photons (Ryer, 1998; Van de Voorst, 1997). These photons (ph) can only be absorbed or emitted in indivisible units. The energy (E) of a photon is related to its frequency (v) or wavelength (λ) through the expression $E = hv$ or $E = hc / \lambda$ (where h is Planck's constant and equals to $6.623\ 10^{-34}$ J s, and c is light speed and equals to $2.998\ 10^{8}$ m s^{-1}), such that short wavelength (or high frequency) photons have more energy than longer wavelength photons. Visible light is the part of the electromagnetic spectrum which is visible to the human eye. It lies between ultraviolet and infrared light, *i.e.* between wavelengths of ~380nm (violet) and ~780nm (red). We shall use the term light for visible light in the rest of the manuscript.

Light energy is measured in Watts (W) or in lumen (lm), which is the photometric equivalent of W weighted to match the eye response of the "standard observer" (Foster and Lucas, 1999; Ryer, 1998). *Radiance* is the power measure of luminous energy flux of a light per time unit (W s^{-1}). *Luminance* or photopic flux, expressed in lumens per second (lm s^{-1}), is weighted to match the responsiveness of the human eye, which is most sensitive to yellow/green. Scotopic flux is weighted to the sensitivity of the human eye in the dark-adapted state. *Irradiance* is a measure of radiometric flux per area unit and per time unit, or flux density. Irradiance is typically expressed in W/cm^2 or W/m^2, or in ph/cm^{-2}/s. *Illuminance* is a measure of photometric flux per area unit, or visible flux density. Illuminance is typically expressed in *lux* (lm/m^2) or foot-candles (lm per square foot).

Circadian rhythms

A *rhythm* is a non-random series of events which repeats at a specific period. Day and night alternations led to 24-h rhythmic variations in nearly all organisms. A *circadian rhythm* is a biological activity that oscillates under constant environmental conditions

with a period close to 24h. Internal mechanisms maintain the periodicity of 24h. External conditions entrain the rhythm, and changes in these conditions modify rhythmicity. A rhythm is characterized by its *amplitude*, *period*, with its reciprocal *frequency*, and *phase* or *phase angle*. The period is the time interval between two occurrences of a reference event in successive cycles. The relationship between two synchronized rhythms is expressed in terms of phase angle difference, which represents the time lag between two equivalent reference points of two cycles, such as their crests or troughs. Chronobiology is the scientific discipline interested in circadian rhythms (DeCoursey, 2004)[*].

Circadian influences in humans

The sleep/wake cycle is probably the most obvious, or visible, 24h rhythm in humans. Its rhythmicity is in part due to a circadian process. Sleep pressure increases with time awake and tends to trigger the next sleep episode, but a circadian process progressively increases throughout the day to maintain wakefulness and counteract the homeostatic influence (Daan *et al.*, 1984; Dijk *et al.*, 1992). This circadian drive is maximal at the end of the day, and then decreases steeply at night to allow sleep. Many aspects of sleep physiology such as rapid eye movement sleep, the power density of the sigma band frequency (12.75-15 Hz) of the electroencephalogram (EGG) which characterizes spindles, sleep propensity (*i.e.* the tendency to fall asleep), and wakefulness during sleep episodes, show a strong circadian rhythmicity (Dijk and Czeisler, 1995; Dijk *et al.*, 1997; Dijk and von Schantz, 2005).

Core body temperature (CBT) exhibits a strong endogenous circadian variation of about half a degree (Waterhouse and DeCoursey, 2004). CBT is maximum at the end of the day (at around 2200h), decreases to reach its minimum at the end of the night (at around 0600h), to progressively increase towards its crest (Figure 1.1). It is influenced by behavioral factors such as rest, activity and posture, but its cycle is preserved under constant conditions. Secretions of hormones also show clear circadian rhythms. The most widely used hormonal rhythm in chronobiology is without a doubt the rhythm of melatonin. Its secretion signals the duration of darkness and is closely related to sleep (Arendt, 2003; Dijk and von Schantz, 2005). Its concentration is low during the day,

[*] Circadian rhythm definitions are adapted from DeCoursey, 2004.

increases at night to reach its maximum in the middle of the night, about 2h before the trough in CBT (Figure 1.1). This pattern is preserved in constant condition protocols, reflecting its control by a circadian pacemaker. Cortisol is another hormone under strong circadian control that has received a lot of attention in chronobiology. Its crest is located at around habitual wake-up time. Thereafter cortisol concentration decreases progressively throughout the day to reach a minimum shortly after sleep onset (Waterhouse and DeCoursey, 2004). The autonomic control of heart rate is also among the physiological parameters showing circadian variations (Hu *et al.*, 2004).

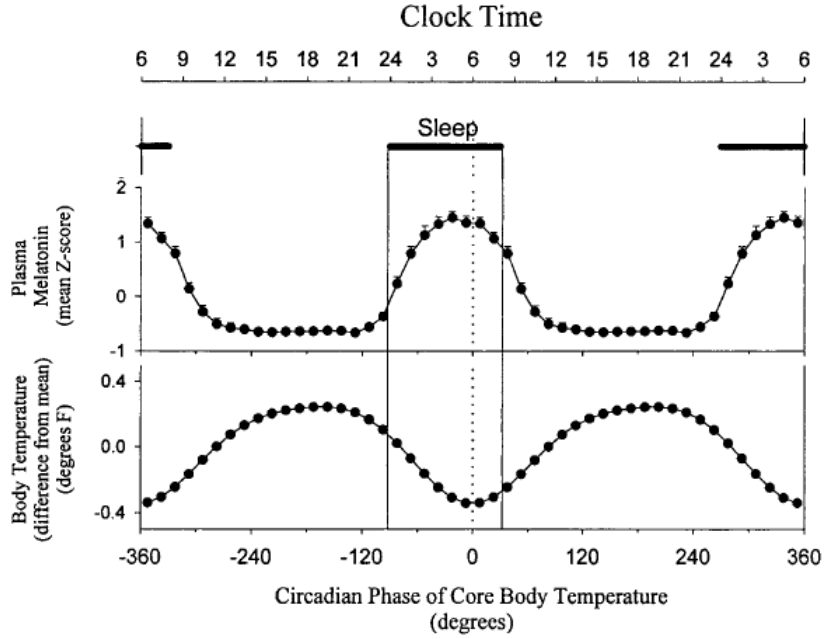

Figure 1.1: Circadian variations in melatonin secretion (***top***) and CBT (***bottom***) centered around the circadian phase of CBT (from Dijk and Lockley, 2002).

In addition to rest and activity, many aspects of behavior receive circadian influences. Subjective alertness, vigilance, attention, mood and performance are dependent on the duration of wakefulness, but they are also strongly regulated by the circadian process (Figure 1.2) (Carrier and Monk, 2000; Dijk *et al.*, 1992; Monk *et al.*, 1997). Subjective alertness and performance levels remain fairly stable throughout the day and progressively decrease if wakefulness is extended into night time. Some recovery of alertness and performance is however observed after CBT minimum, to reach a level intermediate between previous-day and end-of-the-night values, reflecting the influence of a circadian process (Dijk *et al.*, 1992). When the homeostatic influence is removed, subjective alertness, mood and performance variations parallel fairly well

CBT circadian rhythm, *i.e.* variables show maxima at the end of the day and minima at the end of the night (Carrier and Monk, 2000). Circadian variations have been observed in numerous cognitive processes including working memory, reasoning speed, hand dexterity, 2 digit sums, word pair recall, verbal reasoning, serial search, implicit sequence learning, and simple reaction times (Cajochen *et al.*, 2004; Carrier and Monk, 2000; Dijk *et al.*, 1992; Monk *et al.*, 1997; van Eekelen and Kerkhof, 2003; Wyatt *et al.*, 1999). In addition to measures made using responses to tasks, circadian changes in alertness, attention and performance are reflected in variations in waking EEG, mainly in high frequencies (theta or 4.5-to-8Hz band, alpha or 8-to-12Hz band, and beta or 20-to-32Hz band) (Cajochen *et al.*, 2002).

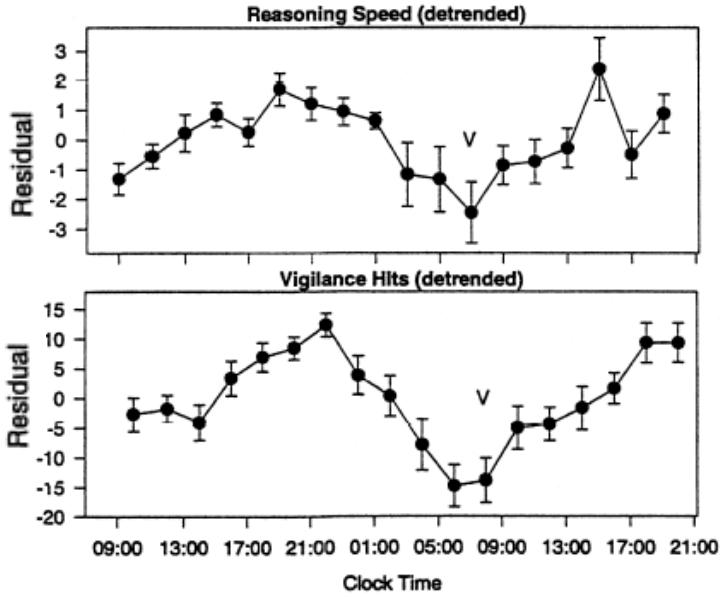

Figure 1.2: Circadian variations in performance (***top***) and vigilance measures (***bottom***) (from Carrier and Monk, 2000). ***V:*** circadian nadir.

Light is the primary synchronizer of human circadian rhythms

Because circadian influences are present in multiple aspects of human physiology and behavior, there is a great interest in understanding the generation and regulation of the circadian system. In the early days of chronobiology, humans were seen as mainly synchronized by social factors. Light was not considered as a major *Zeitgeber* ("time giver"), as it is was widely accepted to be so in animals (Aschoff *et al.*, 1971; Waterhouse and DeCoursey, 2004). This view changed in the 1980's. Bright white light exposure (2500 lux) was shown to be able to suppress the nocturnal rise in melatonin

secretion in humans (Lewy *et al.*, 1980). Subsequent experiments demonstrated that night time bright light exposure (10000 lux) changed the phase of the circadian variations in cortisol and CBT levels independent of sleep timing (Figure 1.3a) (Czeisler *et al.*, 1986). These discoveries were the starting point for numerous studies which confirmed those early results and further unraveled the characterization of light effects on human circadian rhythms.

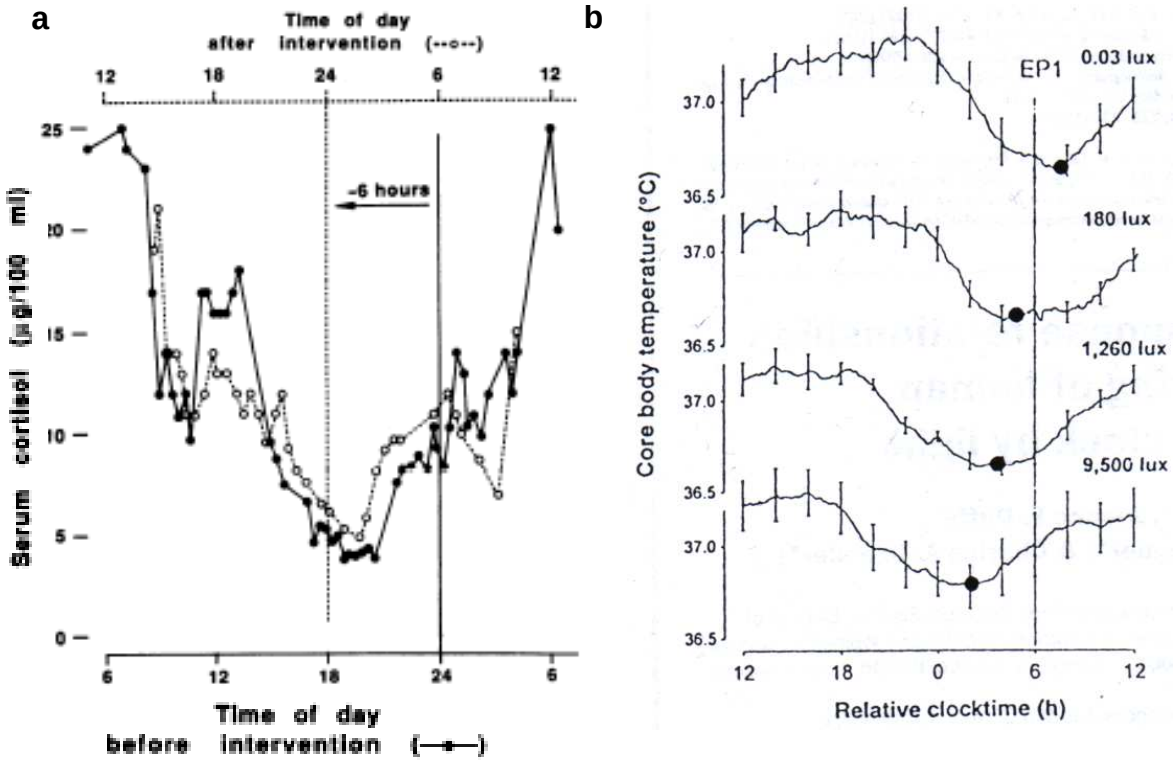

Figure 1.3: Shifts in circadian phase induced by bright light exposures. ***a.*** Cortisol secretion before (black circles – solid line) and after (open circle – dashed line) seven days of evening bright white light exposure (> 7000 lux) (from Czeisler *et al.*, 1986). ***b.*** CBT variations after 3 days of morning light exposure of different intensities. *Horizontal line:* circadian phase measured before light treatment (from Boivin and Czeisler, 1996).

All circadian rhythms have a period slightly different from 24h meaning that readjustment is required to maintain their day and night patterns in phase with environmental days and nights. It continues to be debated whether it is the phase or the period of a circadian rhythm that is altered to assure entrainment (Beersma *et al.*, 1999). Although this issue is difficult to test, the most accepted view is that phase is modified

by exogenous factors and especially by light. Several phase response curves to light exposure established the relationship between the timing of a single or repeated bright light exposures and their effects on circadian phase (Figure 1.4). It clearly defined a phase advancing part of the curve, if light exposure occurred within the hours following CBT minimum, and a phase delaying portion, if light exposure occurred in the hours preceding CBT minimum (Czeisler *et al.*, 1989; Jewett *et al.*, 1991; Jewett *et al.*, 1997; Khalsa *et al.*, 2003; Minors *et al.*, 1991; Van Cauter *et al.*, 1994). The CBT minimum was identified as a critical phase for circadian entrainment: circadian amplitude was abolished if repeated night time bright light exposures were centered around CBT minimum, leading to large phase shifts of unpredictable directions (Czeisler *et al.*, 1989; Jewett *et al.*, 1991).

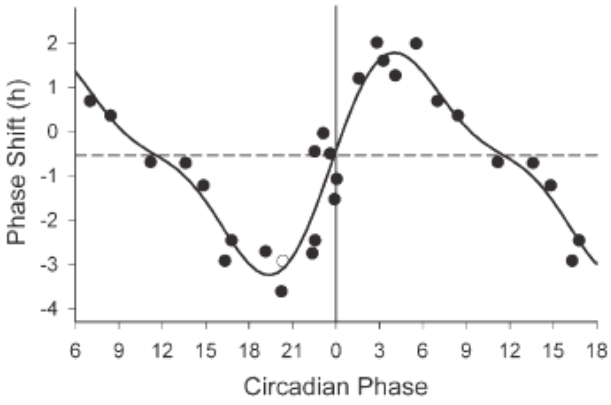

Figure 1.4: Phase response curve to a single 6.7h bright light exposure (10000lux) (from Khalsa *et al.,* 2003). Phase advances (positive values) and phase delays (negative values) are induced depending on the whether light exposure occurred before or after circadian phase in CBT.

Although bright light exposures were first thought to be necessary to influence circadian rhythms, sensitivity to indoor light levels was demonstrated subsequently (Boivin and Czeisler, 1998; Boivin *et al.*, 1996; Gronfier *et al.*, 2007; Jewett *et al.*, 1991). Furthermore, dose-response relationship between phase shifts and light intensities has been established (Figure 1.3b) (Boivin *et al.*, 1996). This dose-response relationship follows a sigmoid logarithmic function (Figure 1.5) meaning that most of the phase shifting effect occurs between 10 and 1000 lux. This sigmoid relationship also implies that a 100 lux night time light exposure induces half of the phase shifting effect elicited by a 10,000 lux light exposure of identical timing and duration (Zeitzer *et al.*,

2000; Zeitzer *et al.*, 2005). The circadian system also integrates photic information over long periods of times. Intermittent light exposures are very effective in suppressing melatonin secretion and induce almost equivalent phase shifts in the CBT rhythm as compared to continuous light (Brainard *et al.*, 1997; Rimmer *et al.*, 2000).

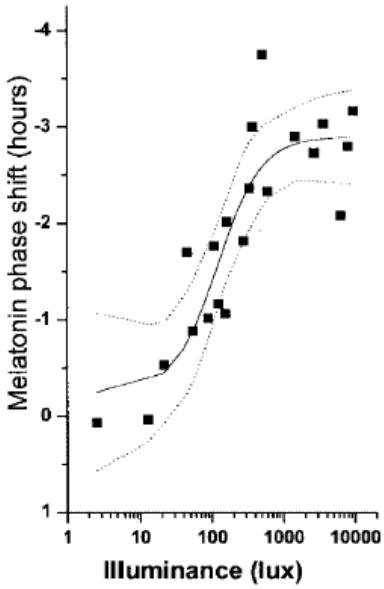

Figure 1.5: Illuminance response curve of the phase shift induced in melatonin secretion by white light exposure (logarithmic scale) (from Zeizter *et al.*, 2000).

If experimental conditions are strictly controlled, a candle light level of 1.5 lux during the day is sufficient to maintain circadian rhythms in phase with a 24h day and night cycle, but not if this cycle is 23.5h or 24.6h long (Wright *et al.*, 2001). In addition to establishing the limits of light entrainment of circadian rhythms, these latter data further demonstrated that, despite inter-individual variations, the intrinsic circadian period in human was close to 24h. Although, in animals, it was widely accepted that the circadian period was close to 24h, the human period was harder to establish and was first estimated to be closer to 25h (Aschoff *et al.*, 1971). Errors in period measures were mainly due to the underestimation of the effects light. Indeed, in well controlled experimental conditions, where influences of external synchronizers are removed, especially those of light, human circadian rhythms oscillate with a stable period slightly longer that 24h (~24.2h) (Czeisler *et al.*, 1999). This period is similar for several endogenous circadian markers (CBT, melatonin, and cortisol levels) and light exposure induces very similar phase shift across these markers (Czeisler *et al.*, 1999; Shanahan and Czeisler, 1991). A single clock must therefore govern all these circadian rhythms. Overwhelming evidence indicates that the master circadian clock is located in mammals

in the suprachiasmatic nuclei (SCN) of the hypothalamus (Reppert and Weaver, 2002) (see Chapter 2).

Light exposure is responsible for inter-individual differences in sleep-wake cycle rhythm

Changes in the interaction between homeostatic and circadian regulations occur with age. Aging is associated with a decrease in sleep continuity, in the amount of slow wave sleep, and in performance, and with a greater sensitivity to the circadian pacemaker reflected notably in a reduced ability to sleep during the day after sleep deprivation (Buysse *et al.*, 2005; Gaudreau *et al.*, 2001). The amplitudes of circadian rhythms of several parameters such as sleep propensity or subjective sleepiness are also reduced with age. The mechanisms responsible for these changes are however unclear. Middle-aged (40-60 year old) and elderly (>60 year old) people have an advanced circadian phase compared to younger individuals. However, this advance does not appear to be related to a change in circadian period, which is stable between age groups (Czeisler *et al.*, 1999), nor to differences in daily light exposure (Kawinska *et al.*, 2005). A recent report suggests that advances in circadian phase could be the result of a decreased ability of older people to delay their circadian rhythms in response to normal room light intensities (Duffy *et al.*, 2007). This can be related to the reduction in light transmission through the lens and in pupil size that occurs with age (Brainard *et al.*, 1997; Charman, 2003). The reduction in transmission occurs especially for the shorter wavelengths of the visible spectrum.

The advance in circadian phase with age is associated with an evolution toward morningness, or morning chronotype (Duffy *et al.*, 2001). Even though all human beings have a circadian period close to 24h, they do not all prefer to live at the same time of the day. This diurnal preference is referred to as *chronotype* (Horne and Ostberg, 1976). Evening individuals prefer go to sleep later and wake up later, whereas morning types prefer early schedules. Extreme chronotypes, getting up and going to bed very early or very late, have interested scientists because they were thought to represent extremes in the relationships between endogenous circadian rhythms and activity. The mechanism underlying the intrinsic differences in diurnal preference remains however to be established. It appears that, in some individuals, extreme chronotype is determined

through a different phase angle between the circadian drive and the sleep/wake cycle, while in others, (homeostatic) differences in the accumulation and dissipation of sleep pressure would be involved (Duffy *et al.*, 1999; Duffy *et al.*, 2001; Mongrain *et al.*, 2005; Mongrain *et al.*, 2006; Taillard *et al.*, 2003). Similarly, for some extreme chronotypes, daily light exposure seems to be partly responsible for the phase advance tendency present in morning types and the phase delay tendency in evening types (Goulet *et al.*, 2007). However, for many other extreme chronotype individuals, differences in light exposure do not appear to explain variations in time of day preferences. Interestingly, a recent study revealed that chronotype changed with latitude, suggesting a influence of daylight on general diurnal preference (Roenneberg *et al.*, 2007).

Non-photic synchronizers of the human clock

As for animals, light is the main synchronizer of human circadian rhythms. For example, in controlled conditions, inverting the rest-activity cycle and modifying the timing of food intake induces weak phase shifts, whereas, if bright white light is administered, individuals adapt quickly to the new imposed rest-activity cycle (Duffy *et al.*, 1996). Behavior is nevertheless important for circadian regulation as it can determine when light can reach the central nervous system, *e.g.* when we sleep, we close our eyes (Dijk & Lockley). Other data clearly show that light is not the only *Zeitgeber* and that other non-photic factors have impacts on circadian regulation in normal sighted and blind individuals (Klerman *et al.*, 1998; Mistlberger and Skene, 2005). Melatonin administration is able to entrain circadian rhythms in blind individuals (Lockley *et al.*, 2000; Sack *et al.*, 2000). Physical exercise during the habitual rest period alters endogenous circadian rhythms both in humans and rodents (Buxton *et al.*, 1997b; Buxton *et al.*, 1997a; Mrosovsky, 1991). Meals and social interactions are also able to do so (Mistlberger and Skene, 2005). Finally, exogenous melatonin administration can affect circadian regulation of its own secretion, but also of cortisol, sleep, and heart rate (Cajochen *et al.*, 1998; Rajaratnam *et al.*, 2003; Rajaratnam *et al.*, 2004; Vandewalle *et al.*, 2007a).

Acute nonvisual effects of light irradiance in humans

Several studies were interested in the illumination episodes or the periods immediately following them and demonstrated acute effects of light on nonvisual or non-image forming (NIF) functions. The suppression of melatonin secretion was the first demonstration of an acute nonvisual response to light (Lewy *et al.*, 1980) and has been replicated repeatedly (Figure 1.6b) (Brainard *et al.*, 1997; Cajochen *et al.*, 2000; Hebert *et al.*, 2002; Lewy *et al.*, 1980; Zeitzer *et al.*, 2005). Evening and night time bright light exposures acutely affect CBT and prevent its night time decrease for several hours after the exposure (Figure 1.6c) (Badia *et al.*, 1991; Cajochen *et al.*, 1992; Cajochen *et al.*, 2000; Dijk *et al.*, 1991). Although general sleep architecture is not modified, the power of lower frequencies of slow wave sleep and their dynamics are altered by a preceding evening light exposure (Cajochen *et al.*, 1992; Dijk *et al.*, 1991). Sleep latency at night is also prolonged by evening light exposure (Cajochen *et al.*, 1992; Carrier and Dumont, 1995; Dijk *et al.*, 1991), whereas it is reduced by a morning illumination (Carrier and Dumont, 1995). Waking EEG correlates of alertness are strongly modified if bright light is administered during the night. Ninety minutes of exposure are sufficient to induce significant increases in power density of higher EEG frequencies, which can be maintained for an entire night if light levels are sustained (Badia *et al.*, 1991; Cajochen *et al.*, 2000). Likewise, sustained wakefulness increases while slow eye movements, which are related to sleepiness, decrease under continuous light exposure (Cajochen *et al.*, 2000; Campbell and Dawson, 1990). Heart rate is also acutely increased by light exposure (Scheer *et al.*, 2004). Similarly, behavioral measures are sensitive to light exposures. Subjective alertness (Figure 1.6a), performance and mood are improved within tens of minutes of a night time light exposure (Badia *et al.*, 1991; Cajochen *et al.*, 2000; Campbell and Dawson, 1990; Daurat *et al.*, 1993; French *et al.*, 1990). Tasks include working memory, visual search, number manipulations, logical reasoning, processing abilities, and vigilance measures (reaction times) (Badia *et al.*, 1991; Campbell and Dawson, 1990).

These acute responses could be detected within a few tens of minutes, but effects depended on light intensities and on the temporal resolution available in the different protocols. As for longer term phase shifts, dose response relationships hold between the acute effects of light and its intensity, and follow a similar logarithmic

sigmoid functions (Brainard *et al.*, 1997; Cajochen *et al.*, 2000; Zeitzer *et al.*, 2005). In addition, dose response functions relate directly to the amount of light entering the central nervous system through the eyes, at least for endocrine functions. Melatonin suppression by light exposure is twice as large when both eyes are exposed rather than one, and suppression is increased if pupil constriction is inhibited (Brainard *et al.*, 1997). The areas of the retina that are illuminated might also influence the acute responses to light irradiance, but conflicting results have emerged (Glickman *et al.*, 2003; Ruger *et al.*, 2005b).

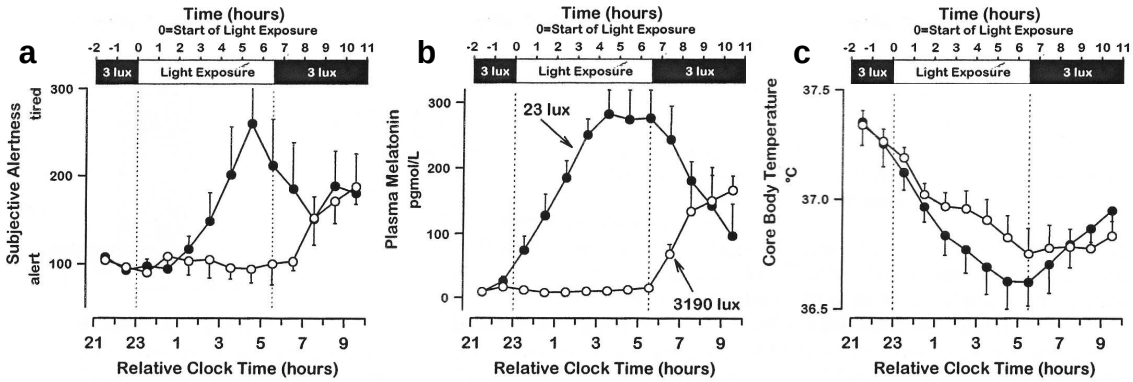

Figure 1.6: Acute effects of a 6.5h night time bright white light exposure (>3000 lux) on ***a.*** subjective sleepiness, ***b.*** melatonin secretion, and ***c.*** CBT (from Cajochen *et al.*, 2000).

Indoor light levels of one or two hundred lux can induce acute effects on circadian variables. This reduces the impact of a subsequent brighter light exposure and can lead to the conclusion that bright light does not affect CBT or performance (Daurat *et al.*, 1993). Hence short term light context influences the effect of a subsequent light exposure. For instance, greater melatonin suppression is observed after a period of darkness than after a period in dim-light for instance (Jasser *et al.*, 2006). Longer term light history is also important: if bright light is avoided for a few days, melatonin suppression by light is enhanced, reflecting some long term light adaptation of the circadian system (Hebert *et al.*, 2002).

Almost every investigation of the effects of light irradiance focused on the effects of light during the biological night. However repeated daytime bright light exposures induce phase shifts in endogenous circadian phase (Hashimoto *et al.*, 1997; Jewett *et al.*, 1997). The induced effects are small, revealing a decreased sensitivity

during the biological day. Light irradiance during the day also elicits acute responses that depend on the time of administration, and on the variables measured (Phipps-Nelson *et al.*, 2003; Ruger *et al.*, 2005a). Daytime bright light exposure does not acutely affect CBT and cortisol secretion but it induces similar effects on behavior than night time exposure. In partially sleep deprived subjects, reaction times and subjective alertness can be improved by daytime bright light exposures.

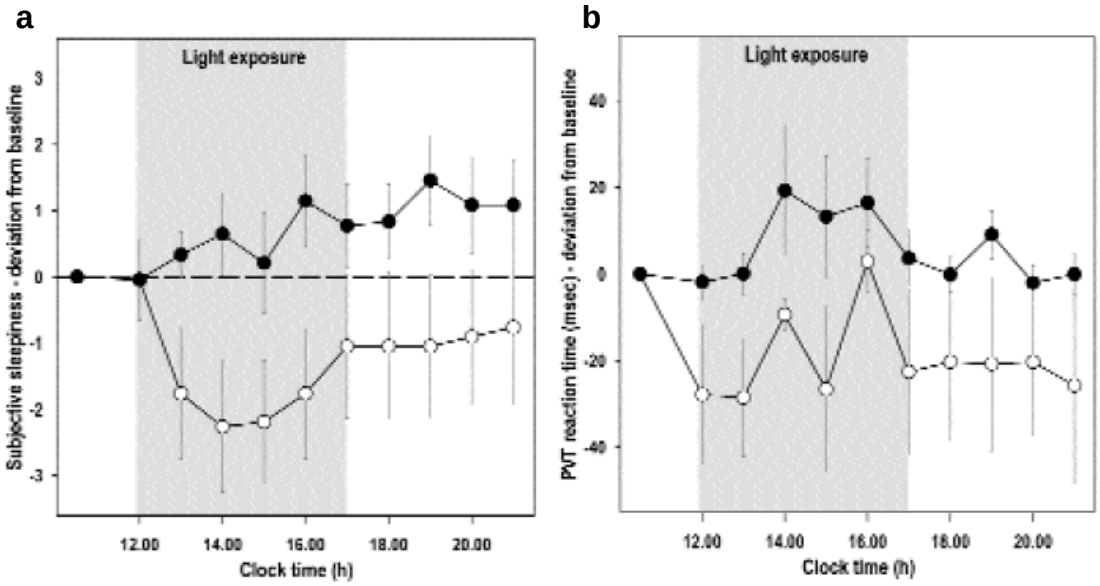

Figure 1.7: Acute effects of a 5h daytime bright white light exposure (~1000 lux) on ***a.*** subjective sleepiness, and ***b.*** reaction times (from Phipps-Nelson *et al.*, 2003).

Light and health

Light is therefore a potent external factor with a broad impact on human physiology and behavior. Its action is detectable in the longer term, but also almost immediately. Understanding light actions on the circadian system could have practical impacts on societal issues such as daytime sleepiness, night shift work, or jet-lag. Daytime sleepiness is a frequent complaint (Groeger *et al.*, 2004; Nugent *et al.*, 2001) which has important consequences not only in terms of quality of life (dissatisfaction with life and anger - see http://www.sleepfoundation.org), but also in relation to education, public health [e.g., traffic accidents (Hakkanen and Summala, 2000; Horne and Reyner, 1995; Pack *et al.*, 1995; Perez-Chada *et al.*, 2005)] and economics (Dervaux, 2005; Leger, 2000). About twenty percent of the working population works at night and is exposed to light at a time where it greatly influences circadian physiology (Dumont and Beaulieu,

2007). This results in misalignments of the biological and environmental days which is often associated with sleep disorders, fatigues and sleepiness. This is similar to the transient jet-lag symptoms experienced when traveling East- or Westwards (Eastman *et al.*, 2005). People suffering from Delayed or Advanced Sleep Phase Syndrome (DSPS and ASPS, respectively) cannot readjust their internal phase to the external world time and live shifted later or early (Wyatt, 2004). Several studies have attempted to reduce the after-effects of night work and jet-lag, or to realign ASPS and DSPS patients using appropriately timed light exposures with varied success rates (Dumont and Beaulieu, 2007; Eastman *et al.*, 2005; Revell and Eastman, 2005; Wyatt, 2004). Transposing experimental protocols to real life environments is however complicated. In addition, it seems important to consider daily pattern of light exposure as a whole to understand the causes of phase misalignment (Dumont and Beaulieu, 2007).

Abnormalities in endocrine circadian regulation are involved in depressive illnesses (Linkowski, 2003), while several psychiatric and neurological disorders are positively affected by light treatment (Wirz-Justice, 2006; Wirz-Justice *et al.*, 2004). Seasonal changes are associated with mood changes in a large portion of the population, and symptoms reach psychiatric significance in patient suffering from Seasonal Affective Disorders (SAD). Light therapy is often proposed as the treatment of choice for SAD and was reported to induce effects different from a placebo effect in several recent studies (Burgess *et al.*, 2004; Glickman *et al.*, 2006). The biological mechanisms involved in SAD are unclear, but seem to be associated with a reduced retinal light sensitivity (Hebert *et al.*, 2004) and a delayed circadian phase in winter (Lewy *et al.*, 2006). Others forms of psychiatric disorders such as bulimia, depression during pregnancy, premenstrual disorders, and bipolar depression are improved following days or weeks of light therapy (Wirz-Justice, 2006). Neuroimaging results have also identified neural correlates of depression and of recovery after light therapy and total sleep deprivation, which is also often employed to treat depression (Benedetti *et al.*, 2007). Elderly people could also benefit from appropriate light exposure. Dementia symptoms appear to be reduced by light therapy, or by increasing ambient light intensity in institutions where patients are often living under very low lighting environments (Van Someren *et al.*, 1997; Wirz-Justice, 2006). Almost all the

mechanisms involved in the beneficial action of light exposure on health factors remains however to be established.

2. Photoreception system, genes and neuroanatomy of nonvisual responses to light

This chapter will first briefly summarize the complexity of the processing of visual information and place our work in context of vision research. This will allow an appreciation of the novel aspects of the chronobiological approaches that will be presented in the other sections of the chapter.

The retina

After passing through the pupil, the lens, and eye humors, light reaches the retina, where the first stages of light processing take place. The retina is a 200μm-thick tissue containing 6 main classes of cells (Figure 2.1, *top right*) (Wassle, 2004). The rod and cone photoreceptors are photon catchers transducing light quanta of all wavelengths into the same electrical outputs (Gegenfurtner, 2003). However, the likelihood of catching a photon of a given wavelength varies for each photoreceptor, rendering them most sensitive to different parts of visible spectrum. This change in spectral sensitivity is the result of the different photopigments present in each photoreceptor type (rhodopsin in rods, and 3 types of iodopsin in the 3 types of cones) (Burns and Lamb, 2003). Rods are abundant (~20 rods for 1 cone) and respond to only a few photons. They are most sensitive to 505nm wavelength and responsible for low light level achromatic vision (*scotopic* vision) (Reeves, 2003). Three types of cones exist in primates, whereas only two are present in other mammals (Wassle, 2004). S-cones are most sensitive to ~430nm radiations (violet), whereas M- and L-cones present a higher sensitivity at respectively ~530nm (green) and ~560nm (yellowgreen) (Solomon and Lennie, 2007). Processing of signals from these three cone types enables high light intensity color vision (*photopic* vision). S-cones (5 to 10% of all cones) have a relatively uniform low retinal distribution (Solomon and Lennie, 2007), M- and L-cones are denser around the fovea, while rod concentration is highest towards the periphery (Wassle, 2004). Photoreceptor outputs are transmitted to the horizontal and bipolar cells, which pass signals onto dendrites of amacrine and retinal ganglion cells (RGC) (Wassle, 2004).

Ganglion cells collect amacrine and bipolar cell signals and transmit them to the brain. The first light information processing is achieved through interactions between these retinal neurons along distinct channels.

Environmental irradiance varies over at least 10 log units (Wassle 2004). However, only a narrow range of intensities can be coded by the nervous system at a given time. Light and dark adaptations are the processes by which the visual system optimizes its sensitivity with respect to external light intensities (Reeves, 2003). Part of the adaptation is achieved by pupil constriction or dilation, but pupil size varies from 2 to 8mm and is therefore only responsible for part of the adaptation process. Adaptation is also realized by switching between scotopic and photopic vision. This process is accompanied by changes in spectral sensitivity between the maximum of the scotopic (505nm) and photopic (555nm) systems. Modifications at the photoreceptor level add to the adaptation possibilities (Burns and Lamb, 2003). Adaptation dynamics depend on the initial and final light levels, but complete dark adaptation can take up to 45min (Reeves, 2003).

Parallel processing for vision

Although interactions between pathways are numerous and complex, vision is generally seen as series of parallel processes leading to conscious perception (Gegenfurtner, 2003).

Retinal circuitry

The retina is one of the most complex parts of the nervous system and is far from being completely understood. Horizontal cells realize lateral contacts between photoreceptors. They appear to sum light signals from several photoreceptors so that light response of neighboring photoreceptors is reduced, in order enhance edges detection and reduce responses to uniform surfaces (Wassle, 2004). RGC are either connected to bipolar cells sensitive to increase (ON channels), or decrease in light inputs (OFF channels), so that bright sites and shadows can be differentiated. M- and L-cones signals can be added (M+L channels) or subtracted (M-L channels) to extract luminance information and green-red composition of a stimulus, respectively. Longer wavelength cones outputs is also summed and then subtracted from S-cones outputs [S-(M+L) channels] to characterize blue and yellow traits (Dacey and Packer, 2003).

RGC can have very large or narrow receptive fields and can receive inputs from single or multiple bipolar cells (Dacey and Packer, 2003; Dacey *et al.*, 2003). ON centre/OFF surround and OFF centre/ON surround RGC respond in opposite ways to changes in the centre and surround of their receptive fields. Similarly to horizontal cells, amacrine cells are involved in these center-surround interactions by sending lateral inhibitory inputs at the RGC level. Opposite responses to color changes are also processed in certain types of RGC, *e.g.* red ON/green OFF, red OFF/green ON, blue ON/yellow OFF, blue OFF/yellow ON. Other features such as motion and contrast are also coded at the RGC level (Wassle, 2004). There are 10 to 15 distinct types of RGC, and information contained in a light spot is funneled into as many parallel channels, which feed into different parts of the retino-geniculo-cortical pathway (Figure 2.1) (Dacey *et al.*, 2003; Wassle, 2004).

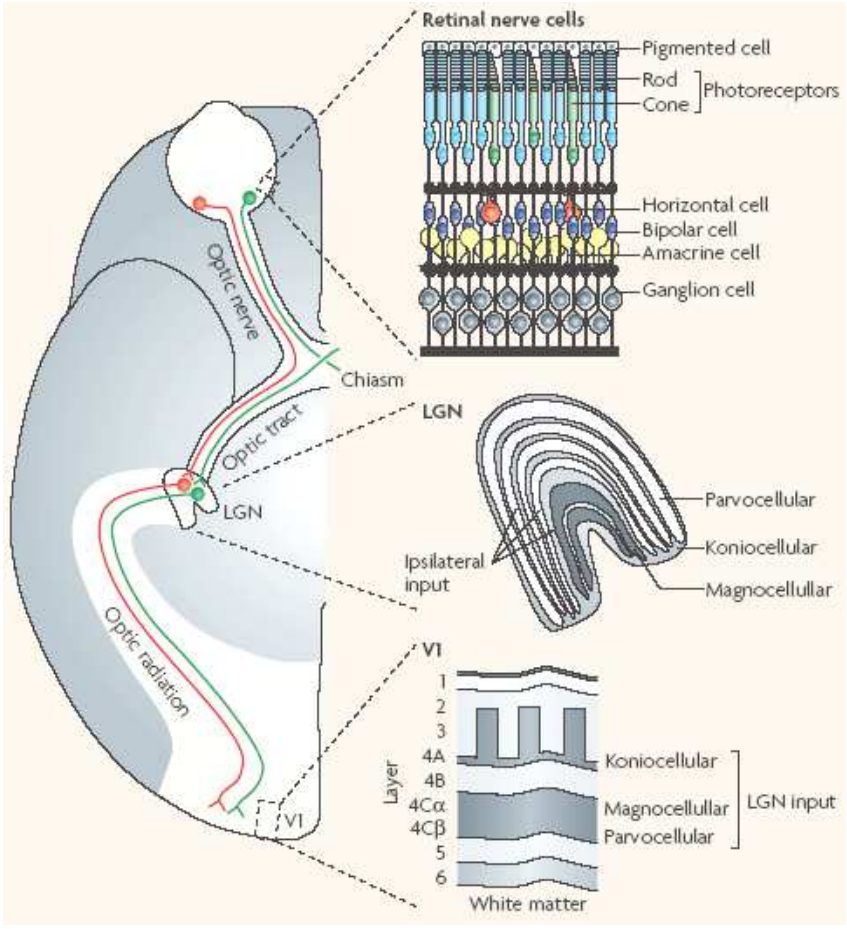

Figure 2.1: ***Left panel:*** Schematic representation of the cerebral pathways linking the retina to the early visual cortex (V1) through the lateral geniculate nuclei (LGN). ***Right panels*****:** scheme of the principal steps with the retina (*top*), the LGN (*middle*), and V1 (*bottom*) (from Solomon and Lennie, 2007).

Thalamic lateral geniculate nuclei

Almost all visual information reaching the occipital cortex is relayed by the thalamic lateral geniculate nuclei (LGN). The LGN is composed of 6 layers and 3 neuron types (Figure 2.1, *middle right*) (Kaplan, 2003). Layer 1, 4, and 6 receive input from the contra-lateral eye. Each layer is composed of P-cells (~80%), of M-cells (~10%), and of K-cells (~10%). M-cells are mostly processing luminance information (L+M channel). P-cells treat red-green information and have a narrower spectral-band sensitivity. They are involved in color discrimination and spatial vision. K-cells were only recently discovered, and deal with blue-yellow information. There are many interactions between the various neuron types in the LGN. Response dynamics differ between cell types, with K-cells showing slowest responses. Some light processing is already taking place in the LGN. For example, M-cells are involved in contrast gain and konio-cellular neurons are sensitive to motion. Light adaptation processes also take place at the LGN level.

Cortical pathways

The LGN signal is fed into the occipital cortex (Figure 2.1, *bottom right*), which is generally divided in a ventral and a dorsal streams (Ungerleider, 1995; Ungerleider and Pasternak, 2003). Both pathways process distinct features; the ventral pathway is seen as dealing with the “WHAT” of a stimulus, whereas the dorsal stream is involved in the “WHERE” of that stimulus (Figure2.2). Cortical processing starts in the striate cortex, or primary visual area (V1), which codes color, orientation, brightness and motion in different subsets of neurons. The ventral pathway primarily recruits first the visual areas 2 and 4 (V2 and V4), then inferior temporal (IT) cortex areas. The ventral stream is crucial for object recognition and codes for features such as color, brightness, orientation, contrast or dimensions of a stimulus. In higher IT areas, more complex features, such as faces, are specifically coded in segregated neuronal populations. The dorsal pathway proceeds from V1 to V2, then to V3 to reach the middle temporal area (MT), *the* motion-sensitive area. Information is sent forward to temporal and parietal areas, such as the superior temporal sulcus or the ventral intraparietal area, to reach area 7a at the top of the dorsal hierarchy. The dorsal stream is essential for appreciating spatial relationships among objects and guidance towards them. In addition to motion,

motion speed and binocular disparity, underlying depth perception, are notably processed in dorsal areas.

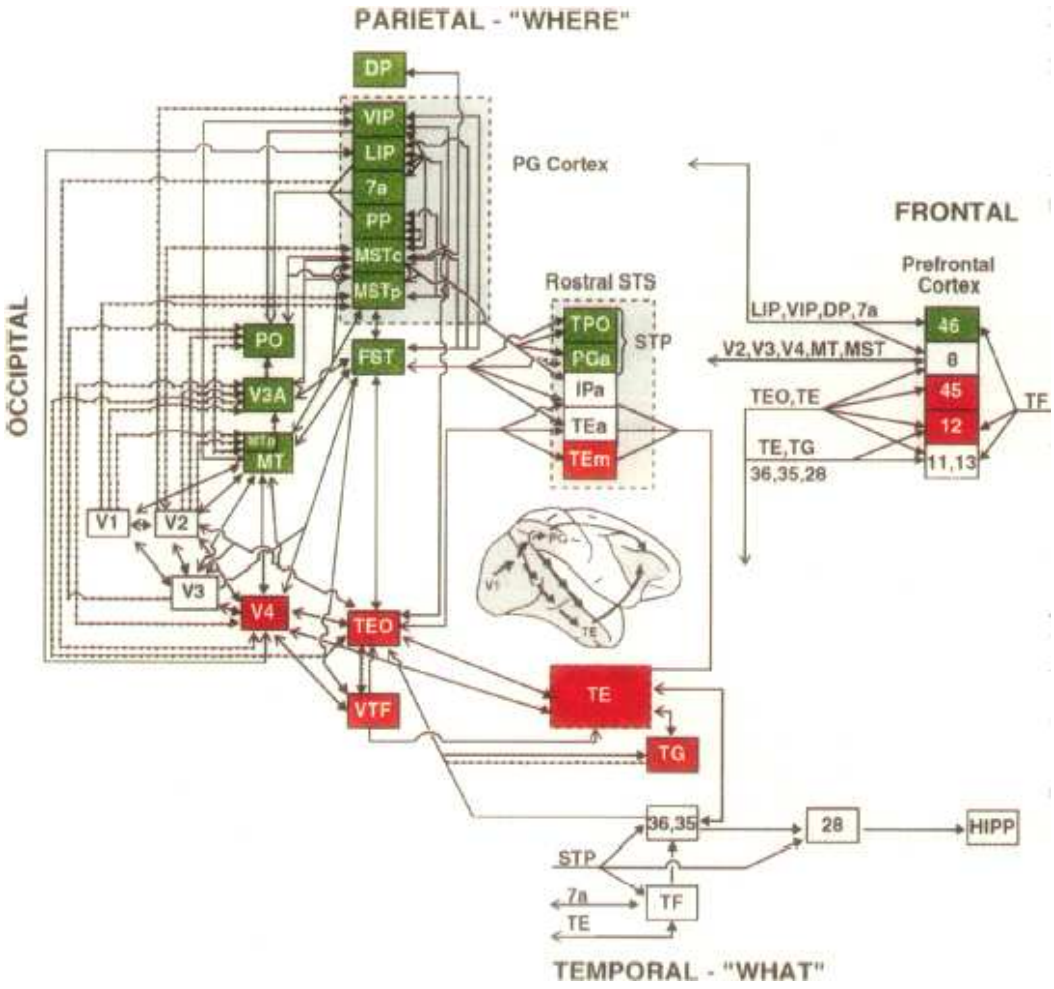

Figure 2.2: Connections between regions of the ventral (**red**) and dorsal (**green**) cortical visual streams (from Ungerleider, 1995). DP: dorsal prelunate area; FST: fundus of superior temporal area; HIPP: hippocampus; LIP: lateral intraparietal area; MSTc: medial superior temporal area: central visual field representation; MSTp: medial superior temporal area, peripheral visual field representation; MT: middle temporal area; MTp: middle temporal area, peripheral visual field representation; PG: inferior parietal area; PO: parieto-occipital area; PP: posterior parietal sulcal zone; STP: superior temporal polysensory area; STS: rostral superior temporal sulcus areas; TEO and TE: inferior temporal areas; TF: parahippocampal area; TG: temporal pole area; VIP: ventral intraparietal area; VTF: visually responsive portion of area TF; V1, primary visual cortex; V2, visual area 2; V3, visual area 3; V3A, visual area 3, part A; V4, visual area 4; 7a: inferior parietal area 7a; 8, 11, 12, 13, 45, and 46: prefrontal Brodmann areas; 28: entorhinal area; 35, 36: perirhinal areas.

Processing goes generally from simple to complex representations while passing from V1 to higher areas, with cell columns progressively integrating several aspects of a stimulus. Bidirectional connections are present between all visual areas (Ungerleider and Pasternak, 2003): feed-forward connections allow bottom-up attention orientation, whereas feedback links permit top-down attention modulation down to early visual areas (Corbetta *et al.*, 1991; Kastner *et al.*, 1999; Koida and Komatsu, 2007; Shulman *et al.*, 1999; Ungerleider and Pasternak, 2003). Both cortical streams are also strongly interconnected and are involved in the processing which occurs in the other stream (Claeys *et al.*, 2004; Seidemann *et al.*, 1999). Both streams reach the temporal lobes, including the hippocampus (Ungerleider and Pasternak, 2003) involved in long term memory (Squire and Zola-Morgan, 1991). Ventral and dorsal projections are found in the prefrontal cortex, which is important to executive functions such as working memory (Collette *et al.*, 2006). Finally the amygdala, strongly implicated in the regulation of emotions (Sterpenich *et al.*, 2006), also receives ventral stream innervations.

All areas of the ventral and dorsal pathways are strongly connected to subcortical structures such as the thalamic pulvinar and brainstem nuclei (Shipp, 2003; Ungerleider and Pasternak, 2003). Brainstem nuclei are thought to have modulatory roles on storage of information and arousal influence in information processing. The inferior pulvinar receives small retinal inputs of unknown function, but pulvinar connections mainly involve the cortex, and subcortical structures such as the superior colliculus (SC) (Casanova, 2003). The pulvinar is involved in arousal and attention regulations (Coull *et al.*, 2004; Foucher *et al.*, 2004; Shipp, 2003), but also plays pure visual roles, such as eye movement regulation and motion processing (Ungerleider and Pasternak, 2003). The connections of the pulvinar with the SC allow retinal information to reach extrastriate areas without passing through V1.

Non-classical photoreception is responsible for the nonvisual effects of light irradiance

Although the neuroanatomy of conscious vision is fairly well established, the mechanisms involved in the nonvisual responses to light are only starting to be elucidated. In humans, the first strong evidence for the involvement of non-classical

photoreception in nonvisual responses to light irradiance came from the study of blind individuals. The demonstration that light exposure can suppress melatonin secretion and shift its circadian phase in some totally blind individuals was surprising and implied that a residual photoreception function was sufficient to transmit light signals through a pathway distinct from vision (Czeisler *et al.*, 1995; Klerman *et al.*, 2002). Experiments in color blind individuals, lacking functional M- or L-cones, demonstrated identical melatonin rhythms and suppressions in response to light exposures when compared to normal individuals. This implied that not all visual photoreceptors are necessary for normal responses to light irradiance (Ruberg *et al.*, 1996). In addition, in normal subjects, shorter wavelengths around the maximum sensitivity of rods induced a greater melatonin suppression than wavelengths maximally stimulating the photopic visual system (Brainard *et al.*, 2001b). Several studies demonstrated that in rodents with degenerated retina with no functional rods and/or cones, normal or close-to-normal circadian and acute responses to light irradiance were maintained (Freedman *et al.*, 1999; Lucas *et al.*, 2001b; Lucas *et al.*, 1999).

Early studies in rodents reported an unusual dose-response relationship in the phase shift responses induced by light exposures and a decreased sensitivity to longer wavelengths which suggested the involvement of a novel photoreception system, if not, of a novel visual pigment (Takahashi *et al.*, 1984). Similarly, melatonin suppression during the night was reported to be most sensitive to monochromatic exposures of wavelengths around 459 and 464 nm (blue light) in humans (Brainard *et al.*, 2001a; Thapan *et al.*, 2001). This sensitivity does not match the classical photoreceptors of the eye. Similarly, pupillary constriction in mice lacking rods and cones was reported to be driven by a photopigment most sensitive to 479 nm wavelengths (Lucas *et al.*, 2001a). This action spectrum was recently reproduced in macaques in the absence of cone and rod inputs and in humans (Gamlin *et al.*, 2007). Night time human cone processing is also influenced by previous light exposure with a maximum sensitivity to 483 nm (Hankins and Lucas, 2002). These action spectrum data strongly suggest that a novel non-classical photoreception system was responsible for the nonvisual effects of light irradiance.

Several human studies compared the nonvisual responses elicited by monochromatic lights geared towards the classical visual system (~555nm), or geared

towards the non-classical photoreception system involved in light irradiance detection (460nm). Acute suppression and shifts (delays and advances) in circadian phase of melatonin secretion were reported to be greatest under shorter wavelength lights (Cajochen *et al.*, 2005; Lockley *et al.*, 2003; Warman *et al.*, 2003; Wright and Lack, 2001; Wright *et al.*, 2004). Limited but significant modifications in sleep architecture were also reported after blue light exposure, as compared to green light exposure and darkness (Munch *et al.*, 2006). Sleepiness, assessed objectively (by waking EEG) and subjectively, heart rate, CBT, expression of the clock gene *PER2*, and a simple reaction time task (measuring vigilance), were most affected by shorter wavelength lights in humans (Figure 2.3 and 2.4) (Cajochen *et al.*, 2006b; Cajochen *et al.*, 2005; Lockley *et al.*, 2006).

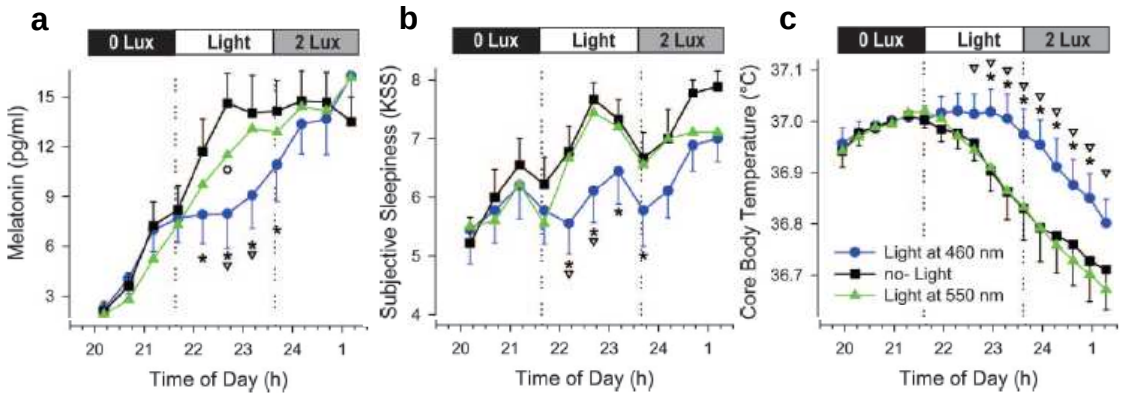

Figure 2.3: Acute responses to blue (460nm) and green (550nm) 2h evening light exposures (2.8 x 10^{13} ph/cm^2/s) (from Cajochen *et al.*, 2005). ***a.*** melatonin secretion; ***b.*** subjective sleepiness; ***c.*** CBT.

Acute effects on reaction times, subjective sleepiness, and melatonin secretion, were detected after 30 min of light exposure and lasted no more than 30 min after cessation of the light exposure. However, the temporal resolution of these studies was limited to 30 min (Figure 2.3a,b). The acute effects on heart rate and CBT had a greater latency and outlasted the light exposure for longer periods of time (Figure 2.3c). In addition, longer wavelengths elicited acute and longer term responses on melatonin secretion and affected reaction times 30 min after green light exposure was initiated (Figure 2.4) (Lockley *et al.*, 2003; Lockley *et al.*, 2006). Furthermore, cortisol was not differentially affected by the wavelength of light and skin temperature appeared to be equally modulated by shorter and longer wavelengths (Cajochen *et al.*, 2005; Lockley *et*

al., 2003; Lockley *et al.*, 2006). These results might suggest specific effects of light irradiance for different parameters of circadian physiology and behavior, and may imply different photoreceptor contributions to these specific effects. A recent study suggested a different light sensitivity for some behavioral aspects (Revell *et al.*, 2006). However, the results of the latter study are inconclusive and only included subjective sleepiness measures. At high intensities green light exposure has been shown to improve behavioral parameters (Horne *et al.*, 1991), demonstrating that, as for white light, if intensity is sufficient, all types of light can induce acute effects.

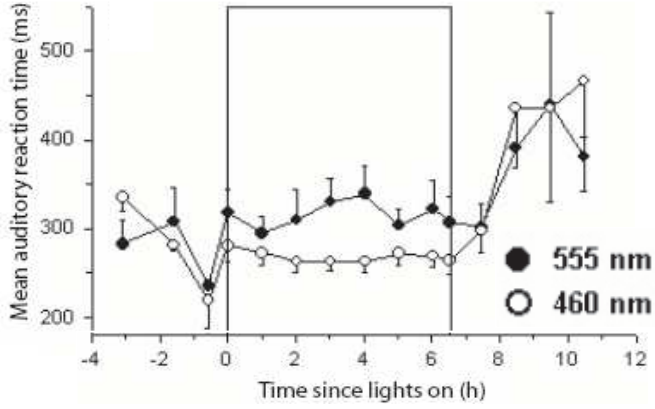

Figure 2.4: Acute effect of a blue (460nm) or green (555nm) 6.5h night time light exposures (2.8 x 10^{13} photons/cm^2/s) on reaction times (from Lockley *et al.*, 2006).

Melanopsin, a novel photopigment in a novel photoreception system driving responses to light irradiance

A novel opsin, melanopsin, was discovered, notably in the human eye, and proposed as a candidate photopigment responsible for the nonvisual responses to light (Provencio *et al.*, 1998; Provencio *et al.*, 2000). Melanopsin is expressed in a subset of intrinsically photosensitive RGC with large receptive fields, distinct morphology, and neurotransmitters, and a low and uniform distribution throughout the retina. These cells constitute the majority of the retinohypothalamic tract (RHT), which conveys information from the retina to the SCN (Berson, 2003; Berson *et al.*, 2002; Gooley *et al.*, 2001; Hannibal *et al.*, 2002; Moore *et al.*, 1995; Provencio *et al.*, 2002; Warren *et al.*, 2003). The development of mutant mice in which the gene for melanopsin, or the genes for melanopsin and rods and cones, were deleted, revealed that melanopsin was

indeed involved in nonvisual responses to light, such as *c-fos* gene induction in the SCN, pupillary constriction, and shifts in locomotor activity circadian rhythm (Lucas *et al.*, 2003; Panda *et al.*, 2003; Panda *et al.*, 2002; Ruby *et al.*, 2002; Semo *et al.*, 2003). In addition, in melanopsin knock-out mutant mice, the RGC which normally express melanopsin and are sensitive to light become insensitive to light (Ruby *et al.*, 2002).

However, debates continued on whether melanopsin acted as a photopigment or as a photoisomerase (enzyme) (Foster and Bellingham, 2002), and other molecules were proposed as the photopigments mediating responses to light irradiance. For instance, chryptochromes, members of plant blue light receptors, and circadian photoreceptor in *Drosophilia*, were shown to be necessary for normal responses to light irradiance in several studies on rodents (Miyamoto and Sancar, 1998; Selby *et al.*, 2000; Thompson *et al.*, 2001; Thresher *et al.*, 1998; Van Gelder *et al.*, 2003a). Simultaneous studies reported that the expression of melanopsin in non-photosensitive cells rendered them photosensitive with a maximum sensitivity around 480nm in 2 studies (Panda *et al.*, 2005; Qiu *et al.*, 2005) and closer to 420nm in another one (Melyan *et al.*, 2005). This definitively established melanopsin as a novel type of photopigment recruited by the photoreception system involved in the transmission of irradiance light information (Figure 2.6). These studies also demonstrated that melanopsin was both a photopigment and a photoisomerase capable of regenerating itself. Cryptochromes for their part are essential molecules in the machinery regulating circadian rhythms in mammals, but not in nonvisual photoreception (Griffin *et al.*, 1999; Lucas and Foster, 1999; Reppert and Weaver, 2002; van der Horst *et al.*, 1999).

Although nonvisual responses to light irradiance, such as phase shifts in locomotor activity and pupillary constriction, were reduced in the absence of melanopsin in rodent mutants, they were not completely absent (Figure 2.7) (Lucas *et al.*, 2003; Panda *et al.*, 2002; Ruby *et al.*, 2002; Semo *et al.*, 2003). Only if melanopsin, cone, and rod gene expressions were prevented, were all responses to light irradiance lost (Hattar *et al.*, 2003). These results revealed that melanopsin is not the sole photopigment involved in the generation of the nonvisual responses to light. In rodents, response sensitivity of SCN neurons to flashes of scotopic or photopic light levels were shown to be compatible with classical photoreceptor inputs to the circadian master clock (Aggelopoulos and Meissl, 2000), and transgenic ablation of rods altered

circadian rhythmicity (Lupi *et al.*, 1999). Other rodent studies revealed that the RHT did not only consist of melanopsin expressing RGC axons (Gooley *et al.*, 2003; Hattar *et al.*, 2006; Sollars *et al.*, 2003). This revealed that other RGC, receiving inputs from rods and cones, project to the SCN and influence circadian regulation.

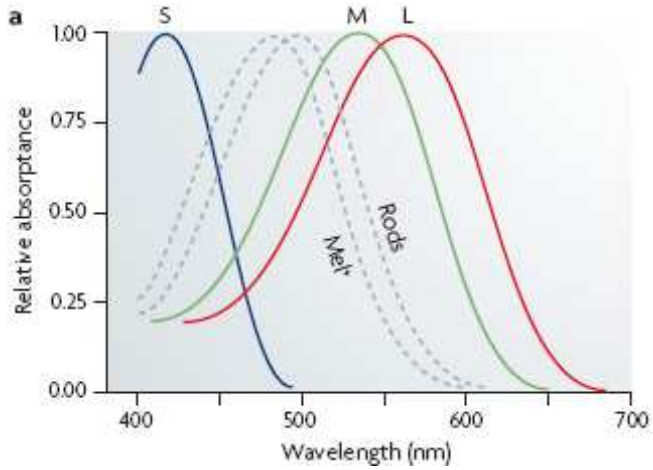

Figure 2.6: Schematic wavelength sensitivity of *S*- (blue), *M*- (green), and *L*-cones (red), and of rods (right dashed curve) and melanopsin expressing RGC (left dashed curve – *Mel*$^{+}$) (from Solomon and Lennie, 2007).

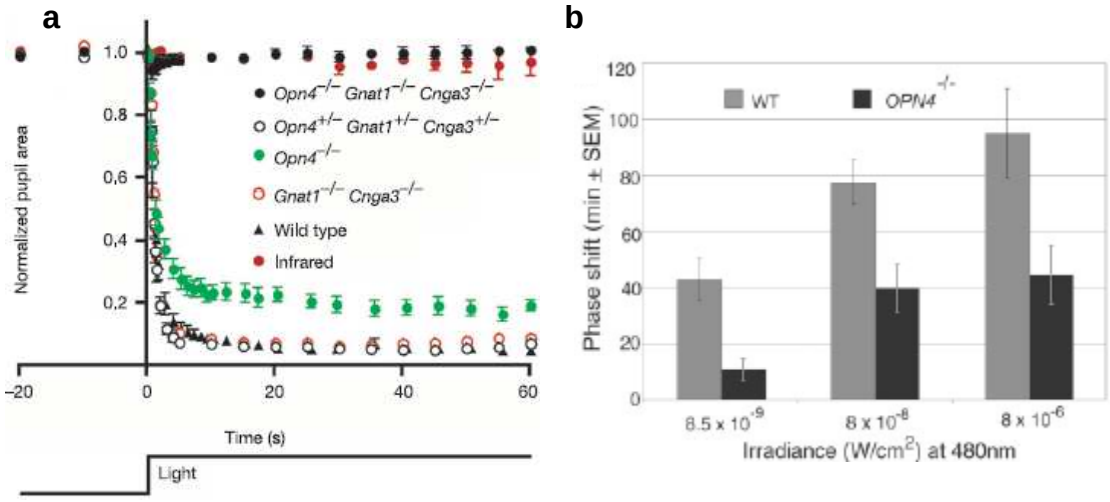

Figure 2.7: Melanopsin involvement in nonvisual responses to light exposure.
a. pupillary constriction in wild type and mutant mice with nonfunctional melanopsin (*Opn4* $^{-/-}$), rods (*Gnat1* $^{-/-}$), and/or cones (*Cnga3* $^{-/-}$) (from Hattar *et al.,* 2003).
b. Phase shifts in locomotor activity induced by light exposure in wild type (WT) and melanopsin knocked-out (*OPN4* $^{-/-}$) mice (from Panda *et al.*, 2002).
Note that responses are still detected in the absence of melanopsin.

Human data also suggested that the classical photoreceptors could support circadian responses to light, at least in part (Rea *et al.*, 2001; Zeitzer *et al.*, 1997). A system giving, different weights to the classical photoreceptors than in vision, was proposed to match the wavelength sensitivity of nonvisual responses to light in humans (Rea *et al.*, 2002). This proposition was later modified to account for the evidence for melanopsin involvement, but still involved S-cones contribution (Figueiro *et al.*, 2004). This contribution was later confirmed in macaques, where S-cones were shown to trigger inhibitory OFF responses in melanopsin expressing RGC, whereas M- and L-cones and rods elicited activating ON responses, demonstrating the presence of neural connections between these photoreceptors and melanopsin RGC (Dacey *et al.*, 2005). Two melanopsin expressing RGC populations presenting dendrites in distinct strata of the retina were observed in macaque and rodent retina (Dacey *et al.*, 2005; Hattar *et al.*, 2006). They seem to present the same response to S- and M+L-cones stimulations, but it is unknown whether they differ at other levels. Further, cone signals were recently shown to contribute to pupillary constriction in macaques (Gamlin *et al.*, 2007). Rods were also shown to interact with melanopsin expressing RGC (Doyle *et al.*, 2006). Modeling of M-cones contribution to nonvisual responses to light, through genetic ablation of these photoreceptors in rodents, showed that they were implicated in nonvisual responses to light and that their contribution dynamically varied across the course of the first 15 min of light exposure (Dkhissi-Benyahya *et al.*, 2007). In addition, integration of photic information arising from flashes of light in rodents was shown to be very different from the integration observed after prolonged light stimulations (Vidal and Morin, 2007). This further suggested that the relative contributions of the different retinal photoreceptors changed in the course of an illumination, especially early in the exposure.

Response dynamics to light stimuli also differs at the photoreceptor level. Rod and cone responses to light are typically time-locked to the stimuli, *i.e.* neural inputs start or cease a few milliseconds after light is turned on or off. In addition, rapid attenuation of rod and cone firing occurs in response to a constant stimulus (Dacey et al., 2005). When cones are inhibited, intrinsic light response of the melanopsin expressing RGC cells is sluggish and does not attenuate: firing is detected seconds after initiation of the stimulus and is maintained for minutes after the exposure,

demonstrating that these cells can account for the long integration time of the nonvisual system (Figure 2.8c) (Berson et al., 2002; Dacey et al., 2005). However, inputs from rods and cones enable melanopsin to respond quickly to light, within a few ms, similarly to rods and cones (Figure 2.8a,b) (Dacey *et al.*, 2005; Wong *et al.*, 2007). Differences in response dynamics are also detected when investigating adaptation to ambient light levels in the classical and non-classical photoreceptors: adaptation is achieved in tens of minutes in the visual system (Reeves, 2003), whereas it takes place on a time scale of hours in melanopsin expressing RGC (Wong *et al.*, 2005).

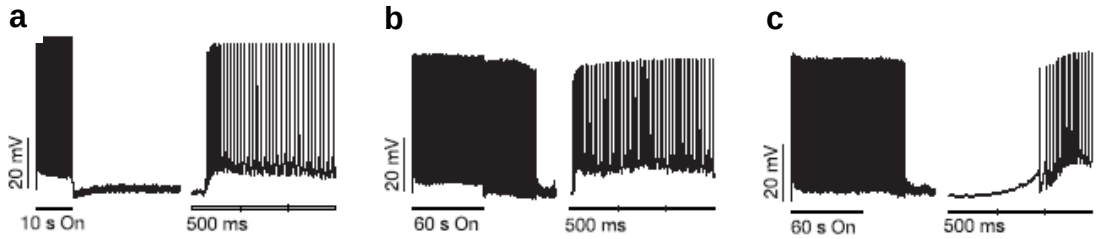

Figure 2.8: Intrinsic rod and cone mediated responses to light exposure in melanopsin expressing RGC (from Dacey *et al.*, 2005). ***a.*** rod mediated response to very low light level exposure (2 x 10^8 ph/cm^2/s); ***b.*** cone mediated response to light (5 x 10^{13} ph/cm^2/s); ***c.*** intrinsic response of melanopsin expressing RGC cell after blockade of cone inputs.

A model was proposed in which at low light levels rods inhibited melanopsin RGC responses, to reflect the higher light level required to induce nonvisual responses (Rea *et al.*, 2005). At high intensities this inhibition would be removed and contributions from cones could further induce response in melanopsin RGC. This model is difficult to verify in humans, but illustrates the complexity of interactions between retinal photoreceptors, and of the contribution of classical photoreceptors to nonvisual response to light. It also emphasized a possible prominent role of S-cones, which, as suggested in macaques (Dacey *et al.*, 2005), could greatly contribute to melanopsin expressing RGC responses, at least in the early part of the illumination (later, attenuation occurs in classical photoreceptors). However the model suggests an activating role of S-cones (Rea *et al.*, 2005) while they were showed to have an inhibitory role in the RGC expressing melanopsin (Dacey *et al.*, 2005). Conversely, melanopsin RGC were shown to influence vision. Melanopsin expressing RGC project

to areas typically involved in vision in macaques, such as the LGN and olivary pretectal nuclei (OPN) (Dacey *et al.*, 2005), and modulate visual processing in rodent retina (Barnard *et al.*, 2006). Finally, a novel type of cones exclusively expressing melanopsin has been discovered, but it has not been attributed a function yet (Dkhissi-Benyahya *et al.*, 2006).

In the past 7 years or so, substantial evidences pointing to the unique characteristics of the circadian photoreception system has accumulated. Why natural selection chose blue light to mediate responses to irradiance changes remains unknown. Because blue light is predominant at dawn and dusk, authors have hypothesized that a greater sensitivity to shorter wavelengths would help synchronizing circadian rhythms to the external time (Foster, 2005). Support for this idea come from data suggesting that melatonin onset and offset of secretions are under the control of two distinct oscillator mechanisms that detect light-to-dark and dark-to-light transitions separately, and would adjust melatonin secretion according to the environmental seasonal change in day length (Wehr *et al.*, 2001).

The presence of two different photoreception systems using the same photoreceptors in different ways, implies that light levels assessed by photon density values should be used to assess the specific effects mediated by the visual and non-classical photoreception systems (Foster and Lucas, 1999). The lux scale is equated for visual sensitivity (Ryer, 1998) and does not correspond to the photon catcher behavior of photoreceptors, only eliciting responses to entire light quanta (Gegenfurtner, 2003), whereas power measures do not take into account the change in energy with the photon wavelengths.

Gene transcription/translation loops generate circadian rhythms and respond to light

Some aspects of the circadian rhythm of cortisol, such as the position of the nadir of secretion, were shown to be very similar in monozygotic, but not in dizygotic twins (Linkowski *et al.*, 1993). These results constituted early evidences suggesting that, similar to most organisms, there was a genetic control of circadian rhythms in humans.

Several genes are implicated in the generation of circadian rhythmicity in mammals, such as *Clock*, *Bmal1*, and those of the *Period* family, *Per1*, *Per2*, and *Per3*,

and *Cryptochrome* family, *Cry1* and *Cry2* (Ko and Takahashi, 2006). The products of these genes are involved in several positive and negative feedback loops regulating their own transcription in a circadian manner. Briefly, CLOCK and BMAL1 form a heterodimer that activates the transcription of the *Per* and *Cry* genes, as well as nuclear receptor genes which inhibit *Bmal1* expression. PER and CRY translocate back to the cell nucleus, where CRY inhibits transcription of *Clock* and *Bmal1* as well as the nuclear receptor genes, releasing their inhibition on *Bmal1* (Reppert and Weaver, 2002; Van Gelder *et al.*, 2003b). One cycle of this feedback loop lasts about 24h and, because clock proteins are also transcription factors for other genes, they impose a circadian expression in multiple downstream systems (Ko and Takahashi, 2006). Disruptions of the feedback loops (through genetic modifications) produce major circadian abnormalities. Novel clock genes continue to be discovered. Figure 2.9 illustrates the complexity of the recent advances in the genetics of circadian rhythms. It is however still unclear how a cellular molecular cycle generates a circadian rhythm output from the whole SCN population.

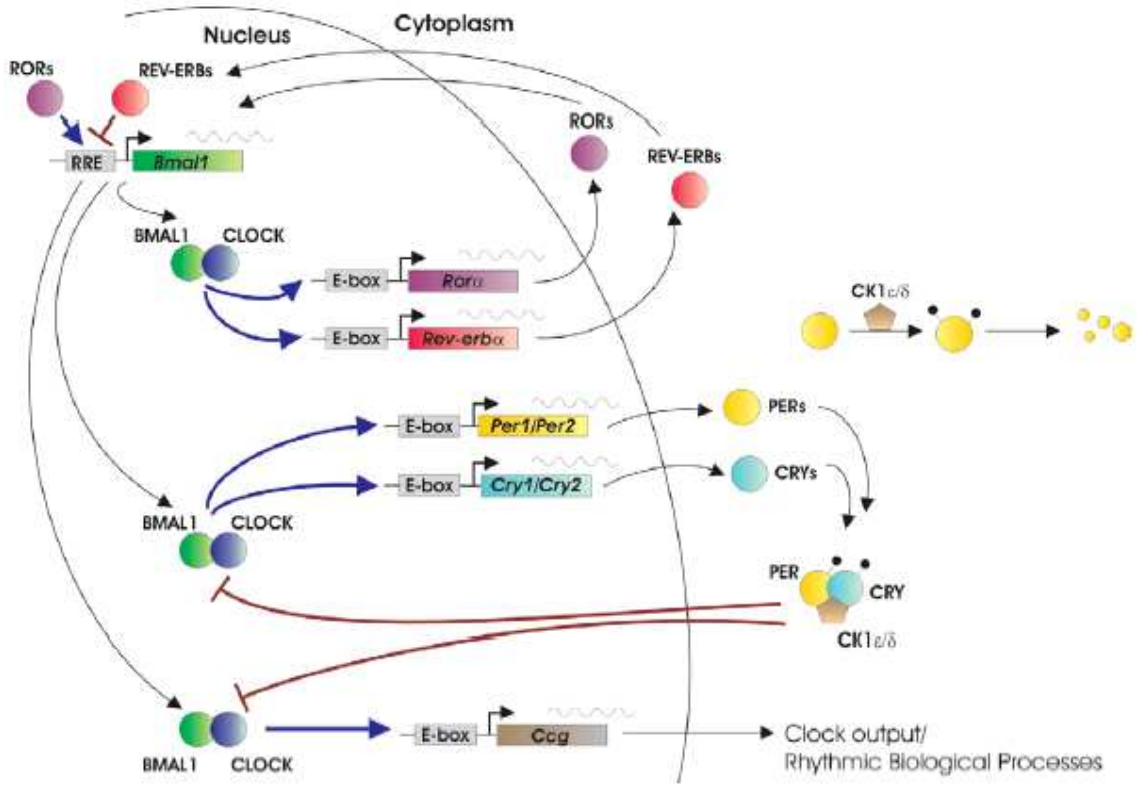

Figure 2.9: Transcriptional and translational feedback loops involved in circadian rhythms generation (from Ko and Takahashi, 2006).

Much remains to be discovered about how light exposure modifies circadian rhythms through its effect on clock genes. The sparse data show that the light irradiance signal reaches the SCN, through neurotransmitter released mostly from melanopsin expressing RGC, and affect gene expressions (Reppert and Weaver, 2002; Van Gelder *et al.*, 2003b). Light rapidly induces *Per1* expression in rodent SCN neurons at the beginning or the end of the night, whereas light induction of *Per2* is only important early in the night and undetectable later. These differences in time of day inductions by light exposure between *Per1* and *Per2* could be related to the phase response curves to light based on physiological measures (Reppert and Weaver, 2002). In humans, *PER2* light-induced expression was detected after exposure to a 2h blue monochromatic light (460nm) but not after a green (550nm) monochromatic light exposure, demonstrating that non-classical photoreception was involved in this effect (Cajochen *et al.*, 2006b). The last member of the *Period* family, *Per3*, does not seem to be directly affected by light exposure (Tarttelin *et al.*, 2003). Increases in PER1 and PER2 protein expression enhance CRY cytoplasm concentration, which seems critical for affecting circadian phase (Reppert and Weaver, 2002). Light exposure also induces expression of other genes acting outside the feedback loops, such as *c-fos* (Dkhissi-Benyahya et al., 2000).

Clock genes have received much attention for their role in circadian disorders such as ASPS and DSPS, and in diurnal preferences in humans. Positive associations have been found between extreme chronotypes and clock gene polymorphisms such as in *PER2* (Carpen *et al.*, 2005), *PER3* (Archer *et al.*, 2003; Pereira *et al.*, 2005), and *CLOCK* (Katzenberg *et al.*, 1998). However some studies do not report such associations [*PER1* (Katzenberg *et al.*, 1999); *CLOCK* (Robilliard *et al.*, 2002)]. Mutations in *PER2* and *PER3* have also been associated with ASPS (Toh *et al.*, 2001) and DSPS (Ebisawa *et al.*, 2001), respectively.

Recently, human *PER3* polymorphisms were reported to affect sleep architecture and performance after 24h of continuous wakefulness (Viola *et al.*, 2007) and to influence mood disorders (Artioli *et al.*, 2007). This adds to *PER3* implications in DSPS and extreme evening chronotype, and shows that this clock gene is an important component of the circadian molecular system.

What is the brain circuitry involved in nonvisual effects of light?

Oscillations in clock gene expressions are not only present in the SCN (Reppert and Weaver, 2002; Saper *et al.*, 2005a). Many brain areas (Abe *et al.*, 2002) and organs, such as the liver or lungs (Reppert and Weaver, 2002; Yamazaki *et al.*, 2000), show intrinsic circadian rhythms in protein and RNA levels that may control local activity. The maintenance and synchronization of all these peripheral circadian rhythmicities are mainly achieved by the SCN (Buijs and Kalsbeek, 2001; Reppert and Weaver, 2002). It seems to be the only structure capable of maintaining prolonged synchronized circadian oscillations *in vitro*, whereas the other brain areas or organs either do not cycle in isolation or are unable to maintain a synchronized rhythm for long durations (Reppert and Weaver, 2002). SCN transplantations, between hamsters with a short endogenous circadian period and hamsters with a 24h period, demonstrated that circadian rhythmicity was imposed by the transplanted SCN (Ralph *et al.*, 1990). However, not all circadian aspects are restored by this transplantation, indicating that chemical and synaptic communications to and from the SCN are necessary to regulate circadian rhythms (Buijs and Kalsbeek, 2001; Matsumoto *et al.*, 1996; Reppert and Weaver, 2002). These communications have been subjected to intense investigations, which will be briefly summarized.

Outputs from the SCN follow three major pathways (Figure 2.10) (Saper et al., 2005b). One pathway runs dorsally and rostrally along the third ventricle, into the medial preoptic area (MPO) of the hypothalamus and then up into the paraventricular nucleus of the thalamus (PVT). This PVT pathway has also been connected to the prefrontal cortex (Sylvester *et al.*, 2002). The functionality of this connection has not been demonstrated but it could serve in part circadian modulations of higher cognitive functions. A second pathway goes to the retrochiasmatic area and capsule of the ventromedial nucleus (VMH). The third pathway is going to the subparaventricular zone (SPZ). Part of the axons pass through the SPZ to reach the dorsomedial hypothalamic area (DMH). This third pathway is the most important in term of the proportion of projections it includes, and of the number of parameters it has been shown to affect.

Projections from the VMH, SPZ and DMH to cholinergic and aminergic neurons, as well as to orexin producing cells have been demonstrated (Deurveilher and Semba, 2005). Many of these projection sites are part of the wide network that regulates arousal (Jones, 2003) but its remains to be determined which of the indirect projections they receive from the SCN are functionally regulating arousal. Thus far circadian regulation of sleep-wake and locomotor activity is thought to be mainly mediated by the pathway linking the SCN, SPZ and DMH as demonstrated by specific lesions of these nuclei (Saper *et al.*, 2005b). A functional link between the SCN and the locus coeruleus (LC) through the DMH has been demonstrated (Aston-Jones *et al.*, 2001). This nucleus is the major source of norepinephrine of the organism, which promotes and regulates wakefulness, and projects to a multitude of regions throughout the brain (Aston-Jones and Cohen, 2005).

In addition, the SCN was shown in rats and in humans to project directly to orexin producing neurons in the lateral hypothalamic area (LHA), which contributes to the regulation of wakefulness (Abrahamson *et al.*, 2001). The ventrolateral preoptic area (VLPO) is also a direct target of the SCN. It is thought to be part of the switch regulating the transition between sleep and wakefulness, and projects to numerous areas involved in arousal regulation including the LC and LHA (Saper *et al.*, 2005a). However these direct SCN projections are weak and it is the DMH that provides a strong indirect link between the SCN, and the LHA, and VLPO (Chou *et al.*, 2003; Saper *et al.*, 2005a). The DMH has also been reported to be important in the circadian regulation of feeding behavior (Gooley *et al.*, 2006). Body temperature regulation appears to be mediated by the direct projections form the SCN to the SPZ, and not to go through the DMH (Saper *et al.*, 2005b). The SPZ also seems involved in circadian regulation of locomotor activity. Melatonin regulation appear to be mainly regulated by indirect connections between the SCN and the pineal gland (Saper *et al.*, 2005b). The SCN project directly to the dorsal paraventricular nucleus of the hypothalamus (PVH), which connects to the columns of the upper thoracic spinal cord that contact sympathetic preganglionic neurons that control pineal melatonin secretion.

Several circadian rhythms which exhibit close temporal relationship are therefore actually mediated through partially distinct pathways. This is thought to allow greater adaptability of the organisms to their environment (Saper *et al.*, 2005b). This

great diversity in the pathways leaving the SCN is reflected in the heterogeneity in the rhythmic activities and in the responses to light present in the SCN. Gene expression and electrical activity was shown to vary from neuron to neuron, and to depend on the neural connections of the different parts of the SCN (de la Iglesia *et al.*, 2004; Saeb-Parsy and Dyball, 2003; Schaap *et al.*, 2001). Furthermore, although most SCN neurons are activated by light exposure, many are inhibited (Meijer *et al.*, 1998). In addition to present broad projections throughout the brain, the SCN receives numerous afferents (Krout *et al.*, 2002). Hypothalamic, limbic, thalamic and brainstem nuclei project to the SCN. These projections could modulate circadian regulation of homeostatic functions, and be responsible for the observed variation in SCN activity with changes in vigilance states (Deboer *et al.*, 2003).

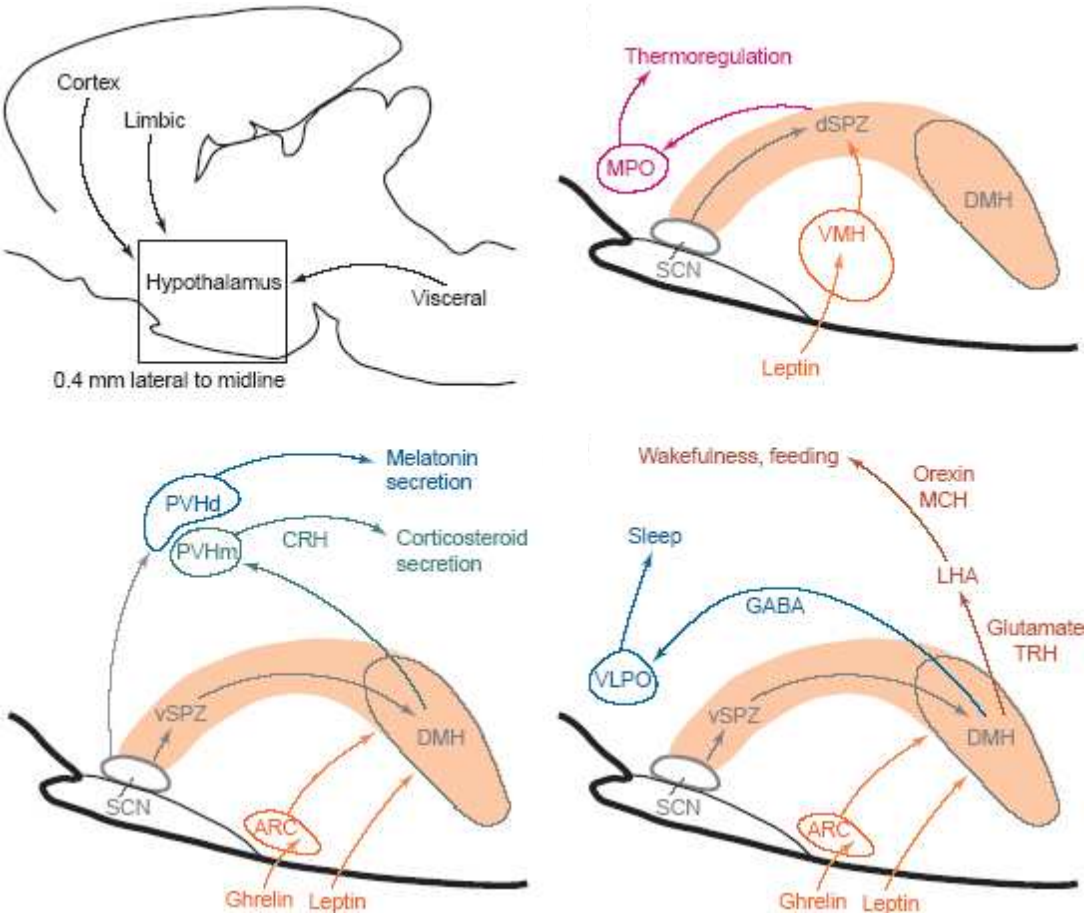

Figure 2.10: Major output pathways of the hypothalamic SCN (from Saper *et al.,* 2005b). See text for abbreviations other than: *ARC*: arcuate nucleus; *PVHd*: PVH dorsal; *PVHm*: PVH medial; *CRH:* corticotrophin releasing-hormone; *MCH:* melanin-concentrating hormone; *TRH:* thyrotropin-releasing hormone.

The SCN receives strong and distinct innervations from the eye through the RHT (Gooley *et al.*, 2001; Sollars *et al.*, 2003). Its cell activity is sensitive to light exposure in a dose dependent manner and the effects of light depend on the time of day (Meijer *et al.*, 1998). Expression of the early gene *c-fos* in the SCN is also induced by light, again in a dose dependent manner, revealing the capacity of the SCN to integrate light inputs (Dkhissi-Benyahya *et al.*, 2000). The SCN clearly appears therefore as one of the early mediators of the effect of light irradiance on physiology and behavior. The intergeniculate leaflet (IGL) of the thalamus also receives strong retinal innervations (Morin and Pace, 2002; Muscat and Morin, 2006). The immediate early gene *c-fos* is even more strongly induced by light exposure in the IGL than in the SCN. The IGL has strong connections with the SCN to which it conveys photic and non-photic information through the geniculo-hypothalamic (GHT) pathway. Further, IGL lesions reduce *c-fos* expression in response to light in the SCN, suggesting a role for the IGL and GHT in SCN photic response to light (Muscat and Morin, 2006). Similarly to the SCN, the IGL projects to and receives afferents from numerous nuclei involved in arousal regulation in the brainstem and hypothalamus such as the VLPO, but also the SC and OPN, involved in eye movements and pupillary constriction, respectively (Morin and Blanchard, 2005).

It is unknown which of the SCN and IGL projections and what other brain areas are involved in mediating the acute and longer term nonvisual responses to light irradiance. The characterization of the brain circuitry involved in eliciting these responses has only recently begun, and much of what is known is limited to the retina and the direct projection of RGC expressing melanopsin (Figure 2.11). There are only a few thousands of these cells in the retina (Dacey *et al.*, 2005; Wassle, 2004) but their projection sites are surprisingly numerous, at least in rodents (Gooley *et al.*, 2003; Hattar *et al.*, 2006; Hattar *et al.*, 2002). They project to other hypothalamic sites than the SCN, such as the VLPO, SPZ, and LHA. Melanopsin RGC also target the IGL, the OPN, as well as other pretectal nuclei, the lateral habenula (LHb), a relay site between the limbic and striatal areas and the midbrain, and the amygdala, a structure involved in emotion regulation, and more specifically the medial amygdala (MA), which seems to integrate signals from the olfactory system. Projections of melanopsin expressing RGC are also found in structure involved in vision such as the LGN and SC. Projections to

the OPN and LGN were described in macaques (Dacey *et al.*, 2005), but the other target sites still need to be confirmed in primates.

Melanopsin RGC targets are often interconnected, supporting that there is a brain irradiance network distinct from the network recruited for vision (Gooley *et al.*, 2003). Most projections in rodents reach the SCN, IGL, and OPN which suggests a prominent role for these three structures, which are strongly interconnected (Hattar *et al.*, 2006). The multiple connections of the melanopsin RGC reveal that nonvisual responses to light could affect the many brain functions sustained by their targets, and further support the importance of understanding the mechanisms of action by which light exposure leads to nonvisual responses.

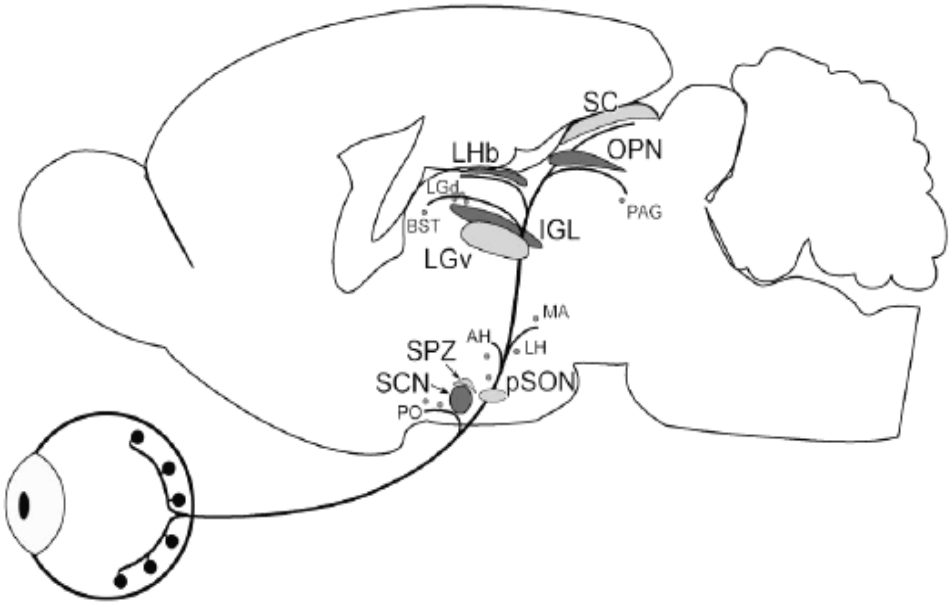

Figure 2.11: Main projections of the melanopsin expressing RGC in mice (from Hattar et al., 2006). See text for abbreviations other than: *AH:* anterior hypothalamic area; *BST*: bed nucleus of the stria terminalis; *LH* = LHA; *PAG*: periaqueductal grey; *PO*: preoptic area; pSON: peri-supraoptic nucleus.

What about the human brain?

Although the evidences presented so far seem to show that there is a good comprehension of the brain system implicated in nonvisual responses to light, we are just starting to understand its complexity. The complexity of the photoreceptor interactions is a good example. In addition, most of the works determining these neural circuits and molecular mechanisms involved in nonvisual responses to light were

carried out in nocturnal rodents. The human system might not be identical. Investigating human brain function (in its entire volume) from a chronobiology perspective is therefore of primary interest. Light exposure is a powerful external stimulus which induces acute disruptions in physiology and allows insight in nonvisual brain responses.

3. Nonvisual responses to light in humans and neuroimaging – Questions addressed in this thesis

At the time this PhD thesis was initiated, a single study had been carried out to investigate nonvisual brain responses to light exposure using functional neuroimaging. This study was held in Liège at the Cyclotron Research Center. The main results of the study will be enounced before stating the objectives of this thesis.

Nonvisual responses to light exposure in the human brain during the circadian night (Perrin et al., 2004)

Nonvisual brain responses to a bright polychromatic light exposure were assessed using positron emission tomography (PET). Scans we recorded in darkness (< 0.01 lux) following 17 min, 16.5 min, 0.5 min and 0 min of bright light exposure (> 8000 lux) between 0030h and 0430h (Figure 3.1a). The nonvisual response to light was independently assessed by a significant suppression of melatonin secretion (Figure 3.1b) and a decrease in the decline of subjective alertness (Figure 3.1c).

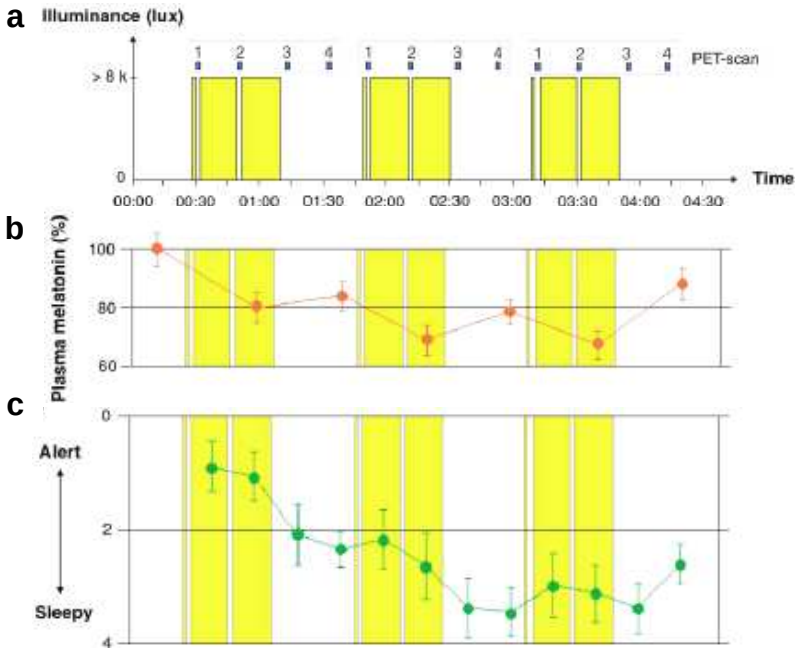

Figure 3.1: Experimental design (***a***), melatonin secretion profile (***b***), and subjective alertness (***c***) results of the PET study investigating the nonvisual effect of bright white light exposure (> 8000 lux) (from Perrin *et al.,* 2004).

Subjects (N = 13) performed an auditory oddball paradigm task during scan acquisitions (Kiehl and Liddle, 2003). EEG was recorded throughout each scan to evaluate the modulation of the P300 evoked potentials elicited by the detection of deviant tones during the task (Halgren *et al.*, 1998). Performance (count of the deviant tones) was high and the P300 amplitudes and latencies were not different across scans, reflecting the proper recruitment of attention by subjects. In order to obtain EEG recordings free of eye-movement artifacts, subjects were requested to fixate a small red diode placed in front of their eyes. This introduced a visual component to the task.

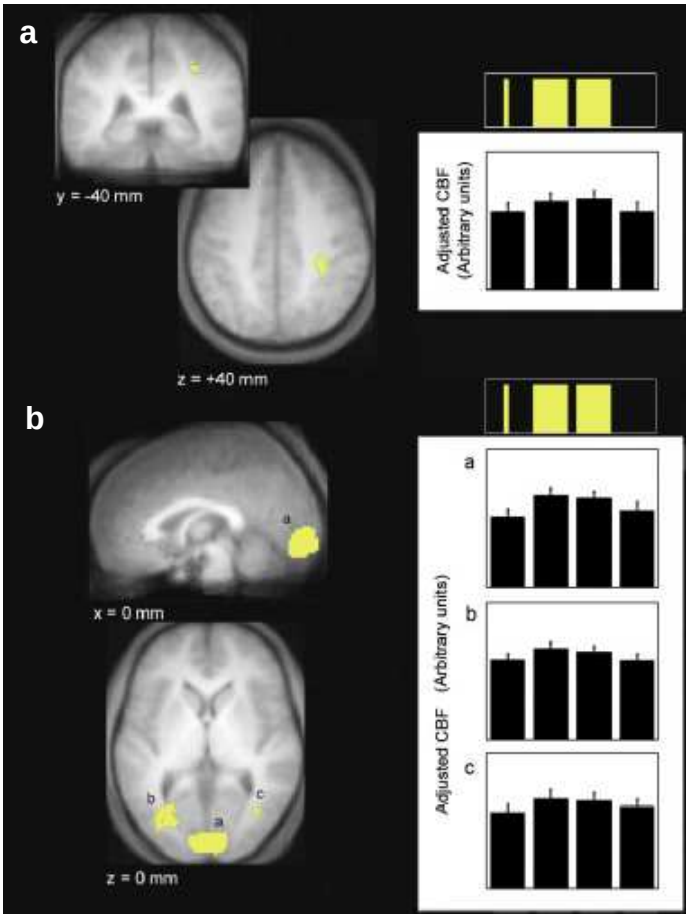

Figure 3.2: Increases in brain activity induced by the bright white light exposure (> 8000 lux) in the following 30 to 120 seconds of darkness. ***Left****:* significant activity modulations on the mean structural image of all subjects; ***right****:* activity estimates of the different brain areas. ***a.*** right IPS; ***b.*** striate (*a; top and lower panels*) and extrastriate cortex (*b and c on lower panel*) (from Perrin *et al.,* 2004).

Light exposure significantly increased regional cerebral blood flow (rCBF) in the striate cortex, bilateral extrastriate cortex, and in the right intraparietal sulcus (IPS)

(Figure 3.2). The IPS is part of the top-down network modulating attention (Corbetta and Shulman, 2002). Therefore the light-induced modulation of activity in this area probably reflects the attentional effect of light detected at the behavioral level. Analyses of the connectivity between the different regions revealed that the connections between the IPS and the visual areas strengthened as light exposure duration increased. This is compatible with an enhanced top-down modulation of early visual areas by parietal regions.

The PET data also revealed that the hypothalamic area was significantly deactivated following light exposure (Figure 3.3). This is compatible with the sustained decrease in activity recorded in rodent SCN following a bright white light exposure (Meijer *et al.*, 1998).

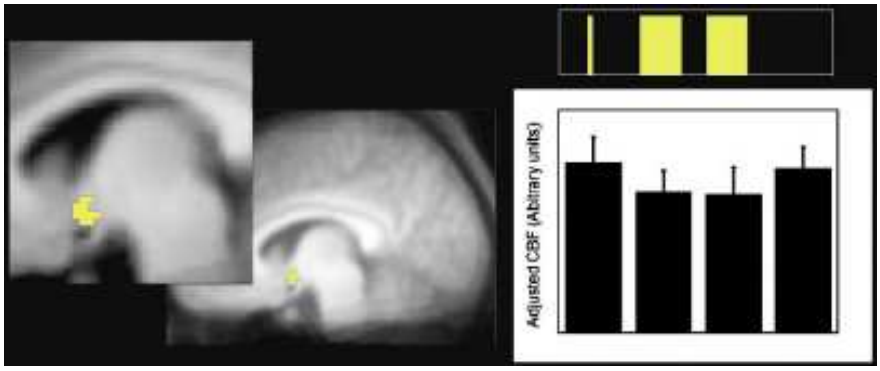

Figure 3.2: Decrease in hypothalamic activity in the following 30 to 120 seconds of darkness following bright white light exposure (> 8000 lux). ***Left****:* significant activity modulation on the mean structural image of all subjects; ***right****:* activity estimates (from Perrin *et al.,* 2004).

In summary this study was the first to identify neural correlates of a nonvisual brain response to light exposure in human. It showed that short light exposures (from a human chronobiology point of view) of less than 20min were sufficient to induce detectable significant modulations of brain activity related to a mixed auditory and visual task in parietal, occipital and hypothalamic areas in the few tens of seconds of darkness following the illumination.

The literature reviewed in the preceding chapter and the results of the PET study described above set the bases of the present PhD thesis, which addressed several experimental questions in three different investigations.

First experimental questions: *Can nonvisual effects of white light exposure be detected during the day in humans? What are the dynamics of daytime nonvisual brain responses to light?*

Most studies investigating the effects of light exposures were carried out at night in order to maximize the effects induced and the sensitivity of the designs. However, light is naturally surrounding us during the day. It is therefore of great interest to determine the nonvisual effects of light at a time the great majority of individuals are exposed to it. A few studies demonstrated nonvisual responses light exposure during the day (Hashimoto *et al.*, 1997; Jewett *et al.*, 1997; Phipps-Nelson *et al.*, 2003; Ruger *et al.*, 2005a). However the brain mechanisms involved were unknown. In line with the previous PET investigation, the first experiment of this thesis aimed at demonstrating that regional brain function could be affected by bright white light exposure (>7000lux) during the day, and at identifying neural correlates of an alerting effect of light.

Subjects performed again an auditory oddball paradigm task, but it was devoid of any visual requirements. Brain activity related to the task was recorded before, during and after a 21 min light exposure. In order to control for the effect of the repetition of sessions, subjects also performed the task in continuous darkness on another day. The analyses did not consider the illumination periods because they were contaminated by classical visual responses. Nonvisual effects of light exposure were therefore observed in the darkness periods following the exposures.

Dynamic changes in nonvisual responses to light are poorly characterized in humans, mainly because of the limited time resolution of the protocols employed. To gain insight in that matter, we introduced a novel neuroimaging technique to the chronobiology field: functional magnetic resonance imaging (fMRI). It benefits from a much better time resolution and allows the characterization of precise brain processing mechanisms. Therefore the first experiment of this thesis also aimed at determining some aspects of the brain dynamics of an alerting effect of light.

A third question was addressed: is it possible to separate the direct nonvisual effects of light on brain responses from the indirect changes related to the enhanced alertness induced by a bright white light exposure? An analysis taking into account the changes in subjective sleepiness in the different subjects was dedicated to this question.

The results of this first experiment are presented in chapter 5.

<u>Second experimental questions</u>: *Can a cognitive brain function other than attention be affected by daytime monochromatic light exposures in human? Do nonvisual brain responses elicited by light depend on the wavelength of the exposure?*

A few studies demonstrated effects of light on behavior, and there are only sparse data supporting nonvisual responses to light related to higher cognitive processes (Badia *et al.*, 1991; Cajochen *et al.*, 2000; Campbell and Dawson, 1990; Daurat *et al.*, 1993; French *et al.*, 1990). In addition, only one study showed an effect of monochromatic light exposure on behavior using a simple reaction time task (Lockley *et al.*, 2006). The second experimentation of this thesis sought first to establish that daytime light exposure could affect several aspects of human cognition by using an auditory working memory task, instead of the previous attention task (auditory oddball). It also aimed at showing that nonvisual brain responses to light vary according to the wavelength of the exposure by illuminating participants with blue (470nm) or green (550nm) monochromatic lights of equal photon densities (3×10^{13} ph/cm^2/s).

Subjects performed an auditory *2-back* task before, during and after being exposed to an 18-minute monochromatic light. Subjects came twice to the lab on 2 separate days. They were exposed to blue light on one day and to green light on the other. Because light exposures were identical in terms of photon density, major bias arising from visual responses to light were prevented when comparing sessions recorded during the illuminations. Therefore, this experiment allowed the comparison of monochromatic light effects during the exposures.

The results of this experiment are reported in chapter 6.

<u>Third experimental questions</u>: *Is it possible to detect brain areas involved in the establishment of nonvisual responses to light using exposure lasting a few tens of seconds only? What are the relative contributions of the different parts of the visible spectrum and, by inference, of the underlying retinal photoreceptors, to nonvisual brain responses to short light exposures in human?*

All retinal photoreceptors mediate nonvisual responses to light (Hattar *et al.*, 2003). Data published after the initiation of this PhD thesis demonstrated connections between classical photoreceptors and melanopsin RGC (Dacey *et al.*, 2005). In addition, a report published after the termination of this third experimentation actually modeled M-cone relative contribution to nonvisual responses to light and revealed that it progressively decreased as the illumination was pursued (Dkhissi-Benyahya *et al.*, 2007).

The third experimentation of this thesis used monochromatic lights aimed at S-cones (violet light – 430nm), melanopsin RGC (blue light – 473nm), and M-cones (green light – 527nm) to characterize their relative contributions to nonvisual brain responses to light. This protocol took the big step forward of using light exposures lasting 50s only, which is very short from a human chronobiology point of view. This was achieved for several reasons. First we hypothesized it would allow the detection of brain areas affected by light early in the exposure. These areas would therefore most probably be involved in the initiation of the nonvisual brain responses to light, and would presumably include subcortical and brainstem structures. Second, short light exposures permit the assessment of the relative contributions of different photoreceptors at a time signals arising from cones are less attenuated than after long uniform light exposures. Third, short light exposures can be alternated within a single acquisition session. This within-session design increases the sensitivity of the analyses as compared to the more conservative between-session comparisons carried out in the previous experiments. Again major bias, arising from the classical visual responses induced by the different exposures, were prevented by comparing illumination of equal irradiance.

The results of this experiment are presented in chapter 7.

4. Methodology

Experimental settings

Several physical constrains have to be taken into account when designing magnetic resonance imaging (MRI) experiments. First and most importantly, the scanner generates an important magnetic field (60,000 times the Earth magnetic field for a 3 Tesla (T) MR scanner, such as the one used in this thesis). The room containing the scanner is therefore built as Faraday cage to prevent magnetic variations arising from outside the cage to disturb data acquisition. No ferromagnetic objects can be present in the Faraday cage as they could be attracted by the magnetic field and cause serious safety hazards. Although of no potential danger, non ferromagnetic objects can disturb the local magnetic field and should not be placed close from the data acquisition area. Second, the MR scanner used in this thesis is specifically design for head studies, and is therefore shallower than whole-body MR scanners. The head-coil (*i.e.* the antenna detecting the signal recorded) only leaves a few centimeters in front of the participant's eyes. Third, gradient switching required for functional MRI data acquisition generate a loud noise (>100 dB). Participant's ears are therefore protected and specific audio systems have to be employed for auditory tasks.

We used a bright (70,000lux at light source level) cold white light source (PL900, Dolan-Jenner Industries, Boxborough, MA, USA) placed outside the Faraday cage (Figure 4.1c). Light intensity could be computer controlled. A 6-meter-long and 1-inch-diameter metal free optic fiber (Figure 4.1e; Dolan-Jenner Industries, Boxborough, MA, USA) ran along the participant's bodies to carry light from the source to the diffusers (Figure 4.2). The diffusers were specifically designed at the University of Liège for the purposes of this thesis (Figure 4.1a). It consisted of mounted mirrors (Figure 4.1b) turning the light beam arriving parallel to the participant's bodies to illuminated the eyes (Figure 4.2). The 4 x 5.5 cm diffusing frame was placed 3 to 4 cm away from the eyes and ensured uniform illumination of most of the visual field. Ultra-violet (UV) light filters (Edmund Optics Inc., York, UK) were placed in the diffusers to prevent UV hazards. Monochromatic lights were produced by placing narrow band-pass filters (full width at half maximum (FWHM): 10nm; Edmund Optics Inc., York, UK)

between the light source and the optic fiber. Changes in colors were achieved by a computer controlled filter wheel (Figure 4.1d; AB301-T, Spectral Products, Albuquerque, NM, USA) placing filters in front of the light beam (Figure 4.1c). Light level could not be assessed directly in the MR scanner and was therefore calibrated outside the Faraday cage using a radiometer (Q203, Macam Photometrics Ltd., Livingston, UK). Relative poly- and monochromatic light spectra were determined using a spectrometer placed at the diffuser level (AvaSpec-2048, Avantes, The Netherlands).

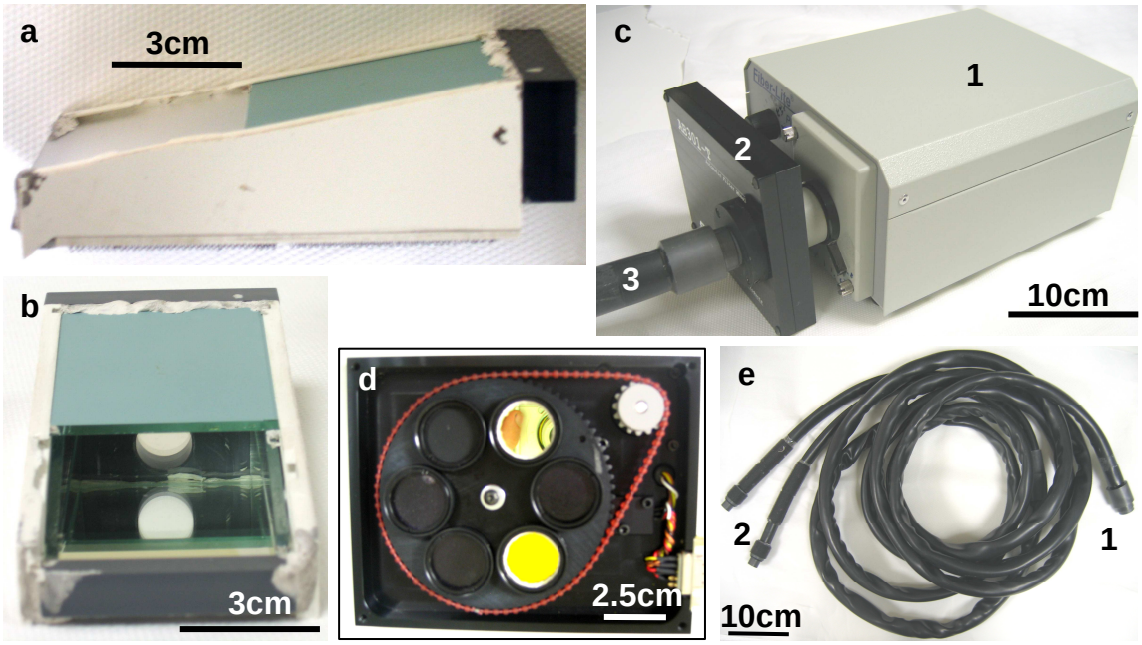

Figure 4.1: Lighting equipment. ***a.*** Lateral view of a diffuser. ***b.*** Above view of a diffuser; the diffusing piece of plastic which is positioned in front of the participant eye has been removed to show the inside mirrors. ***c.*** Light source (*1*), filter wheel (*2*), optic fiber (*3*). ***d.*** Inside view of the filter wheel mounted with 2 narrow band-pass filters. ***e.*** fiber optic; light source end (*1*), and split diffuser end (*2*). Approximate scales are indicated on each picture.

In the first and second experiments, a single eye was exposed to light while the other was continuously monitored using an infra-red eye tracking devise (Model 504, Applied Science Group, Bedford, MA, USA). Data were visualized online and video-taped to ensure participants had their eyes open during the light exposure. Space limitation did not allow assessment of gaze position and pupil size. For the third experiment, we chose to illuminate both eyes to increase the amount of light reaching

the brain within the short 50s illuminations. Space limitation did not allow the use of the eye tracking devises. However, both previous experiments revealed that all participants kept their eyes open during the illuminations. We are therefore very confident that this was also the case in the third experimentation, as participants were instructed that the eye tracking system was used, and that one eye was continuously monitored (a "dummy" infra-red mirror was placed above the diffusers).

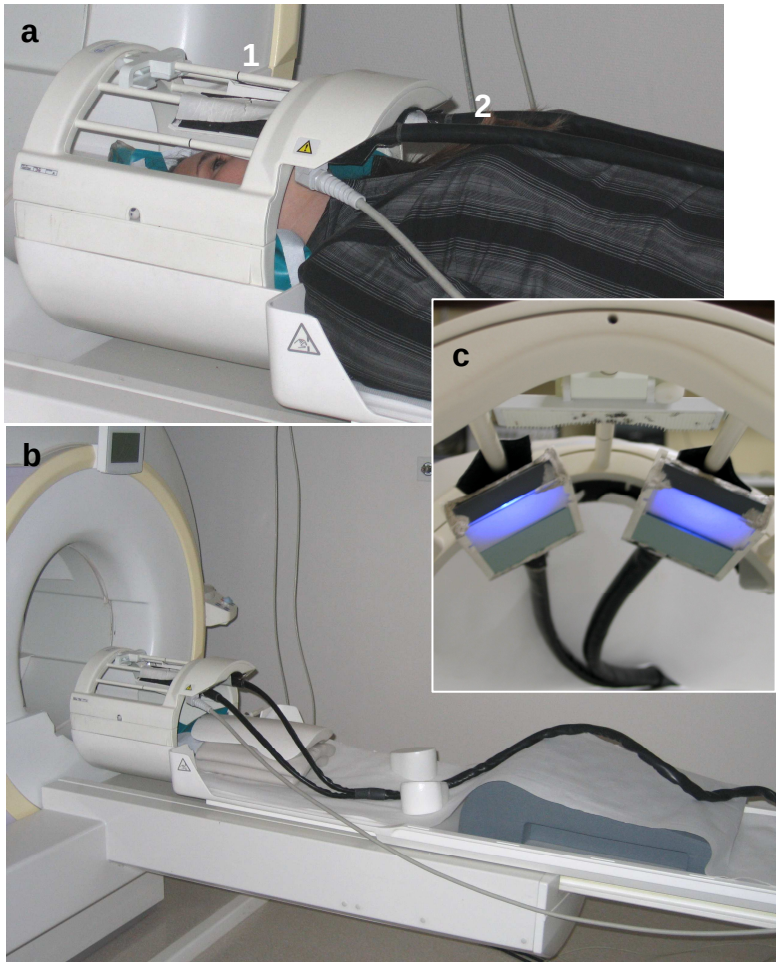

Figure 4.2: Lighting equipment and MR scanner. ***a.*** Participant position; the optic fiber (*2*) runs along the body to the diffusers (*1*) placed in front of the eyes. ***b.*** View of the MR scanner showing the fiber optic running to the head-coil onto which the diffusers are placed. ***c.*** View of head-coil showing the diffusers connected to the split end of the optic fiber (blue light -473nm- is diffused).

Auditory tasks were built using Cogent 2000 toolboxes (http://www.vislab.ucl.ac.uk/Cogent/) implemented in Matlab 6.1 (Mathworks Inc., Sherbom, MA, USA) on a 2.8-GHz XEON DELL personal computer (Round Rock, TX,

USA). Sounds were transmitted to the participants using MR CONTROL amplifier and headphones (MR Confon, Magdeburg, Germany). Participants' responses were required in all three experiments and were obtained using a MR compatible keypad designed at the Cyclotron Research Centre. The computer the task was run on, was synchronized with the MR scanner and precisely logged the time of occurrence of each stimulus, scans and participant's response. Regressors precisely modeling the different aspects of the protocol could therefore be computed and utilized in the fMRI data analysis.

Magnetic resonance imaging principles

Magnetic resonance imaging first requires the production of a strong homogenous magnetic field. Atomic nuclei, principally the proton (*i.e.* nuclei of the hydrogen atoms), align themselves with the field, resulting in an overall magnetization of the tissues. The rate at which nuclei reach equilibrium in the magnetic field, is called the longitudinal relaxation rate T1 (Kastler, 1997a). Proton nuclei precess (~ rotate) around the magnetic field at a specific frequency. The phase of all proton nuclei are however random so that the resultant of the precession is null. This random phase equilibrium state is disturbed by the application of a brief radio frequency (RF) electromagnetic pulse that creates a transient phase coherence in all proton nuclei. The resulting transient magnetization can in turn be detected by the head coil (or antenna) as a radio signal. It is this signal that is turned into an image. The radio signal exponentially fades away within a few milliseconds. The time of this decay is referred to a transverse relaxation time T2* (Kastler, 1997a). The value of T2* depends on two phenomenon. First, precessing nuclei induce small magnetic variations in their surrounding that induce slightly different rates of precession in the other nuclei. Nuclei phase differences increases and results in an extinction of the local magnetization induced by the RF pulse. This local effect varies according to the composition of tissues or fluids. Second, dephasing dynamics depend on the inhomogeneities of the external main magnetic field

The radio signal recorded after several RF pulses is reconstructed into an image. Spatial reconstruction is achieved by inducing spatially varying local magnetic fields that result in different precession frequencies. Three orthogonal magnetic field gradients are applied to the examined volume (*e.g.* the brain), resulting in a different precession frequency and phase for each proton nuclei (Kastler, 1997b). The detected signal can

therefore be characterized according to three dimensional coordinates. A given T2* value correspond to a specific encoded value so that variations in T2* resulting from tissues composition differences are reflected in the intensity of the signal displayed in the images.

Functional MRI principles

Functional MRI uses echo planar imaging (EPI) which allows the recording of the signal arising form the whole brain volume in a few seconds only (Kastler, 1997c). Although this time resolution is poor as compared to the EEG, the combination of both a relatively fast acquisition time and a very good spatial resolution (usually a few mm) made fMRI a very popular technique for the study of brain function. The brain volume is recorded a slice at a time (about 100ms per slice). For functional images, slices are usually a few mm thick and signal recorded from a surface of a few square mm are pooled to form a data point. This volume unit is referred to as *voxel*. The spatial resolution of functional EPI acquisitions is not optimal but this relative weakness is greatly overcome by the mapping of functional data onto precise anatomical images (*coregistration*, see below). Whole brain volume signal (scan) is recorded every repetition time (RT). The EPI set on the 3T MR scanner employed in this thesis used a RT of 2130 ms, and thirty two 3 mm-thick slices resulting in a spatial resolution of 3 x 3.4 x 3.4 mm (= voxel size).

The EPI settings optimally record variations in radio signal arising from changes in the oxygen level within the blood. This blood oxygen level dependent (BOLD; Figure 4.3) fMRI signal models haemodynamic changes that indirectly arise from changes in local cell activity (Glaser *et al.*, 2004)[*]. When neural populations fire, metabolism increases in neuron and glial cells and induces a local increase in rCBF, blood volume, and oxygen consumption. Oxyhaemoglobin level rises after 1-3s because of an increase of blood supply that is in excess as compared to the local oxygen requirements (an initial decrease in oxyhaemoglobin may occur initially). Oxyhaemoglobin reaches a maximum concentration (*peak*) approximately 3-9s after the initiation of the activation, to then decrease below the baseline level (*undershoot*), before returning to baseline level 20 to 25s after the initial activation.

[*] See pp. 824-6

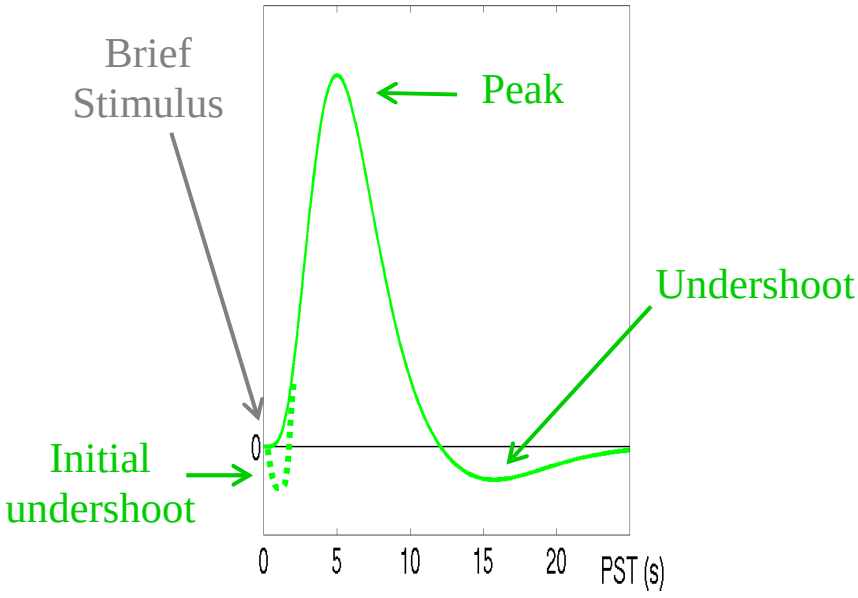

Figure 4.3: Schematic representation of a BOLD response. ***PST****:* post stimulus time (seconds) (After Phillips C., 2004)

The magnetic state of the iron atoms of haemoglobin changes depending on whether the molecule is in the oxy- or deoxyhaemoglobin state. Increase in deoxyhaemoglobin concentration decreases T2* relaxation time. The BOLD signal is therefore a reflection of the local blood ratio in oxy- and deoxyhaemoglobin which is indirectly dependent on the activity level of the surrounding brain cells (Glaser *et al.*, 2004)[*].

Processing of fMRI data

We used Statistical Parametric Mapping (SPM - http://www.fil.ion.ucl.ac.uk/spm) implemented in Matlab to analyzes fMRI data (Figure 4.4). Data of the first 2 experiments were computed with SPM2 implemented in Matlab 6.1 whereas data of the third experiment were computed using SPM5 implemented in Matlab 7.1. However, most evolutions of the software between SPM2 and SPM5 did not concern fMRI data analyses. Data processing may vary from one user to the other and from one protocol to the other. Only the procedures used in this thesis will be described.

[*] See pp. 824-6

Preprocessing

Before any statistical analysis is computed, fMRI data undergo several preprocessing stages. The first step of the preprocessing aims at realigning all data onto the first brain volume acquired, using rigid body registration method [for a complete description of this method see (Ashburner and Friston, 2004b)]. This first step takes into account the movements of the participant from one scan to the next. Although usually limited, these movements change the origin of the signal recorded in the three dimension space of the scanner. Six vectors are generated at this stage, one for each movement direction (3 translations and 3 rotations). Each vector contains values that represent the displacement of each scan as compared the first scan.

Functional images are then precisely matched, or coregistered, to a high resolution anatomical image of the subject (Friston, 2004)[*]. This image is recorded in a separate session, using acquisition settings privileging spatial resolution.

Functional and high resolution anatomical images are then normalized to a standard space in order to allow between subject comparisons [for a complete description of the method see (Ashburner and Friston, 2004a)]. This standard space is similar to the 3 dimensional space set by Talairach and Tournoux (Talairach and Tournoux, 1988) which used three orthogonal planes approximately corresponding to 3 major brain subdivisions to assign 3 dimensional coordinates to every parts of the brain. However, Talairach and Tournoux (1988) based their description on a dead brain in which displacement of the neural tissues had occurred. The standard space now used in SPM is a mean brain of about 350 anatomical images recorded at the Montreal Neuroscience Institute (MNI) and is referred to as the MNI space.

In the last preprocessing step, fMRI data are smoothed (using a Gaussian Kernel; FWHM 8mm) in order to improve the signal-to-noise-ratio and reduce residual inter-individual differences (Friston, 2004). Smoothing also renders data more suitable for the statistical analysis which is based on a random Gaussian field theory.

General linear model, design matrix, and regressors

The statistical analysis uses a general linear model (GLM) to describe the signal X in a voxel i of each acquired brain volume j in terms of a linear combination of the

[*] See p. 604

regressors *R* of the design matrix, plus a constant term *K*, and an error term *ε*, representing the unexplained variability:

$$X_{i,j}=\beta_{1,i} \times R_{1,j} + \beta_{2,i} \times R_{2,j} + \beta_{3,i} \times R_{3,j} +\ldots+ \beta_{y,i} \times R_{y,j} + K_i + \varepsilon_i$$

where $\beta_{y,i}$ is a parameter estimate of the voxel *i* and represents the relative contribution of the R_y regressor to the signal recorded in voxel *i* (Kiebel and Holmes, 2004)[*]. It is estimated using a method of ordinary least squares, which aims at reducing most the sum of the squared differences between the actual and the fitted values.

All conditions of the experiment are modeled in columns of a design matrix that contains all relevant factors of the experimental design and relates them to the preprocessed fMRI data (Kiebel and Holmes, 2004)[†]. A column is a continuous regressor that contains either stick or block functions that indicate the precise time of each trial type and its duration, and are convolved with the haemodynamic function to match the characteristics of the BOLD signal recorded (Kiebel and Holmes, 2004)[‡]. Trials can last several scans (blocks) or be instantaneous (events; duration = 0s). The design matrix attempts to comprehensively describe the experimental design, and also comprises a mean term. Regressors include therefore the conditions of interest (*e.g.* correct responses of the participant) but also those of no interest (*e.g.* wrong responses) and the realignment parameters computed during preprocessing (*NB*: realignment parameters are not convolved with the haemodynamic response). Regressors may take particular shapes if the underlying brain mechanism is thought to follow a pattern different from classical blocks or events. For instance, in the third experiment, we hypothesized that light information stimulation could build-up through a 50s illumination period and introduced "sawtooth-like" parameters, convolved with the haemodynamic response, that modeled any brain activity that would increase progressively to return to baseline after each illumination (see Figure 7.1c).

Regressors may also represent a parametric modulation of a trial type, *i.e.* a trial the amplitude of which changes in the course of the acquisition of the data (Kiebel and Holmes, 2004)[§]. The most typical parametric modulation is the effect of time. Brain

[*] See p. 726
[†] See p. 751
[‡] See p. 752
[§] See p. 753

activity may decrease with time if boredom occurs, or may increase with time if a stimulation progressively builds-up. In the first two experiments of this thesis, linear changes in activity with time were modeled. Although these changes do not always reveal significant variations, they model part of the data variance, reduce the residual error and therefore increase the sensitivity of the analysis. Quadratic changes in brain activity with time were also modeled in the first two experiments allowing the detection of faster modulations of brain activity.

Statistical inferences

Once the solution of the GLM is found, parameter estimates of all voxels can be entered in statistical tests (*t*, *F*) using linear contrasts (*c*) (Penny and Holmes, 2004)[*]. These contrasts are applied at each voxel and constitute contrast images that can be inspected to detect significant voxels. Linear contrasts can include a single regressor. The resulting contrast image will then represent the main effect of this regressor. Contrasts can also compute the difference between regressors. The resulting images will then represent the difference between the regressors. The statistical tests computed take into account the size of the effect ($c^T \times \beta_i$) but also its variance.

Summary statistics images are fed in a second level analysis (random effects analysis) taking into account inter-subject variability and allowing inferences on the general population from which the subjects were drawn (Penny and Holmes, 2004)[†]. Summary statistics images are further smoothed (using a Gaussian Kernel; FWHM: 6mm) before being fed into the random effects analysis. Statistical inferences are carried on the parameter estimates computed at the random effects.

The brain volume is composed of more than 100,000 voxels. The likelihood of obtaining voxels significantly affected by an experimental condition by chance is therefore high (*e.g.* with $p = 0.001$, we would obtain at least 100 significant voxels by chance). Data are corrected for multiple comparisons to prevent this type of error. The correction method takes into account the spatial correlation between voxels of the fMRI data to computes the number of independent measures of the data set, and define the *Z*-value threshold required to reach significance (Brett *et al.*, 2004). If based on the literature, a significant activation can be expected in a given location, correction for

[*] See p. 844
[†] See p. 843

multiple comparisons can be computed on a small (generally spherical) volume (of generally of 10 mm radius) around the *a priori* location of interest. If no *a priori* are available for a given location it has to survive the more conservative multiple comparison correction over the entire brain volume to be considered significant (Brett *et al.*, 2004)[*].

A basic assumption of classical statistics is that the data variance is identical and identically distributed across factors. When this assumption is falsified, a correction for non-sphericity is applied, for instance when regressors of a design matrix are correlated (Glaser and Friston, 2004)[†]. This was the case in the first experiment where voxel values were modeled by linear and quadratic time modulators at the random effects level.

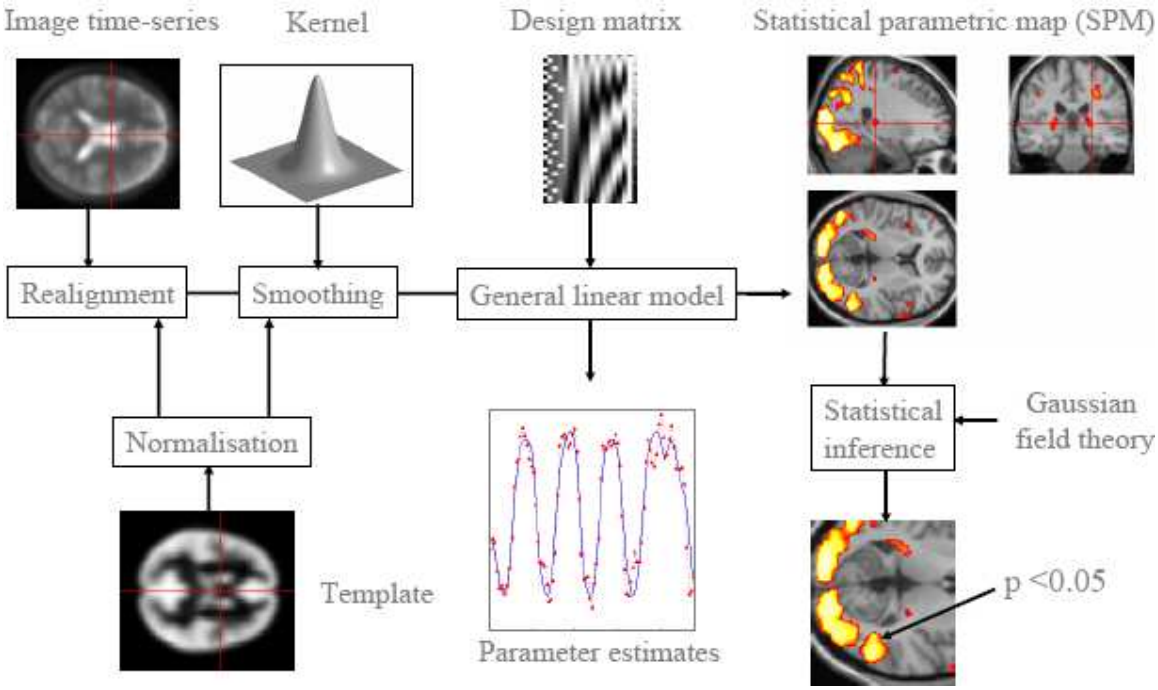

Figure 4.4: Schematic representation of the preprocessing and statistical analysis of fMRI data using SPM (Friston, 2004)

Masking procedure

In order to test whether the significant voxels of a given contrast *C1* are also significant in another contrast *C2*, *C1* can be masked by all the voxels of *C2* that have a higher

[*] See pp. 875-6
[†] See p. 781

value than a predefined threshold (usually the mask is composed of all voxels having Z score leading to a $p < 0.05$ *uncorrected*). The masking procedure can leave all the voxels that are common to both *C1* and *C2* (inclusive mask), or that are only present in *C1* (exclusive mask).

Bayesian inferences

If voxel *i* is shown significant in a given contrast C, it can be stated that it was significantly affected by the experimental condition, in case of a main effect, or that its activity was significantly different between two conditions, for differential contrasts (i.e. the null hypothesis is rejected). However if voxel *i* is not shown significant, one cannot state that voxel *i* was not affected by the experimental condition or that it was similarly modulated by two conditions. Posterior probability maps (PPM) enabling conditional or Bayesian inferences about regionally specific effects can be computed to help resolving this issue (Friston and Penny, 2003). These maps reflect the posterior probability of each voxel of presenting a value superior to a predefined threshold, given the data. The value of this threshold takes into account the variability of the whole data set. Using Bayesian inferences, it is therefore possible to state that voxel *i* was very unlikely to be affected by the experimental condition if PPM value of voxel *i* is low. It could also be possible to state that voxel *i* was more likely to be significant in *C1* than in *C2* if PPM values are superior in *C1*.

Bayesian inferences were only possible at the random effect level in SPM2. In SPM5, PPM can be computed at the fixed effect level and fed in a random effects analyses on PPM of all subjects. Bayesian inferences were carried out in the first two experiments of this thesis, which used SPM2.

5. Daytime light exposure dynamically enhances brain responses

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Introduction and summary

In humans, light enhances both alertness and performance during night and day time (Badia *et al.*, 1991; Cajochen *et al.*, 2000; French *et al.*, 1990; Phipps-Nelson *et al.*, 2003) and influences regional brain function (Perrin *et al.*, 2004). These effects do not correspond to classical visual responses but involve a nonvisual, or non-image forming, system, which elicits greater endocrine, physiological, neurophysiological and behavioral responses to shorter wavelength lights than to wavelengths geared toward the visual system (Brainard *et al.*, 2001a; Cajochen *et al.*, 2005; Hankins and Lucas, 2002; Lockley *et al.*, 2003; Lockley *et al.*, 2006; Munch *et al.*, 2006). During daytime, the neural changes induced by light exposure, and their time courses, are largely unknown. Using fMRI, we characterized the neural correlates of the alerting effect of daytime light by assessing the responses to an auditory oddball task (Halgren *et al.*, 1998; Kiehl *et al.*, 2001; Kiehl and Liddle, 2003; Stevens *et al.*, 2000), before and after a short

[*] The supplemental materials published online with this article are included in the body of this chapter and in appendix 1.

exposure to a bright white light. Light-induced improvement in subjective alertness was linearly related to responses in the posterior thalamus. In addition, light enhanced responses in a set of cortical areas supporting attentional oddball effects, and prevented decreases of activity otherwise observed during continuous darkness. Responses to light were remarkably dynamic. They declined within minutes after the end of the light stimulus, following various region-specific time courses. These findings suggest that light can modulate activity of subcortical structures involved in alertness, thereby dynamically promoting cortical activity in networks involved in ongoing nonvisual cognitive processes.

Materials and Methods

Subjects

Participants were healthy, right-handed, non-smokers, moderate caffeine and alcohol consumers, and were not on medication (N = 19; 11 females; age: 20-25 [median: 21]; body mass index: 17.9-26 [median: 21.1]). A semi-structured interview established the absence of medical, traumatic, psychiatric, or sleep disorders. None had worked on night shifts during the last year or traveled through more than 1 time zone during the last 2 months. Extreme morning and evening types, as assessed by the Horne-Ostberg Questionnaire (Horne and Ostberg, 1976), were excluded. None complained of excessive daytime sleepiness as assessed by the Epworth Sleepiness Scale (Johns, 1991) and of sleep disturbances as determined by the Pittsburgh Sleep Quality Index Questionnaire (Buysse *et al.*, 1989). Participants gave written informed consent. The study was approved by the Ethics Committee of the Faculty of Medicine of the University of Liège.

Volunteers followed a constant sleep schedule for 7 days before the first experiment day and until the second, 2 days later. Compliance to the schedule was assessed using wrist actigraphy (Cambridge Neuroscience, UK) and sleep diaries. In order to record 2 volunteers on the same day at the same circadian time, volunteers were requested to follow one of 2 sleep schedules differing by 1.5h (2300h - 0700h ± 30min, or 0030h - 0830h ± 30min). They were requested to refrain from caffeine and alcohol-containing beverages and intense physical activity for 3 days before participating.

Protocol

Subjects were scanned during 6 consecutive 8-minute sessions during which they performed an auditory oddball task (Figure 5.1a,b).

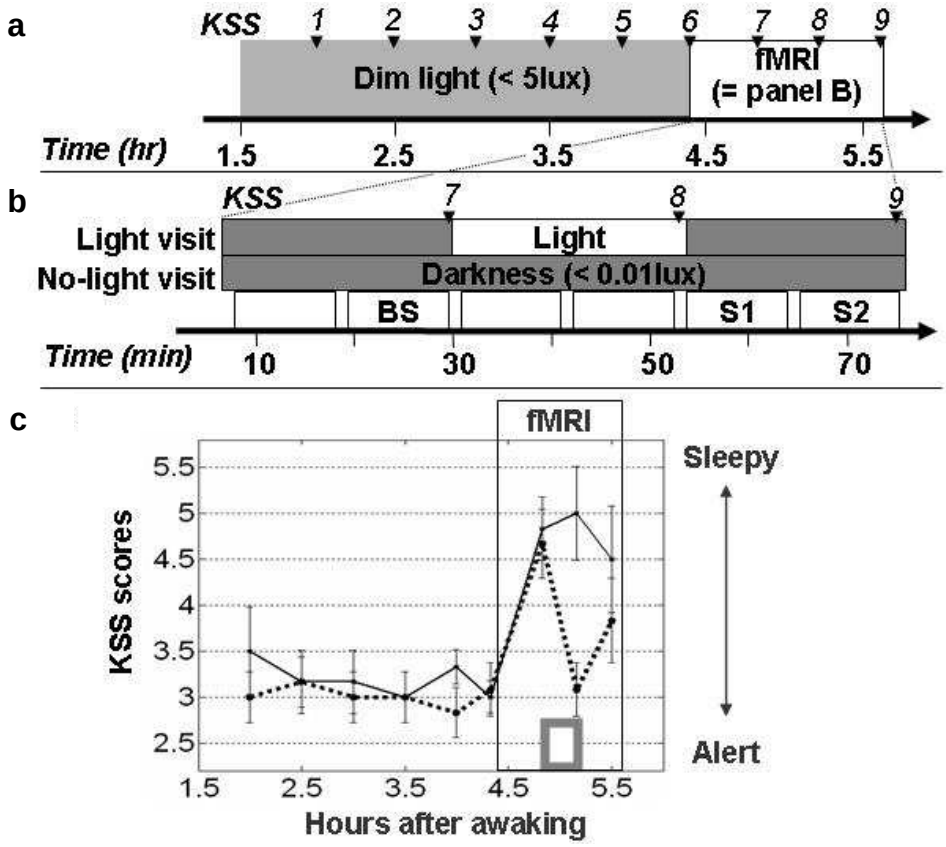

Figure 5.1: Experimental design and subjective alertness evolution.

a. General timeline relative to scheduled wake time (hrs). *Arrows*: KSS 1-9.

b. Timeline of the fMRI acquisition of both days (enlarged fMRI box of panel a). *Empty rectangle:* 6 oddball sessions. *BS:* Baseline session; *S1-2:* post-exposure sessions 1 and 2. Time in minutes after entering the scanner. *Arrows:* KSS 7-9.

c. Mean subjective alertness of subjects (± SEM). *Solid line:* day without light exposure. *Dotted line:* day with light exposure. *Gray rectangle:* light exposure period. *Empty rectangle:* fMRI period. Time relative to scheduled wake time (hrs).

The auditory oddball task is devoid of any visual processing and elicits reproducible brain responses (Kiehl and Liddle, 2003). Data were acquired before (2 sessions; < 0.01 lux), during (2 sessions; > 4.16 x 10^{15} ph/cm²/s, or > 7000 lux) and after (2 sessions; < 0.01 lux) one eye was exposed for 21 minutes to a bright white light.

Light exposure occurred approximately 5h after habitual wake up time. The same protocol was followed on another day, but no light was administered. The order of the day with and without light was counterbalanced over subjects.

During each experiment day, they first stayed in dim-light (< 5lux) to eliminate effect of previous outdoor illumination for 3h during which they rated their vigilance on the Karolinska Sleepiness Scale (KSS) (Akerstedt and Gillberg, 1990) every 30 minutes. Three additional KSS scores were obtained right before the light exposure, at the end of it and at the end of the experiment. Volunteers received a small standardized snack in the middle of the 3h preparatory period. Interaction with subjects during data acquisition was limited to standardized sets of sentences. No feedback was given on performance. Subjects were trained on a shortened version of the protocol at least a week before the experiment.

Oddball task

Subjects were required to count the odd tones, and respond to them by pressing a key as fast as possible. 300 auditory stimuli per session were presented. They consisted of frequent (600 Hz) and odd tones (400 Hz), presented ~90% and ~10 % of the time in pseudo-randomized order. Each tone was 600 ms long; stimulus onset asynchrony was 1000 ms between tones and at least 4200 ms between odd tones. Tones were produced using COGENT 2000 (http://www.vislab.ucl.ac.uk/Cogent/) and were transmitted to the subjects using MR CONTROL audio system (MR Confon, Germany). On both days, volume level of both tones was set by the volunteer before the first session.

Light exposure

One eye was exposed to light (spectrum: Figure 5.2). The other was monitored using infrared eye-tracking system (Applied Science Group, MA). The eye-tracking signal was video-taped, and examined to ensure volunteers had their eyes open at all time and were looking toward the light during the illumination. The exposed eye and the order of the day with and without light exposure were counterbalanced. Light was transmitted by a metal-free optic fiber from a source (PL900, Dolan-Jenner, MA) to a diffuser ensuring uniform illumination through a 4 x 5.5 cm frame. Due to space constraints within the head coil, the diffuser box was close to the subject's eye (3 to 4 cm). Slight variations in subject morphology or in the position the diffuser box can modify the light intensity

reaching the eye by a few hundreds of lux. This is the reason why we state the light intensity was > 7000 lux. This figure represents the lower bound of light intensity used in this study. For some subjects, we computed that light intensity levels were closer to 8000lux.

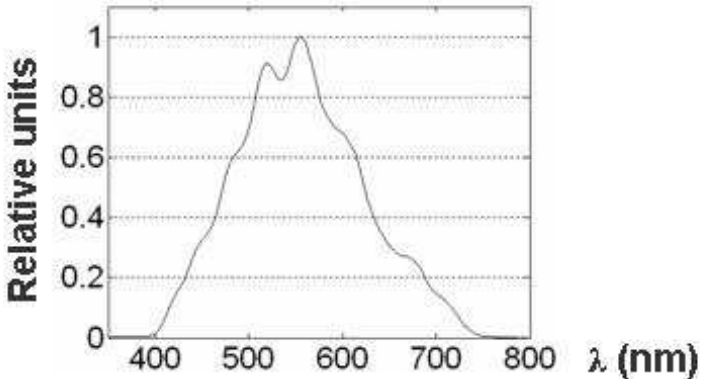

Figure 5.2: Spectrum of the white light used for the illumination. Intensity is given relative to the maximum arbitrary units for every wavelength (λ).

Demographic data

Three subjects reaching mean reaction time higher than 500ms for more than one session of the same visit were excluded. They did not conform to the instructions requiring to respond as fast as possible. As melatonin level is low during daytime (Dijk and Lockley, 2002), we relied on the known alerting effect of light (Badia *et al.*, 1991; Cajochen *et al.*, 2005; Cajochen *et al.*, 2000; Phipps-Nelson *et al.*, 2003), as assessed by the KSS (Akerstedt and Gillberg, 1990), to ascertain an effect of light. Subjects were considered as responders if a reliable response to light was deemed detected, i.e. if, when subtracting the KSS score obtained at the end of the baseline session (before light exposure period) from the KSS score obtained at the end of light exposure period, the score of the day with light exposure was larger than the score of the day without light exposure. Four non-responders were excluded from the analysis looking for the effects of light exposure (including behavioral analysis). Twelve responders (7 females; age: 20-25 [median: 22]) were included in this analysis. The last analysis, testing the effects of light on the correlates of alertness, incorporated the 4 non-responders (10 females, age: 20-25 [median: 21]).

Functional MRI scan acquisition

fMRI time series were acquired using a 3T MR scanner (Allegra, Siemens, Germany). Multislice T2*-weighted images were obtained with a gradient echo-planar sequence using axial slice orientation (32 slices; voxel size: 3.4x3.4x3 mm^3; matrix size 64 x 64 x 32; repetition time = 2130ms; echo time = 40ms; flip angle = 90°). The 4 initial scans were discarded to allow for magnetic saturation effects. Sessions consisted of 232 scans. Head movements were minimized using a vacuum cushion. A structural T1-weigthed 3D MP-RAGE sequence (TR 1960ms, TE 4.43ms, TI 1100ms, field of view (FOV) 230x173 cm^2, matrix size 256x256x176, voxel size: 0.9x0.9x0.9 mm) was acquired in all subjects.

Functional MRI data analysis

Functional volumes were analyzed using SPM2 (http://www.fil.ion.ucl.ac.uk/spm). They were corrected for head motion, spatially normalized to an echoplanar imaging template conforming to the MNI space, and spatially smoothed with a Gaussian kernel of 8mm FWHM. Analysis of fMRI data, based on a mixed effects model, was conducted in 2 serial steps, accounting respectively for fixed and random effects. For each subject, changes in brain regional responses were estimated using a general linear model in which the activity evoked by odd tones in each session was modeled by stick functions, convolved with a canonical haemodynamic response function. Two further regressors represented the modulation of brain responses to odd tones by linear and quadratic function of time. Movement parameters derived from realignment of the functional volumes were included as covariates of no interest. High-pass filtering was implemented in the design matrix using a cut-off period of 128 seconds to remove low frequency drifts. Serial correlations in fMRI signals were estimated using an autoregressive (order 1) plus white noise model and a restricted maximum likelihood algorithm.

The effects of interest were then tested by linear contrasts, generating statistical parametric maps. Since no inference was made at this level of analysis, summary statistic images were thresholded at $p_{uncorrected} = 0.95$. Summary statistic images resulting from these different contrasts were smoothed (6mm FWHM Gaussian kernel) and entered in a second-level analysis. This second step accounts for inter-subject

variance in the main effects of light and corresponds to a one-sample *t*-test for brain responses to odd tones. Both time modulators were included in a separate parametric within-subject one-way ANOVA. For the latter analysis, error covariance was not assumed independent between regressors and correction for non-sphericity was used for final inferences (Glaser and Friston, 2004). The resulting set of voxel values for each contrast constituted maps of the T-statistics for the main responses and F-statistics when they were modulated by time, thresholded at p = 0.001. Statistical inferences were performed after correction for multiple comparisons on small spherical volumes (svc; 10mm radius) at $p_{svc} < 0.05$ threshold, around *a priori* locations of activation in structures of interest, taken from published work on attention and oddball tasks in fMRI.

The second analysis tested for the effects of light on alertness and used a singular value decomposition conducted on the 18 KSS scores collected over the 16 subjects. The component related to the highest eigenvalue, i.e. explaining the largest part of the variance, was selected for the analysis. The corresponding eigenvector over subjects was used in a multiple regression at the random effects level, on the contrast (summary statistics) images representing the day by session (post 1 > baseline) interaction. Statistical inferences were conducted as previously described, with priors focused on the neural correlates of alertness.

Before performing any svc, peaks reported in Talairach (Talairach and Tournoux, 1988) space were transformed to MNI space using Matthew Brett's bilinear transformation (http://imaging.mrc-cbu.cam.ac.uk/imaging/MniTalairach - no coordinates were shifted more than 5 mm). Standard stereotactic coordinates of previously published *a priori* locations, used for spherical svc, are as follow.

Locations involved in oddball paradigm and perceptual novelty detection. These locations are as follows: right anterior cingulate gyrus 16 38 10 (Kiehl and Liddle, 2003), left and right precuneus ±11 -52 70, 0 -45 55 (Kiehl *et al.*, 2001), right insula 45 11 5, right superior temporal sulcus 49 -12 -5 (Stevens *et al.*, 2000), right posterior cingulate 0 -22 42 (Huettel *et al.*, 2004), left hippocampus -30 -34 -6, -22 -38 -6 (Strange *et al.*, 1999), right fusiform gyrus 32 -80 –20 (Kiehl and Liddle, 2003).

Locations involved in attention. These locations are as follows: right dorso-lateral prefrontal cortex 20 8 48 (Hopfinger *et al.*, 2000), 53.54 5.25 41.63, 49.49 -1.34 49.99 mm, right intraparietal sulcus 25.3 -61 46.8, 29.3 –52.6 42.9, 23.2 -64.7 35.8, right

fusiform gyrus 23.23 -80.52 -23.72, 25.25 -80.62 -21.34, right insula 31.31 21 14.17, 43.43 10.7 13.62 (Shulman *et al.*, 1999) (transformed in MNI coordinates), 32 24 4 (Hopfinger *et al.*, 2000).

Locations involved in arousal regulation. These locations are as follows: left thalamus pulvinar -4 -24 10 (Foucher *et al.*, 2004), -6 -30 9 (Coull *et al.*, 2004).

Masking procedures

In the day by session (post 1 > baseline) interaction, mean parameter estimates suggested differences in baseline activity between days. To rule out this possible confound, we excluded from the interaction the brain areas in which neural responses differed during the baseline session between both days (exclusive mask at $p_{uncorrected}$ = 0.05). Results remained unaffected, except for responses in the rIPS in which some voxels were excluded. In this area only, baseline differences might in part explain the day by session (post 1 > baseline) interaction effect.

In the day by session (post 1 > post 2) interaction, mean parameter estimates revealed an increase of activity from the 1st to the 2nd post-exposure session of the day without light exposure. To verify that this increase did not rule the interaction effect, we excluded from the interaction brain areas in which a difference was found between days during the 2nd post-exposure session (exclusive mask at $p_{uncorrected}$ = 0.05). This mask did not affect the results.

Bayesian inferences and posterior probability maps

In the random-effect analyses, PPM (Friston and Penny, 2003) and effect size were computed for response to odd tones in the first post-exposure sessions of both days. The day (light > no-light) by session (post 1 > post 2) interaction revealed regions in which activity decreased from the 1st to the 2nd post-light exposure sessions in the light visit. We wanted to verify that these regions presented a higher probability of activation in the 1st post-exposure session of the day with light exposure compared to the same 1st post-exposure session of the day without light exposure, to support the interpretation of the result of the day (light > no-light) by session (post 1 > post 2) interaction.

Results and discussion

Only data acquired in three sessions of darkness (hereafter referred to as baseline and first and second post-light sessions) were considered. Data obtained during sessions with light exposure were discarded because they were contaminated by classical visual responses (Haynes *et al.*, 2004). The very first session was not used because it can be contaminated by physiological events related to recent postural changes (Bonnet and Arand, 1998). The timeframe of the nonvisual light-related effects was examined at two levels in subjects showing an alerting effect of light. First, we report modulation of evoked responses by light exposure; this modulation is expressed between sessions preceding (baseline session) and following (post-exposure sessions 1 and 2) the illumination. Second, we addressed light-dependent modulations of the evoked responses within-sessions, over a shorter timescale. The light-dependent effect here was the time-dependent adaptation of evoked responses within each session. Finally, to establish the relationship between these light-dependent effects and the alerting effects of light exposure, we extended the cohort to include non-responders (subjects who did not exhibit an alerting effect of light) and used a subject-specific measure of this alerting effect to predict the light-dependent effects described above.

The first set of analysis included subjects showing an alerting effect of light. As expected, repeated measure ANOVA on KSS scores of responder subjects with session and day (light > no-light) as within-subject (N = 12) factors revealed main effects of session ($F(8) = 6.19$; $p < 0.00001$), day ($F(1) = 5.60$; $p = 0.037$), and a day by session interaction ($F(8) = 4.30$; $p = 0.00021$; Figure 5.1c). Planned comparisons showed no significant differences between days over the 7 KSS scores prior to illumination ($F(1) = 2.24$; $p = 0.16$), and for the last KSS score ($F(1) = 3.47$; $p = 0.09$). A significant main effect of light condition was detected only for the 8th KSS measures, collected at the end of the illumination period ($F(1) = 19.51$; $p = 0.001$).

Repeated measure ANOVA on the same subjects with session and day as within subject factor did not reveal any session effect for reaction time ($F(5) = 1.86$; $p = 0.12$), and for errors in counting the number of odd tones ($F(5) = 1.62$; $p = 0.17$). No effect of day was found for reaction time ($F(1) = 4.13$; $p = 0.07$) and for counting errors ($F(1) = 0.37$; $p = 0.56$). Likewise, no interaction between the 2 factors could be detected either

for reaction time ($F(5) = 0.46$; $p = 0.81$), and for counting errors ($F(5) = 0.34$; $p = 0.89$). The error rate in the oddball paradigm was very low (0-2 misses/session).

In partially sleep deprived subjects, daytime white light exposure has been reported to improve reaction times (Phipps-Nelson *et al.*, 2003). In contrast, our normally rested subjects were able to maintain steady reaction times during all sessions, despite concurrent fluctuations in alertness. Differences in cognitive task, sleep pressure, and exposure duration probably explain this discrepancy. Moreover, different nonvisual responses might be sensitive to different wavelengths, as suggested with subjective alertness (Revell *et al.*, 2006).

For fMRI data, a significant day (light > no-light) by session (post 1 > baseline) interaction effect was observed in the left hippocampus (-30 -30 -2 mm; $Z = 3.91$; $p_{svc} = 0.011$), right anterior cingulate cortex (10 36 12 mm; $Z = 3.88$; $p_{svc} = 0.011$), left precuneus (-8 -50 72 mm; $Z = 3.82$; $p_{svc} = 0.014$), and right intraparietal sulcus (rIPS; 22 -56 40 mm; $Z = 3.33$; $p_{svc} = 0.049$; Figure 5.3a,d; Appendix 1: supplemental table S5.1). Mean parameter estimates showed that, in these areas, light exposure prevented the progressive decline in responses observed in continuous darkness during the day without light and increased activity as compared to baseline.

In the post-exposure period, a significant day by session (post 1 > post 2) interaction was observed in the right precuneus (8 -54 52 mm; $Z = 3.67$; $p_{svc} = 0.036$) and right superior temporal gyrus (rSTG; 44 -16 -2 mm; $Z = 3.25$; $p_{svc} = 0.038$; Figure 5.3e,f; Appendix 1: supplemental table S5.2). Mean parameter estimates showed that the responses in these regions decreased from the 1st to the 2nd post-exposure session of the day with light exposure, whereas during the day without light exposure, responses increased from the 1st to the 2nd post-exposure session (this latter increase did not rule the interaction effect; see *masking procedure* in *Material and methods*). No significant modulation had been found in the previous day by session (post 1 > baseline) interaction in the rSTG and right precuneus. This may be due to the lack of statistical power of between-session contrasts at the random effects level. In keeping with this suggestion, posterior probabilities of activation (Friston and Penny, 2003) were considerably larger during the 1st post-exposure session of the day with light exposure in both regions (precuneus: $p_{light} = 0.47$, $p_{no\text{-}light} = 0.01$; rSTG: $p_{light} = 0.81$, $p_{no\text{-}light} = 0.05$).

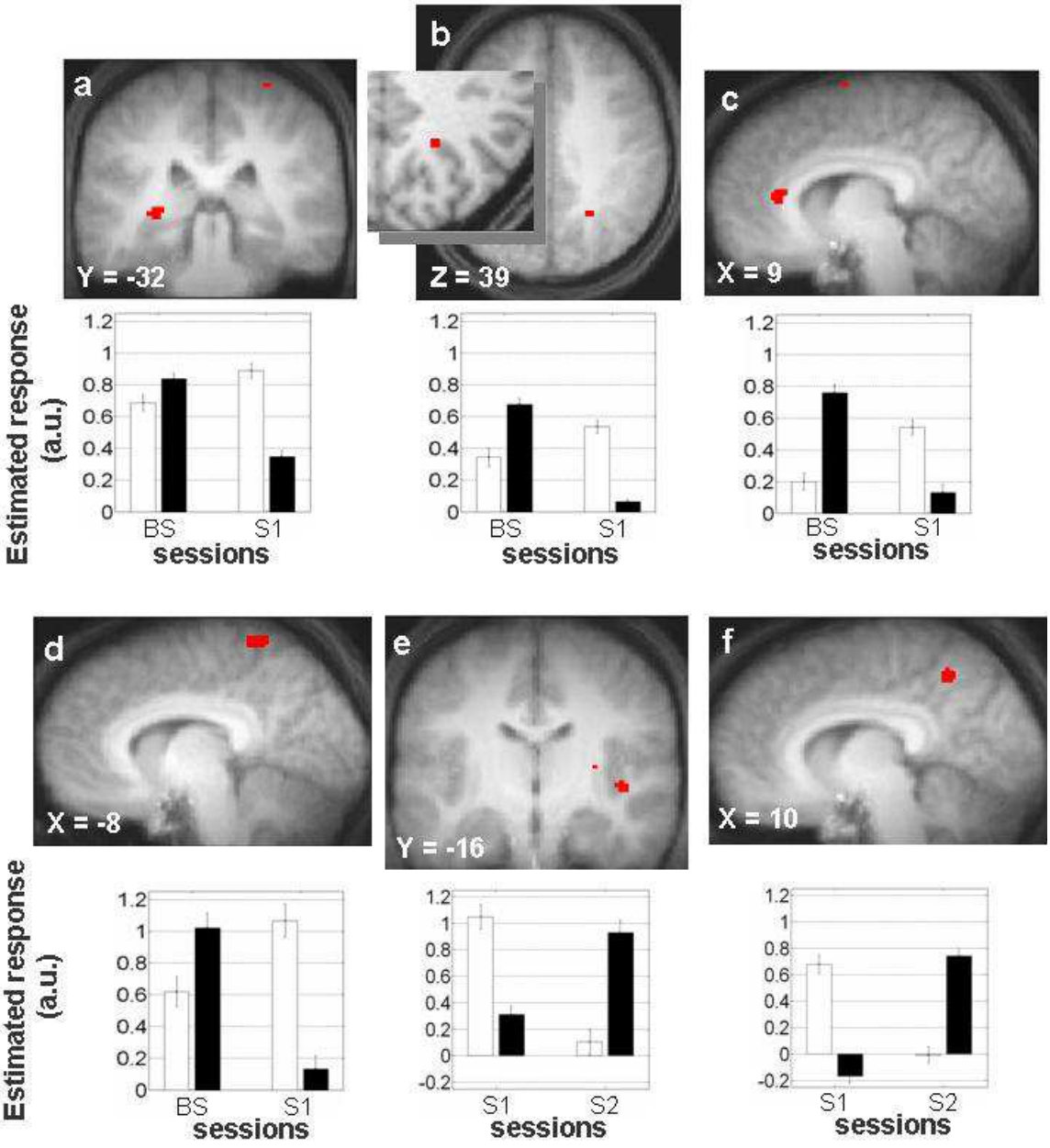

Figure 5.3: Day by session interactions.

Graphs: Mean parameter estimates of the day with light exposure (*empty bars*) and without light exposure (*filled bars*) (a.u. ± SEM). ***BS:*** baseline session; ***S1-2:*** post-exposure sessions 1 and 2. ***a-d:*** day by session (post 1 > baseline) interactions. **e-f:** day by session (post 2 > baseline) interactions. ***a.*** left hippocampus; ***b.*** rIPS (inset: enlarged parietal region in a representative subject); ***c.*** right anterior cingulate; ***d.*** left precuneus; e. rSTG; ***f.*** right precuneus. In all figures, statistical results are overlaid to the population mean structural image ($p_{uncorrected} < 0.001$).

Time modulation
(a.u.)
Estimated response
(a.u.)
a
Y = 20
b
X = 29
c
X = 13
d
X = 8
scans
sessions
BS
S1
S2
1
2

Importantly, no significant increase in response was observed in the 2nd post-exposure session ($p_{uncorrected} < 0.001$; Appendix 1: supplemental table S5.3). These findings suggest that the effects of light exposure largely dissipated within 10 minutes after the end of the light exposure, similarly to alertness which was only transiently enhanced by light exposure. Finally, no decrease in brain response was elicited by light exposure.

We then looked for brain areas in which responses would dynamically dissipate within sessions. Such changes would not necessarily give rise to significant changes in activity when averaged over a whole session and would not appear in between-session contrasts. We therefore compared the within-session temporal modulations of brain responses in post-exposure sessions to baseline. Within the set of areas where a significant temporal modulation was detected, we considered only regions in which mean parameters estimates were consistent with an effect of light counteracting the decrease in activity induced by continuous darkness (Figure 5.4, lower panels). In these conditions, any negative modulation of activity by time can arguably be interpreted as a dissipation of the effects following light exposure (Appendix 1: supplemental tables S5.4 and S5.5).

The day by session (post 1 > baseline) interaction computed on brain responses modulated by time identified 5 regions (Figure 5.4): the right insula (40 20 8; $Z = 4.48$; $p_{svc} = 0.002$), right posterior cingulate cortex (8 –26 42; $Z = 3.35$; $p_{svc} = 0.049$),

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Figure 5.4: Day by session interaction computed on the brain responses modulated by time.

Upper panels. Day by session (post 1 > baseline) interactions (red voxels). Panel *a* also shows light condition by session (post 2 > baseline) interactions in green (yellow for overlapping voxels). ***a.*** right insula; ***b.*** right posterior cingulate; ***c.*** rSPL; ***d.*** rDLPFC (left) and right fusiform gyrus (right).

Middle panels. Reconstruction of the modulation of the response in the 1st post-exposure session, of the day with light exposure (arbitrary units) over the course of 230 scans (~ 8 minutes). In panel *a*, the dotted line pertains to the 2nd post-exposure session. Temporal modulation of the BOLD response was reconstructed by the sum of both time modulators weighted by their respective mean parameter estimates.

Lower panels. Mean parameter estimates in the baseline (*BS*) and 1st post-exposure (*S1*) sessions, and 2nd post-exposure session (*S2*) for panel *a*, of the day with light (*empty bars*) and without light exposure (*filled bars*) (a.u. ± SEM).

right superior parietal lobe (rSPL; 14 –44 76; $Z = 4.23$; $p_{svc} = 0.007$), right dorso-lateral prefrontal cortex (rDLPFC; 28 12 42; $Z = 3.50$; $p_{svc} = 0.046$), and right fusiform gyrus (34 -84 -16; $Z = 3.99$; $p_{svc} = 0.009$). In all these regions, responses decreased more quickly after light exposure than during continuous darkness, as compared to baseline. The computed temporal modulation (Figure 5.4, middle panels) shows that responses were never maintained at initial post-light levels for more than 50 scans (~100 seconds). A similar temporal modulation was identified, again in the right insula (40 18 6 mm; $Z = 3.71$; $p_{svc} = 0.019$), by the day by session (post 2 > baseline) interaction (Figure 5.4a, dotted line). These results indicate that the dissipation of the responses to light exposure follows multiple region-specific time courses.

The oddball task engages cognitive processes such as auditory perception, attention and working memory (Halgren *et al.*, 1998; Kiehl *et al.*, 2001; Stevens *et al.*, 2000). Light modulated responses in the right SPL, DLPFC and IPS, each part of the top-down attention network, and in the right insula, anterior cingulate and STG, each involved in the bottom-up reorientation of attention towards low-frequency events (Corbetta and Shulman, 2002; Halgren *et al.*, 1998). Light also induced changes in the left hippocampus, involved in perception, identification and integration of the stimulus, processes in which the superior temporal sulcus and rIPS are also involved (Halgren *et al.*, 1998; Stevens *et al.*, 2000; Strange *et al.*, 1999). The fusiform gyrus, precuneus and posterior cingulate cortex are typically reported in oddball fMRI and their responses were also modulated by light (Huettel *et al.*, 2004; Kiehl *et al.*, 2001; Stevens *et al.*, 2000).

In our final fMRI data analysis, we extended our cohort to cover people who did not show an alerting response to light. In the 4 non-responder subjects, discarded from the analyses characterizing the effect of light exposure, KSS scores did not show any alerting effects of light exposure. We computed a non-parametric Mann-Whitney U-test on 2 independent groups (responders: N = 12; non-responders: N = 4) over subjects' mean reaction times across repetition of oddball sessions in each visit. This test revealed that reaction times of non-responders were faster than responders during the day with light exposure (for the baseline session, 1st post exposure session, as well as very first session and 2nd session of the light exposure period: $Z_{corrected} = 2.18$; $p = 0.03$ – for 2nd post-exposure session as well as 1st session of the light exposure period: $Z_{corrected} =$

1.94; p = 0.06) and in 4 of the 6 sessions of the day without light exposure (baseline session: $Z_{corrected}$ = 2.30; p = 0.019 – 2nd post-exposure session, as well as the very first session and the 2nd session of the light exposure period: $Z_{corrected}$ = 1.81; p = 0.08 – 1st post exposure session and 1st session of the light exposure period: $Z_{corrected}$ = 1.21; p = 0.26).

These data suggest that non-responders remained very alert at all times and no effect of light on alertness could possibly be observed. We therefore wanted to establish the relationship between the light-dependent modulation of evoked responses and variation of alertness at the between-subject level. To summarize alertness variations we used the principal eigenvariate (following a principal component analysis of the KSS scores). This eigenvariate is a scalar summary of the degree to which each subject follows the course of the principal eigenvector, which accounted for 68.49% of alertness variance (inset Figure 5.5a).

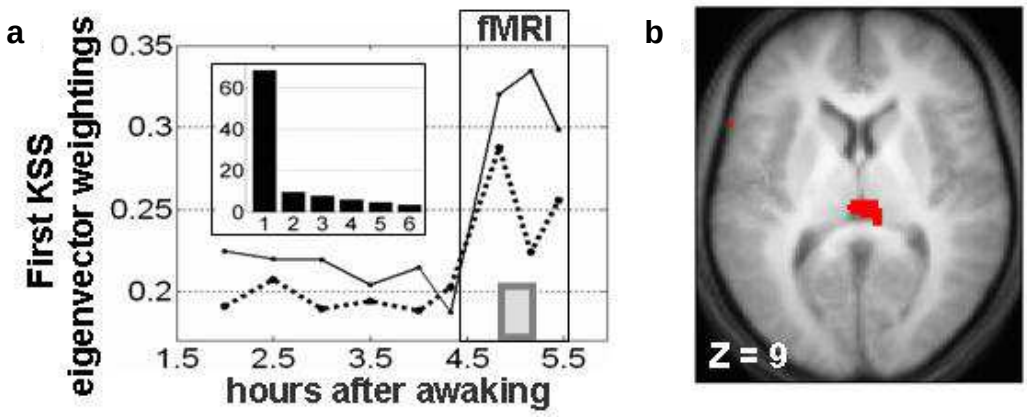

Figure 5.5: Interaction of light and alertness.

a. Profile of the 1st eigenvector of the singular value decomposition on KSS scores. *Solid line*: day without light exposure. *Dotted line*: day with light exposure. *Inset:* Percentage of variance explained by the six first components.

b. Day by session (post 1 > baseline) interaction related to the 1st eigenvector in the pulvinar.

Responses identified in the day by session (post 1 > baseline) interaction were significantly related to the 1st eigenvariate in a single area of the thalamus, in a location compatible with the pulvinar (-2 -24 8; *Z* = 4.11; p_{svc} = 0.003; Figure 5.5b and Appendix 1: supplemental table S5.6), an area distinct from the brain regions reported in the other

analyses. The thalamus is a key structure modulating alertness, involved in the interaction between alertness and attention in humans (Coull *et al.*, 2004; Foucher *et al.*, 2004). This result indicates that the change in thalamic response to odd tones after light exposure is linearly related to alertness variation induced by light exposure, independently of whether light induced a behavioral effect in every subject. Owing to and beyond this alerting effect, responses to the cognitive challenge are increased at the cortical level.

Beside the classical visual system, irradiance information is interpreted in mammals by a nonvisual system (Foster, 2005) that generates a wide range of physiological responses, such as the modulation of alertness (Badia *et al.*, 1991; Cajochen *et al.*, 2005; Cajochen *et al.*, 2000), hormone secretion (Cajochen *et al.*, 2005; Cajochen *et al.*, 2000; Dijk and Lockley, 2002), heart rate, sleep latency, core body temperature (Badia *et al.*, 1991; Cajochen *et al.*, 2005; Cajochen *et al.*, 2000; Dijk and Lockley, 2002), retina neurophysiology (Hankins and Lucas, 2002), pupillary constriction (Lucas *et al.*, 2001a), and gene expression in the SCN (Dkhissi-Benyahya *et al.*, 2000).

The light-induced modulations of brain responses to odd tone detection arguably represent still another type of nonvisual responses. It is unlikely that the classical visual system might interfere with a pure auditory task and modulate the responses elicited by the detection of odd tones, presented in a stream of frequent tones, after the light exposure has ended. In addition, the light-induced modulation of brain responses presents two basic features of nonvisual responses: they are induced by, and they outlast, light exposure. Classical visual response to light typically cease very shortly after the end of the stimulation. Even in the retina, cones or rods respond to light stimulation in a stimulus-locked manner. In contrast, light pulses of a few seconds induce a sustained response which outlasts the light stimulus and declines slowly in melanopsin expressing ganglion cells, photoreceptors in the nonvisual system (Dacey *et al.*, 2005). Both classical and non-classical photoreceptors contribute to nonvisual response in rodents (Hattar *et al.*, 2003). As the white light source covered the whole visible spectrum and included ~3 times more photons in the photopic than in the nonvisual range, classical and melanopsin photoreceptors were differentially stimulated

(Foster, 2005). However, we cannot determine the relative contribution of each type of photoreceptors to the brain responses modulation.

The present results confirm and extend our previous PET results. First, this fMRI study shows that short white light exposure affects brain function also during daytime. Second, event-related fMRI characterizes transient cerebral responses to a cognitive challenge (Josephs and Henson, 1999), which implies that only areas involved in odd tone detection could be identified, whereas PET characterized enduring light-induced changes in functional states of the brain, related or not to the ongoing task. Third, fMRI, due to its better temporal and spatial resolutions, allowed us to show that light exposure elicits effects on brain activity that quickly dissipate following region-specific time courses. While the topography of brain responses depends on the task executed by the participants, the multiple dynamics of the light-induced modulations in regional brain responses might represent a general phenomenon.

Melanopsin expressing RGC project to several hypothalamic regions, including the SCN (Gooley *et al.*, 2003). In rodents, indirect projections from the SCN to cell groups involved in arousal regulation exist in the forebrain and brainstem (Deurveilher and Semba, 2005; Saper *et al.*, 2005a). At present, it is not known which of these projections contribute to the establishment of a cortical response to light exposure. It is likely that the initial nonvisual responses activate brainstem and/or diencephalic structures, which in turn modulate thalamic, then cortical responses. The direct projections of the melanopsin RGC to the LGN (Dacey *et al.*, 2005), if also present in human, might also be the natural pathways followed by irradiance information to influence thalamic and indirectly, cortical activity.

Conclusion

A short exposure to bright light can transiently prevent the sleepiness developed in continuous darkness. At the macroscopic systems level, the alerting effect of light is reflected by an enhanced thalamic activity, which in turn might modulate cortical responses to a cognitive challenge, independently from any visual information. The enhanced brain responses outlast the exposure but quickly dissipate following regionally-specific time courses.

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6. Wavelength-dependent modulation of brain responses to a working memory task by daytime light exposure

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Introduction

Whereas the classical visual system generates images of the external world, another nonvisual system (also referred to as "non-image-forming" system) detects variations in ambient irradiance and elicits a wide range of responses. These responses include long-term modifications of circadian rhythms and acute changes in hormone secretion, heart rate, sleep propensity, alertness, CBT, retinal neurophysiology, pupillary constriction, and gene expression (Badia *et al.*, 1991; Brainard *et al.*, 2001a; Cajochen *et al.*, 2005; Dijk and Lockley, 2002; Dkhissi-Benyahya *et al.*, 2000; Duffy *et al.*, 1996; French *et al.*, 1990; Hankins and Lucas, 2002; Lockley *et al.*, 2003; Lockley *et al.*, 2006; Lucas *et al.*, 2001a). Converging evidence derived from classical physiology techniques, such as determination of wavelengths of maximum sensitivity (action spectra), and molecular genetic techniques, such as genetic ablation of rods and cones in rodents, point to the unique characteristics and neuroanatomical basis of the nonvisual system (Brainard *et al.*, 2001a; Hankins and Lucas, 2002; Lucas *et al.*, 2001a; Thapan *et al.*, 2001). Its

[*] The supplemental materials published online with this article are included in the body of this chapter and in appendix 2.

wavelength of maximum sensitivity is shifted to shorter wavelengths (blue light) compared to the classical visual system in both animals and humans. The nonvisual system depends on input from both RGC expressing melanopsin (Berson *et al.*, 2002; Dacey *et al.*, 2005; Hattar *et al.*, 2002) and the classical visual photoreceptors (Hattar *et al.*, 2003). Melanopsin is a recently discovered photopigment (Provencio *et al.*, 2000) that is most sensitive to blue light at a wavelength ranging from 420nm to 480nm, depending on the study considered (Melyan *et al.*, 2005; Panda *et al.*, 2005; Qiu *et al.*, 2005). The melanopsin expressing RGC transmits irradiance signals to hypothalamic nuclei such as the suprachiasmatic nuclei (SCN), as well as to a number of non-hypothalamic structures (*e.g.* superior colliculi, LGN, medial amygdala), suggesting that the melanopsin dependent photoreception system modulates many brain functions (Gooley *et al.*, 2003; Hattar *et al.*, 2006). However, its action on cortical function has not been studied extensively.

Although it is often stated that light affects behavior and cognition in humans, few studies have been devoted to studying these effects. White light has been shown to improve subjective alertness and performance on simple tasks such as reaction time, digit recall, two letter search and simple problem solving both during night and daytime (Badia *et al.*, 1991; Campbell and Dawson, 1990; French *et al.*, 1990; Phipps-Nelson *et al.*, 2003). To date only 2 neuroimaging studies, using positron emission tomography (PET) (Perrin *et al.*, 2004) and fMRI (Vandewalle *et al.*, 2006) characterized the neural correlates of the nonvisual effects of white light exposure. Two studies have shown that a blue-light sensitive photoreception system modulates the effect of light on alertness and reaction times (Cajochen *et al.*, 2005; Lockley *et al.*, 2006). These latter studies, however, did not include brain imaging and the neural correlates of the effects of blue light remain unknown. Furthermore, there is currently no direct evidence that light exposures of wavelengths close to the maximum sensitivity of the melanopsin-dependent photoreception system (blue ~470 nm), or of the classical three cone photopic system (green 550 nm) elicit different nonvisual brain responses to a complex cognitive task. In the present fMRI study, we aimed at demonstrating that the spectral quality of light influences the activity in brain areas involved in executive functions, even during daytime, a time at which humans are naturally exposed to abundant light.

Materials and Methods

Subjects

Participants were healthy, young subjects (N = 18; 10 females; age: 18-29 [median: 23]; Body Mass Index (BMI): 18.7-29.7 [median: 22.85]). A semi-structured interview established the absence of medical, traumatic, psychiatric, or sleep disorders. Absence of color blindness was assessed by the 38 plate edition Ishihara's Test for Color-Blindness (Kanehara Shupman Co., Tokyo, Japan). All participants were non-smokers, moderate caffeine and alcohol consumers, and were not on medication. None had worked on night shifts during the last year or traveled through more than one time zone during the last 2 months. Extreme morning and evening types, as assessed by the Horne-Ostberg Questionnaire (Horne and Ostberg, 1976), were not included. None complained of excessive daytime sleepiness as assessed by the Epworth Sleepiness Scale (Johns, 1991) and of sleep disturbances as determined by the Pittsburgh Sleep Quality Index Questionnaire (Buysse *et al.*, 1989). All participants had normal scores at the 21 item Beck Anxiety Inventory (Beck *et al.*, 1988) and at the 21 item Beck Depression Inventory II (Steer *et al.*, 1997). They were right-handed as indicated by the Edinburgh Inventory (Oldfield, 1971). Participants gave their written informed consent and received a financial compensation for their participation. The study was approved by the Ethics Committee of the Faculty of Medicine of the University of Liège.

Volunteers followed a 7-day regular sleep schedule before their first visit and kept the same schedule for 2 more days, until their second visit. Compliance to the schedule was assessed using wrist actigraphy (Actiwatch, Cambridge Neuroscience, UK) and sleep diaries. In order to record 2 volunteers on the same day at approximately the same circadian time, volunteers were requested to follow one of 2 sleep schedules differing by 1.5h (2300h-0700h +/- 30min, or 0030h-0830h +/- 30min). Volunteers were requested to refrain from all caffeine and alcohol-containing beverages and intense physical activities for 3 days before participating to the study.

Protocol

Volunteers completed the protocol on two separate days (Figure 6.1). The experimental paradigm was identical on both days, except for the monochromatic light exposure condition (blue or green), the order of which was counterbalanced.

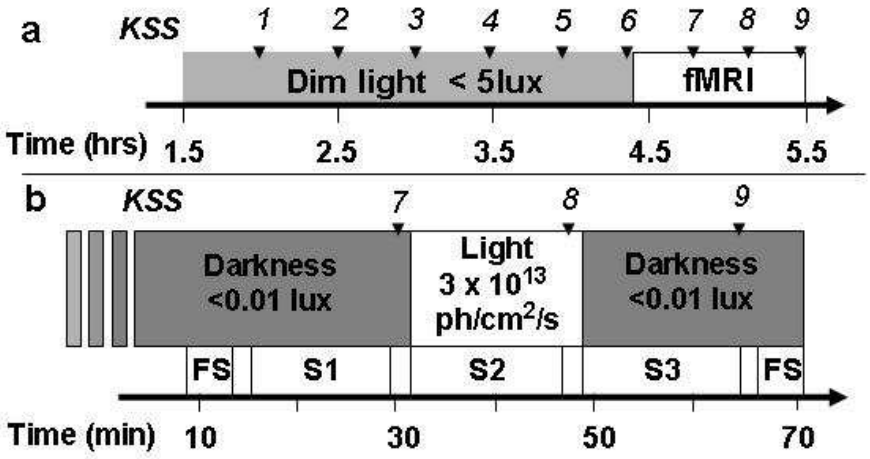

Figure 6.1: Experimental design.
a. General timeline. Time relative to scheduled wake time (hrs). *Arrows:* subjective sleepiness assessment (*KSS 1-9)*.
b. Timeline of the fMRI period. *S1-3:* 2-back sessions 1 to 3. *FS:* flanking sessions. Time in minutes after entering the scanner. *Arrows:* subjective sleepiness assessment (*KSS 7-9)*.

On each day, subjects were first maintained in dim light (< 5 lux) for 3h and then scanned during 3 consecutive sessions which were timed before (session 1; < 0.01 lux), during (session 2) and after (sessions 3; < 0.01 lux) one eye was exposed for 18 minutes (durations varied slightly, see Results) to a blue (470nm) or a green (550nm) monochromatic light. The photon densities of both light exposures were identical (3 x 10^{13} photons/cm²/s) so that blue light stimulation of the melanopsin dependent photoreception system would be equal to the stimulation of the classical photoreception systems elicited by green light during the other visit. Light exposure occurred approximately 5 hours after habitual wake up time, i.e. during the biological day when melatonin secretion is low (Dijk and Lockley, 2002). During every session, participants performed an auditory *2-back* working memory task (Braver *et al.*, 2001), which does

not explicitly depend on visual input, and is reliably executed by a majority of subjects. Subjective alertness scores, as assessed by the Karolinska Sleepiness Scale (KSS) (Akerstedt and Gillberg, 1990), were collected every 30 minutes during the 3h preparatory period and every 20 minutes while in the scanner.

Participants performed the 2-back task during two 3-minute flanking sessions placed at the beginning and at the end of the fMRI acquisition period. The first flanking session allowed enough time for physiological events related to recent postural changes (sitting, walking to the fMRI scanner, standing for a few minutes, and then lay down in supine position) to dissipate (Bonnet and Arand, 1998). The latter events can influence arousal and might have otherwise contaminated our data. The second flanking session took into account potential participants' expectancies about the end of the experiment, which might change their motivational and arousal state. Participants were unaware of the duration of this last flanking session and were told its duration could vary substantially.

During the data acquisition period, all subjects interacted with the same investigator who used a standardized set of sentences between every *2-back* sessions. This protocol was implemented in order to minimize variation in motivational state due to social interactions (*e.g.* encouragement by an investigator which may modify brain responses; cf. Grandjean *et al.*, 2005). No feedback was given on performance. Volunteers received a small standardized snack in the middle of the 3-hour preparatory period preceding fMRI data acquisition. They were trained on a shortened version of the protocol and habituated to the experimental conditions at least a week before the experiment. Subjects had to reach 75% of correct responses on the 2-back task at the end of training to participate to the experiment.

2-back task

Stimuli consisted of 9 French monosyllabic consonants that were phonologically different so that they could easily be identified. Stimuli were 500ms long and inter-stimulus-interval was 3000ms long. For each consonant, volunteers were requested to state whether or not it was identical to the consonant presented 2 stimuli earlier, by pressing a button on a keypad for "yes" and another one for "no". Thirty-four series of 25 to 30 stimuli were constructed with ~30% of positive answers. Inter-series-intervals

lasted 10 to 25 seconds. Series were presented only once per visit and were randomly assigned to one of the scanning sessions. In both visits, the number of series in each session varied as follow: flanking sessions consisted of 2 series, session 1 of 9 series, session 2 of 10 series, and session 3 of 11 series. Stimuli were produced using COGENT 2000 (http://www.vislab.ucl.ac.uk/Cogent/) implemented in MATLAB (Mathworks Inc., Sherbom, MA) on a 2.8 GHz XEON DELL personal computer (Round Rock, TX) and were transmitted to the subjects using MR CONTROL amplifier and headphones (MR Confon, Germany). On both visits, the first session was preceded by a short session during which volunteers had to set the volume level to ensure an optimal auditory perception during scanning.

Light exposure

In a previous fMRI study, we reported that 21 minutes of white light exposure (> 7000 lux) was sufficient to counteract the decrease in alertness and brain activity otherwise observed in continuous darkness (Vandewalle *et al.*, 2006). However, we could not easily separate the changes in responses related to the light-related increase in alertness from the effect of light *per se.* We specifically designed the present study in order to avoid the confounding effects of variation in alertness and performance. First, we used a monochromatic light stimulus with a photon density about a hundred times lower than in our previous fMRI study. Second, only one eye was exposed. Previous investigations demonstrated additivity of binocular compared to monocular illumination (Brainard *et al.*, 1997). Third, the monochromatic light exposure was limited to 18 minutes, a short exposure as compared to previous studies investigating the effect of monochromatic light on behavior (Cajochen *et al.*, 2005; Lockley *et al.*, 2006) and melatonin secretion (Brainard *et al.*, 2001a; Lockley *et al.*, 2003; Thapan *et al.*, 2001). Thus the total number of photons administered in our study is 10 to 15 times smaller than in behavioral investigations (Cajochen *et al.*, 2005; Lockley *et al.*, 2006) and most endocrine studies (Brainard *et al.*, 2001a; Lockley *et al.*, 2003), but not all (Thapan *et al.*, 2001). Using this experimental strategy, we were aiming to characterize the changes in brain responses independent of behavioral changes.

Narrow interference band-pass filters (FWHM: 10nm; Edmund Optic, UK) were used to produce two monochromatic illuminations at 470nm and 550nm. The exposed

eye and monochromatic light exposure were assigned pseudo-randomly in a counterbalanced manner. The light was transmitted by a metal-free optic fiber from a source (PL900, Dolan-Jenner Industries, MA) to a small diffuser placed in front of the subjects' eye. The diffuser was designed for the purpose of this study and ensured a uniform illumination. Light was administered through a 4 x 5.5 cm frame placed 3 to 4 cm away from the eye. Irradiance could not be measured directly in the magnet, but the light source was calibrated and irradiance estimated to be 3×10^{13} photons/cm²/s (840-C power meter, Newport, Irvine, CA). The non-illuminated eye of the subject was monitored at all times using an infra-red eye-tracking system (ASL, Model 504; Applied Science Group, Bedford, MA). The images of the eye-tracking system were monitored on-line, video-taped, and examined in order to ensure that all volunteers included in the analyses had their eyes open at all time and were looking toward the light during the illumination.

Functional MRI data acquisition

Functional MRI time series were acquired using a 3T MR scanner (Allegra, Siemens, Germany). Multislice T2*-weighted fMRI images were obtained with a gradient echo-planar sequence using axial slice orientation (32 slices; voxel size: 3.4x3.4x3 mm^3; matrix size 64x64x32; repetition time = 2130ms; echo time = 40ms; flip angle = 90°). The 4 initial scans were discarded to allow for magnetic saturation effects. There was little variation in the number of scans of the homologous sessions of both visits (1^{st} flanking sessions: 95.3 ± 4.2 (mean ± SD); sessions 1: 408.6 ± 8.3; sessions 2: 454.6 ± 7.1; sessions 3: 506.8 ± 7.6; 2^{nd} flanking sessions: 96.6 ± 3.5). Head movements were minimized using a vacuum cushion. A structural T1-weigthed 3D MP-RAGE sequence (TR 1960ms, TE 4.43ms, TI 1100 ms, FOV 230 x 173 cm², matrix size 256 x 256 x 176, voxel size: 0.9x0.9x0.9mm) was also acquired in all subjects.

Functional MRI data analysis

Functional volumes were analyzed using Statistical Parametric Mapping 2 (SPM2 - http://www.fil.ion.ucl.ac.uk/spm) implemented in MATLAB. They were corrected for head motion, spatially normalized (standard SPM2 parameters) to an echo planar imaging template conforming to the MNI space, and spatially smoothed with a Gaussian Kernel of 8 mm FWHM. The analysis of fMRI data, based on a mixed effects model,

was conducted in 2 serial steps, accounting respectively for fixed and random effects. For each subject, changes in brain regional responses were estimated using a general linear model in which the activity evoked by the 2-back series in each session was modeled by boxcar functions, convolved with a canonical haemodynamic response function. As we reported previously (Vandewalle *et al.*, 2006), the dynamics of the light-induced modulations of brain activity is fast in some areas. Such rapid changes do not necessarily give rise to significant changes in activity when averaged over a whole session and consequently, do not appear in between-session contrasts. We therefore added two further regressors in our analyses, representing the modulation of brain responses to the 2-back series by linear and quadratic time. We used these regressors to compare the within-session modulation of brain responses by (linear and quadratic) time in the different sessions in order to identify any nonvisual brain response that would build-up and dissipate with time after lights were turned on and off, respectively. Movement parameters derived from realignment of the functional volumes were included as covariates of no interest. High-pass filtering was implemented in the matrix design using a cut-off period of 128 seconds to remove low frequency drifts from the time series. Serial correlations in fMRI signal were estimated using an autoregressive (order 1) plus white noise model and a restricted maximum likelihood (ReML) algorithm.

The effects of interest were then tested by linear contrasts, generating statistical parametric maps [(SPM(T)]. Since no inference was made at this (fixed effects) level of analysis, summary statistic images were thresholded at $p_{uncorrected} = 0.95$. The summary statistic images resulting from these different contrasts were then further smoothed (6 mm FWHM Gaussian kernel) and entered in a second-level analysis. This second step accounts for inter-subject variance in the main effects of light (random effects model) and corresponds to a one-sample *t*-test for brain responses to the 2-back series. Both time modulators were included in a separate parametric within-subject one-way ANOVA. For the latter analysis, the error covariance was not assumed independent between regressors and a correction for non-sphericity was used for final inferences (Glaser and Friston, 2004). The resulting set of voxel values for each contrast constituted maps of the T statistics for the main responses and F statistics when they were modulated by time, thresholded at $p_{uncorrected} = 0.001$. Statistical inferences were

performed after correction for multiple comparisons on small spherical volumes (svc; 10 mm radius) at a threshold of $p_{svc} = 0.05$, around *a priori* locations of activation in structures of interest, taken from published work on *n-back* tasks and executive processing, multimodal binding, and from our own work on the effects of white light on brain responses in fMRI.

Before performing any svc, peaks reported in Talairach (Talairach and Tournoux, 1988) space were transformed to MNI space using Matthew Brett's bilinear transformation (http://imaging.mrc-cbu.cam.ac.uk/imaging/MniTalairach; no coordinates were shifted more than 5 mm). Standard stereotactic coordinates of previously published *a priori* locations, used for spherical svc, are as follow:

Locations involved in working memory and executive functions: left IPS -26 -58 47 (Collette *et al.*, 2005), -20 -66 46, -20 -66 48 (Wager *et al.*, 2004), -12 -71 47 mm; right insula 32.32 22.44 5.53 (Cohen *et al.*, 1997) (transformed to MNI space), 40 16 2 (Wager *et al.*, 2004); left thalamus -8 -12 -11; left supramarginal gyrus -38 -50 42 (Wager and Smith, 2003), -40.40 -51.68 45.15 (Cohen *et al.*, 1997) (transformed to MNI space); left middle frontal gyrus -43 24 27 (Braver *et al.*, 2001), -40 22 21 (Cohen *et al.*, 1997).

Locations involved in multimodal activation/cross-modal binding: left thalamus (Bushara *et al.*, 1999) -14 -20 8; right insula 36 24 -4, 38 22 -6 (Bushara *et al.*, 2001); left inferior parietal lobule -44 -38 42 (Bushara *et al.*, 1999).

Location modulated by white light exposure: right insula 40 20 8 (Vandewalle *et al.*, 2006).

Masking procedures

In all analyses, we excluded brain areas that were not recruited by the 2-back task from all the interaction analyses, by masking our results with a map of all regions that showed any positive response to the task (inclusive mask $p_{uncorrected} = 0.9$). In the light condition (blue > green) by session (2 > 1) interaction we applied an exclusive mask for baseline differences (session 1 green > session 1 blue; $p_{uncorrected} = 0.05$) in order to rule out possible confounds arising from these differences. In the light condition (blue > green) by session (2 > 3) interaction we also applied an exclusive mask for differences at the end of the visits (session 3 green > session 3 blue; $p = 0.05$ *uncorrected*), which

ruled out possible confounds arising from these differences. In order to verify which effect contributed to the light condition (blue > green) by session (2 > 3) interaction we employed two independent masks. We applied a mask ($p_{uncorrected} = 0.05$) including areas for which activity decreased from the 2nd to the 3rd session during the blue light condition. Interaction effect in the regions remaining after the application of this mask would be mostly related to the latter decrease in activity in the blue light condition. A second verification employed another mask ($p_{uncorrected} = 0.05$) excluding areas for which activity increased form the 2nd to the 3rd session of the green light condition. Interaction effect in the regions remaining after the application of this mask would not be mostly related to the latter increase in activity in the green light condition.

Bayesian inferences and posterior probability maps

In the random-effects analyses, we aimed at verifying that the absence of significant statistical effects in one contrast in a location of the brain was not merely due to an error of type II (false negative). We computed PPM enabling conditional or Bayesian inferences about regionally specific effects (Friston and Penny, 2003), which provide the posterior distribution of an activation given the data. PPM and effect size were computed for response to the 2-back series in the light condition (blue > green) by session (3 > 1) interaction to verify the absence of remaining light modulation in the post light exposure period. We estimated the posterior probabilities for each of the regions we reported in the light condition (blue > green) by session (2 > 1 and 2 > 3) interactions. PPM were also computed on the second sessions of both visits, in order to check that no activation was present in the occipital cortex during the illumination periods.

Results

Behavior

The length of the 2-back series and the pauses between series varied. Session duration changed therefore a little between visits and subjects. Light exposure in the blue light condition lasted 17 min 45s on average (range: 17 min 20s to 18 min 13s; median: 17 min 49s). Light exposure in the green light condition lasted 17 min 54s on average (range: 17 min 37s to 18 min 28s; median: 17 min 50s). In order to rule out placebo or expectancy effects, we debriefed the participants about their color preferences. Nine

subjects preferred the blue illumination, 8 preferred the green light condition and one had no preference. Therefore, differences in light exposure duration and in expectation or placebo effects are unlikely to have biased the results of this experiment.

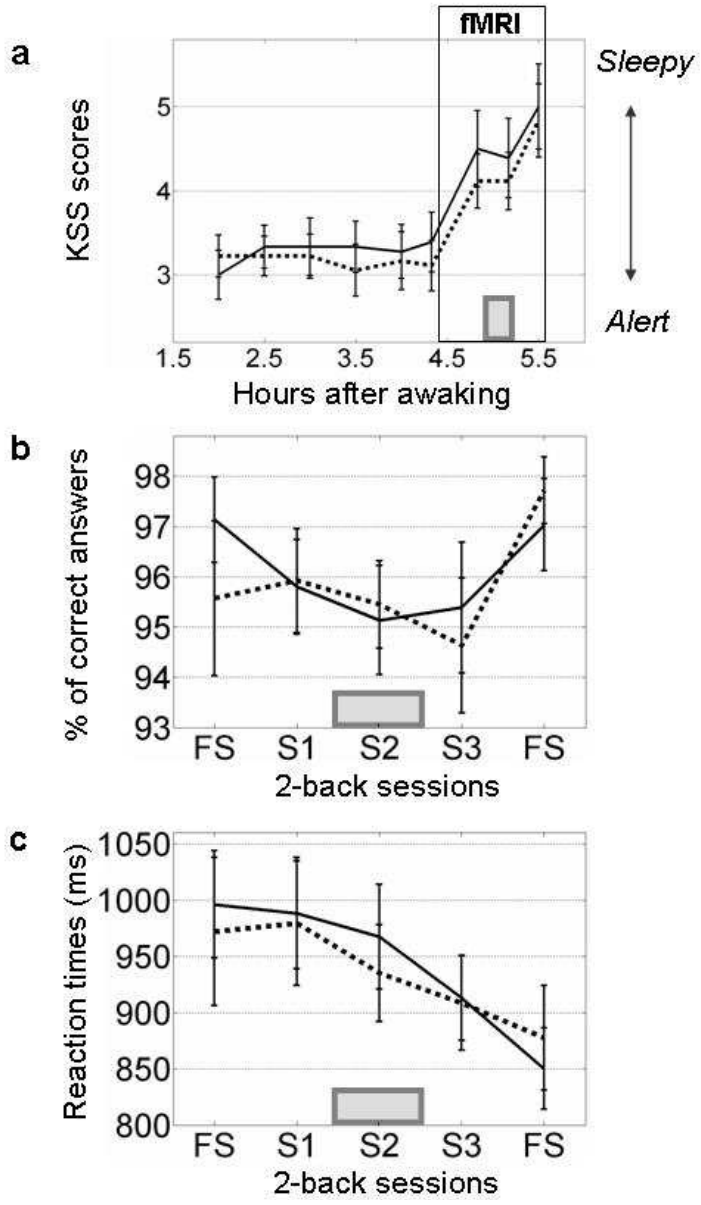

Figure 6.2: Behavioral results.

Solid line: blue light condition; *dotted line:* green light condition; *gray rectangle:* light exposure period.

a. Mean KSS scores (± SEM). *Box:* fMRI period. Time relative to scheduled wake time (hrs).

b. Mean accuracy (± SEM). *S1-3:* 2-back sessions 1 to 3. *FS:* flanking sessions.

c. Mean reaction times (± SEM). *S1-3:* 2-back sessions 1 to 3. *FS:* flanking sessions.

Repeated measure ANOVA on KSS scores (Figure 6.2a) with repetition and day (blue > green) as within-subject factors revealed main effects of repetition (F-value = 9.95; df = 8, 136; p-value < 10-6), but no main effects of day (F-value = 2.07; df = 1, 17; p-value = 0.17), and no day by repetition interaction (F-value = 0.47; df = 8, 136; p-value = 0.87). Although light did not significantly affect alertness, it seemed to counteract the increase in subjective sleepiness observed in KSS scores on both days.

Subjects were instructed to be as accurate as possible and that at least 75% of correct responses were requested. Repeated measures ANOVA on accuracy scores (Figure 6.2b) with session and day (blue > green) as within-subject factors revealed main effects of session (F-value = 4.66; df = 4, 68; p-value = 0.002), but no main effects of day (F-value = 0.29; df = 1, 17; p-value = 0.60), and no day by session interaction (F-value = 0.85; df = 4, 68; p-value = 0.50). Although subjects were not instructed to be as fast as possible, reaction times were analyzed. Repeated measure ANOVA on reaction times (Figure 6.2c) with session and day (blue > green) as within-subject factors revealed main effects of session (F-value = 19.51; df = 4, 68; p-value < 10-6), but no main effect of day (F-value = 0.04; df = 1, 17; p-value = 0.84), and no day by session interaction (F-value = 0.75; df = 4, 68; p-value = 0.56).

FMRI data

Therefore, as intended, when fMRI data were considered, any difference in brain activity between visits could only be attributed to the behavioral differences between light conditions. We first aimed at characterizing the wavelength-specific time courses of brain responses from sessions 1 to 3. We therefore computed 2 separate light

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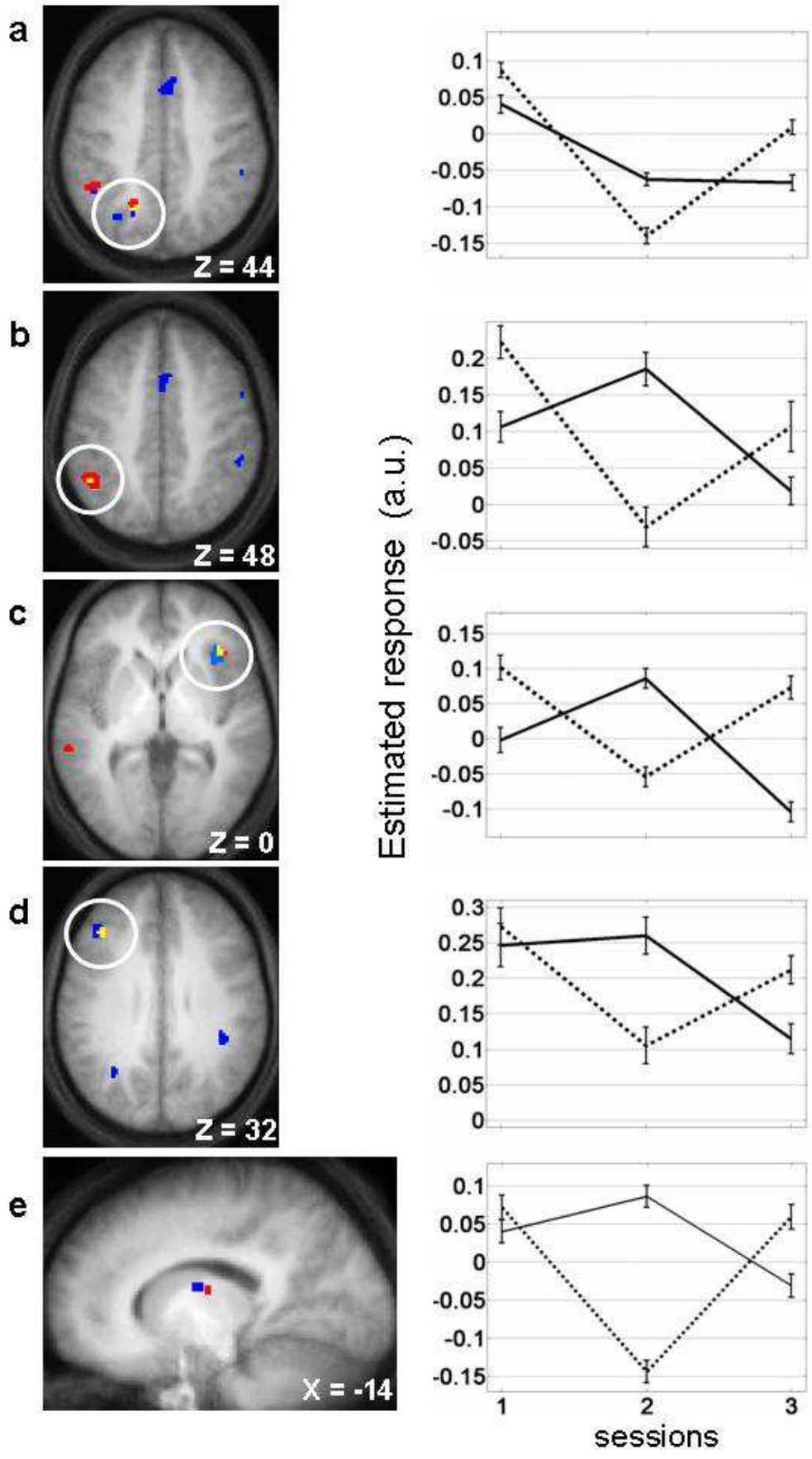
Figure 6.3: Comparison of the brain modulations observed during blue light condition (470nm) and green light condition (550nm).

a. left intraparietal sulcus; ***b.*** left supramarginal gyrus; ***c.*** right insula; ***d.*** left middle frontal gyrus; ***e.*** left thalamus.

***Left panels*:** responses are displayed over the mean structural image of all subjects ($p_{uncorrected}$<0.001). The light condition (blue > green) by session (2 > 1) interaction is displayed in red. The light condition by session (2 > 3) interaction is displayed in blue. Overlaps are in yellow.

***Right panels*:** mean parameter estimates in the 1st, 2nd and 3rd sessions (a.u. ± SEM). *Solid line:* blue light day; *dotted line:* green light day.


a
Z = 44
b
Z = 48
c
Z = 0
d
Z = 32
e
X = -14
Estimated response (a.u.)
0.1
0.05
0
-0.05
-0.1
-0.15
0.2
0.15
0.1
0.05
0
-0.05
0.15
0.1
0.05
0
-0.05
-0.1
0.3
0.25
0.2
0.15
0.1
0.05
0
0.1
0.05
0
-0.05
-0.1
-0.15
1
2
3
sessions

conditions by session interaction contrasts. The first one compared the differences of brain activity found in both light conditions when comparing the illumination periods (sessions 2) to the baseline sessions (sessions 1) [light condition (blue > green) by session (2 > 1) interaction], while the second one evaluated the differences of brain activity obtained between light conditions when comparing the illuminations to the post-exposure periods (sessions 3) [light condition (blue > green) by session (2 > 3) interaction]. Both interactions revealed significant differences in the left intraparietal sulcus (IPS), left supramarginal gyrus, left middle frontal gyrus (MFG), right insula, and in the left thalamus (Table 6.1 and Figure 6.3; Appendix 2: supplemental table S6.1 and S6.2). The activity estimates (right panels Figure 6.3) showed that blue light exposure prevented the progressive decline in brain responses observed during green light exposure (from 1st to 2nd sessions). As a rule, blue light exposure increased regional responses, as compared to baseline, except in the left IPS.

Activity estimates also revealed that the responses in these regions decreased from the 2nd to the 3rd session during the blue light condition, whereas they increased from the 2nd to the 3rd session of the green light condition. Further analyses (see *Masking procedures* in *Materials and methods*) revealed that in the right insula, left supramarginal gyrus, and left MFG, the significant effects were essentially due to the decrease in response during the post exposure period of the blue light condition. In contrast, in the left IPS and thalamus, the effects were largely influenced by the increase in activity after the green light was switched off.

We then assessed whether the differences in the effects of the light conditions persisted after the light exposures. However no significant difference in brain activity was identified in the contrast comparing the post-exposure sessions to the baseline sessions, suggesting that no differential effects of light conditions remained during the post-exposure period, as compared to baseline. Accordingly, probabilities of activation, as inferred by Bayesian statistics (Friston and Penny, 2003), were low (<22%) in the all 5 areas for which we detected an effect of light exposure during the illumination period.

Importantly, no regions were significantly more deactivated by blue than green light exposure during or after the illumination period, as compared to baseline (Appendix 2: supplemental table S6.3). Likewise, no brain areas were more activated by

blue light as compared to green light exposure after as compared to during the illumination.

Table 6.1. Comparisons of the responses to blue and green light exposures (MNI coordinates)

	Light condition (blue > green) by session (2 > 1) interaction.					**Light condition (blue > green) by session (2 > 3) interaction.**				
Brain regions	***x***	***Y***	***z***	***Z-score***	***p-value (svc)***	***x***	***Y***	***z***	***Z-score***	***p-value (svc)***
Left IPS	-18	-60	44	4.03	0.004	-34 -20	-62 -64	344 2	4.30 3.45	0.027 0.023
Left supramarginal gyrus	-46	-50	48	3.58	0.016	-44	-50	38	3.93	0.005
Left Thalamus	-14	-14	16	3.16	0.049	-10	-4	16	4.16	0.002
Left MFG	-38	32	34	3.63	0.014	-40	32	28	4.20	0.002
Right Insula	40	28	0	3.31	0.033	38	28	0	3.77	0.008

Collectively, our results speak for specific time-limited enhancement in brain responses during blue, as compared to green, light exposure. We point out that blue light exposure has been reported to induce greater pupillary constriction than green light exposure and is consequently associated with reduced light input to the retina (Cajochen *et al.*, 2005). Although we could not assess pupil size in the present study, it is very likely that, if pupillary constriction differed between light conditions, constriction would have been greater under blue light exposure. Consequently, any superiority of blue light in modulating brain responses is unlikely to be related to the effect on pupil size.

Noteworthy, no difference between light conditions were found in the occipital cortex for any of the comparisons. Bayesian statistic inferences confirmed that the probability of activation never exceeded 2% in the occipital cortex in both light

conditions during the illumination period. This finding speaks against the involvement of the classical visual system in the observed effects.

Finally, we did not identify any brain areas where responses changed with time within each session, and differently between light conditions (see *Functional MRI data analysis* in *Materials and methods*). This absence of temporal modulation implies that the light-related differences in brain activity reported above appeared almost immediately after lights were switched on and dissipated very quickly after lights were turned off.

Discussion

The present results demonstrate that brain responses to a complex cognitive task are modulated by light exposure in a wavelength-dependent manner. When compared to a green light exposure of identical photon density, a short exposure to a 3×10^{13} ph/cm^2/s blue light on a single eye during daytime is sufficient to induce almost immediate changes in brain activity. These changes persist for the duration of the exposure, but cease when light is switched off. These findings cannot be accounted for by any measurable difference in alertness or performance, nor by any order or placebo effects. In addition, because the experimental design contrasted two narrow-band monochromatic lights, our findings suggest that the melanopsin dependent photoreception system contributed to modulate these responses.

The light-induced modulation of brain responses were located in structures typically involved in executive functions (Cabeza and Nyberg, 2000; Cohen *et al.*, 1997; Collette *et al.*, 2006). The left MFG, supramarginal gyrus and IPS have been repeatedly implicated in *n-back* tasks. The insula and the thalamus, both in the left and right hemispheres, have been involved in several aspects of working memory (Cabeza and Nyberg, 2000). Areas are mostly located in the left hemisphere in keeping with the left lateralization of verbal working memory (Braver *et al.*, 2001; Collette *et al.*, 2006). The thalamus is a key structure modulating arousal, reported in studies exploring the interplay between alertness and cognition (Coull *et al.*, 2004; Foucher *et al.*, 2004). Additionally, the right insula, left parietal cortex, and thalamus are also involved in visuo-auditory cross-modal binding (Bushara *et al.*, 2003; Bushara *et al.*, 1999; Downar

et al., 2000) and would respond during the performance of an auditory task under visual stimulation.

We previously reported that white light exposure induced nonvisual responses outlasting the illumination period (Vandewalle *et al.*, 2006). In contrast, in the present study, the monochromatic light exposures we used elicited immediate changes in brain responses, which did not outlast the exposure and dissipated swiftly. This reveals a new aspects of the dynamics of the nonvisual responses to light, which, except for pupillary constriction (Lucas *et al.*, 2001a), are typically assumed to develop over tens of minutes (Brainard *et al.*, 2001a; Cajochen *et al.*, 2005; Lockley *et al.*, 2003; Lockley *et al.*, 2006; Thapan *et al.*, 2001). The swift dynamics observed in the present study are probably related to the low dose of light administered.

Our design implies that the melanopsin dependent photoreception system contributed to modulate brain responses to the cognitive task (Brainard *et al.*, 2001a; Dacey *et al.*, 2005; Hankins and Lucas, 2002; Lucas *et al.*, 2001a; Melyan *et al.*, 2005; Qiu *et al.*, 2005; Thapan *et al.*, 2001). The melanopsin dependent photoreception system is known to transmit irradiance signal to numerous subcortical structures including the SCN, site of the master circadian clock, the VLPO, involved in sleep regulation, the superior colliculus and the lateral LGN, both part of the classical visual system, the IGL, implicated in circadian photoentrainment, the medial amygdala, involved in reproduction behavior modulation, the OPN, implicated in pupillary constriction, the lateral habenula, etc. (Hattar *et al.*, 2006). These structures are connected to many other major physiological systems; it is therefore difficult to designate a unique pathway mediating our effects. Likewise, indirect projections from the SCN to cholinergic, orexin and aminergic cell groups involved in arousal regulation exist in the forebrain and brainstem (Abrahamson *et al.*, 2001; Aston-Jones, 2005; Deurveilher and Semba, 2005; Saper *et al.*, 2005b) and might be responsible for the increased responses observed in the thalamus. In addition, direct projections of the melanopsin RGC to the LGN have been reported in primates (Dacey *et al.*, 2005) and might represent the pathway followed by irradiance information to influence thalamic activity, if they are also present in humans.

Because performance and alertness did not differ across days in the present study, light-induced cortical and subcortical response changes occurred independently

from behavioral modifications. It can also be argued that they are very likely to occur very early in the cascade of events elicited by melanopsin dependent responses, since modulation appeared almost instantaneously. Our previous fMRI studies, which used bright white light exposure in an attentional paradigm, also reported significant effects of light on thalamic and insular activity in the period of darkness following the illumination (Vandewalle *et al.*, 2006). Collectively, these data suggest that the thalamus and the anterior insula are key structures in mediating the effects of light on brain activity related to different cognitive functions during and after the exposure.

Although our design used a wavelength close to the peak sensitivity of the melanopsin dependent photoreception system (470 nm) and the data are consistent with an involvement of the melanopsin system, we are not in a position to assess the specific contribution of each photoreceptor. Short, medium-, and long cones were reported to input to the melanopsin pathway (Dacey *et al.*, 2005) and all classical photoreceptors were shown to be necessary for a complete nonvisual response to light in rodents (Hattar *et al.*, 2003). A recent human study also reported a novel type of cones expressing exclusively melanopsin (Dkhissi-Benyahya *et al.*, 2006). Lights of various spectral compositions and dose response protocols should specifically address this question.

Our protocol also revealed intriguing brain deactivations during green light exposure followed by a subsequent increase in activity. Current knowledge about the effects of green light exposures only allows very speculative interpretations of these findings. On the one hand, the effects of green light are reminiscent of those we observed during continuous darkness in a previous experiment. We reported that the repetition of an auditory oddball task in continuous darkness induced a temporary deactivation in several brain areas that were counteracted by bright white light (Vandewalle *et al.*, 2006). On the other hand, although, to our knowledge, no report supports this hypothesis, it is tantalizing to suggest that green light exposure would have a genuine effect on brain responses, different from blue light exposure. In such perspective, the deactivations we observe would be the result of a specific process induced by 550nm light exposure. Future experiments should be specifically designed to separately assess the effects blue and green light exposures.

The vast majority of studies on the effects of light exposure mediated by the melanopsin dependent photoreception system took place at night and/or after extended wakefulness episodes (Badia *et al.*, 1991; Brainard *et al.*, 2001a; Cajochen *et al.*, 2005; Campbell and Dawson, 1990; Lockley *et al.*, 2003). The few studies carried out during daytime imposed partial sleep deprivation to increase sleepiness and thereby maximize the sensitivity of their design (Phipps-Nelson *et al.*, 2003; Ruger *et al.*, 2005a). As light exposure occurred during the day in well rested subjects, our data have a broader impact. The spectral composition of common artificial light is geared towards the classical photopic system and does not consider the contribution of light to nonvisual functions. Future research should establish the optimal light regime (wavelength, duration, photon density, light history) required to efficiently enhance human cognition during daytime, especially for demanding tasks (*e.g.* education) or professions (*e.g.* military, healthcare professional, police, spaceship or plane crews).

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7. Brain responses to violet, blue and green monochromatic light exposures in human: prominent role of blue light and of the brainstem.

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Introduction

Light processing has been studied extensively in the context of circadian biology which emphasizes nonvisual (or non-image-forming) effects of environmental light (irradiance). These nonvisual effects include the synchronization of the circadian clocks, suppression of melatonin, regulation of sleep, as well as improvements of alertness and cognition (Brainard *et al.*, 2001a; Cajochen *et al.*, 2005; Dijk and Lockley, 2002; Lockley *et al.*, 2003; Lockley *et al.*, 2006; Munch *et al.*, 2006). We have shown that nonvisual responses related to alertness and cognition are associated with changes in regional brain activity detected by positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) (Perrin *et al.*, 2004; Vandewalle *et al.*, 2006; Vandewalle *et al.*, 2007b). A number of recent studies, using a wide variety of methodologies, revealed that acute or longer term human nonvisual responses are most sensitive to monochromatic lights of wavelengths between ~ 460 and 480nm (Brainard *et al.*, 2001a; Cajochen *et al.*, 2006b; Cajochen *et al.*, 2005; Dkhissi-Benyahya *et al.*, 2007; Gamlin *et al.*, 2007; Lockley *et al.*, 2003; Lockley *et al.*, 2006; Lucas *et al.*,

[*] The supplemental materials submitted with this article are included in the body of this chapter and in appendix 3.

2001a; Munch *et al.*, 2006; Thapan *et al.*, 2001; Vandewalle *et al.*, 2007b). This is much shorter than the overall maximum sensitivity of the photopic system (~555nm) and does not coincide with the maximum sensitivity of any of the classical photoreceptors (rods: ~505nm; S-cones: ~430nm; M-cones: ~530nm; L-cones: 560nm) (Buck, 2003; Solomon and Lennie, 2007).

A fifth retinal photopigment, melanopsin, was recently discovered (Provencio *et al.*, 2000) and shown to be expressed in retinal ganglion cells (RGC) that are directly light sensitive (Berson *et al.*, 2002), with a maximum sensitivity between 420 to 480nm (Melyan *et al.*, 2005; Panda *et al.*, 2005; Qiu *et al.*, 2005). Melanopsin expressing RGC are implicated in nonvisual responses to light (Berson *et al.*, 2002; Panda *et al.*, 2002). They project to numerous brain structures in rodents (Gooley *et al.*, 2003; Hattar *et al.*, 2006), including hypothalamic nuclei, such as the SCN and the VLPO, as well as many non-hypothalamic structures including the OPN, and amygdala, but also areas typically involved in vision such as the LGN and the superior colliculi. In addition, melanopsin RGC project to the LGN and OPN in Macaques (Dacey *et al.*, 2005). These neuroanatomical pathways provide a mechanism by which irradiance changes could affect many brain functions, *i.e.* circadian entrainment, pupillary constriction, arousal, attention, and emotion regulation, as well as vision (Cajochen *et al.*, 2005; Dacey *et al.*, 2005; Dkhissi-Benyahya *et al.*, 2007; Lockley *et al.*, 2003; Lockley *et al.*, 2006; Lucas *et al.*, 2001a; Vandewalle *et al.*, 2006; Wirz-Justice *et al.*, 2004). However, classical visual photoreceptors are necessary to induce complete nonvisual responses to light (Hattar *et al.*, 2003). In addition, RGC which do not express melanopsin, and presumably are not photosensitive, project to the SCN, intergeniculate nuclei (IGL) of the thalamus and VLPO, suggesting that signal arising from the classical retinal photoreceptor reaches these structures (Gooley *et al.*, 2003; Sollars *et al.*, 2003). Nevertheless, the respective roles of the different retinal photoreceptors have not been completely assessed.

Rod and cone responses to light are typically time-locked to the exposure, *i.e.* neural inputs start and cease within a few ms after light is turned on and off, respectively. In addition, quick attenuation of rod and cone signals occurs in the presence of a constant light stimulus (Dacey *et al.*, 2005). Intrinsic light responses of the melanopsin expressing RGC are much more sluggish and do not show attenuation: they

are only detected seconds after the onset, and firing is maintained for minutes after the end of the light exposure. This feature suggests that these cells are able to account for the long integration time of the nonvisual system (Berson *et al.*, 2002; Dacey *et al.*, 2005). However, melanopsin expressing RGC receive inputs from rods and the three classes of cones, which enable them to instantaneously respond to light exposure, and suggest an important role for rods and cones in the nonvisual response to light early in the exposure (Dacey *et al.*, 2005). Accordingly, relative efficacy of different wavelengths indicates that M-cones contribute importantly to the initiation of the response in rodents, but later the melanopsin expressing RGC are the dominant contributor (Dkhissi-Benyahya *et al.*, 2007). Similarly, wavelength sensitivity of rat SCN neuron responses to light flashes suggested a contribution of rods and all cones to the SCN response to brief light exposures (Aggelopoulos and Meissl, 2000).

A role for S-cones in nonvisual response was suggested in humans by data showing a greater increase in subjective alertness under violet light exposure (420-440nm) (Revell *et al.*, 2006). However, most human studies investigating the mechanisms of nonvisual responses to light employed monochromatic exposures targeting melanopsin RGC and M- and L-cones (Cajochen *et al.*, 2005; Lockley *et al.*, 2006; Munch *et al.*, 2006; Vandewalle *et al.*, 2007b). Reassessment of S-cones contribution to nonvisual responses to light using a violet light specifically targeting these photoreceptors remains to be done. In addition, nonvisual responses to different wavelengths in humans have only been characterized after long duration exposures (at least tens of minutes), *i.e.* after substantial attenuation of rod and cone signals. Thus, the relative contributions of all retinal photoreceptors in early nonvisual responses to light are largely unknown in humans.

Furthermore, besides the known projections of RGC expressing and not expressing melanopsin to brain structures involved in nonvisual functions, most the brain mechanisms and pathways mediating nonvisual responses to light exposure are unknown. In rodents, the SCN and thalamic IGL receive light irradiance information almost immediately and appear therefore to be strongly implicated in eliciting nonvisual responses to light (Meijer *et al.*, 1998; Morin and Blanchard, 2005). The SCN and IGL project to many brain structures involved in arousal regulation (Morin and Blanchard, 2005; Saper *et al.*, 2005b) and a functional indirect connection between the SCN to the

brainstem locus coeruleus (LC) has been established (Aston-Jones *et al.*, 2001). This may be the pathway followed by light to modulate alertness. However beyond these early subcortical and brainstem structures, the brain mechanisms involved in generating physiological or behavior nonvisual responses to light are not characterized in animals.

In humans, using PET and fMRI, we identified neural correlates of the alerting effect of a bright white light exposure (> 7000lux), delivered at night or during the day in brain areas such as the IPS, hippocampus, thalamic pulvinar, insula, and hypothalamus (Perrin *et al.*, 2004; Vandewalle *et al.*, 2006). More recently we demonstrated that brain activity related to a working memory task is maintained (or even increased) by blue (470nm) monochromatic light exposure, whereas it decreases under green (550nm) monochromatic light exposure (Vandewalle *et al.*, 2007b). These effects were detected in areas implicated in working memory such as the thalamus, insula, IPS, and middle frontal gyrus (MFG). These studies were carried out using prolonged light exposures (17 to 21 min). The brain areas first affected by light exposure and involved in triggering nonvisual responses to light are therefore largely unknown in humans.

In the present study, we used fMRI to specifically assess early nonvisual effects of light over the entire brain. We used alternating violet (430nm), blue (473nm), or green (527nm) monochromatic light exposures of equal photon density to investigate the processing of stimuli preferentially triggering S-cones, melanopsin expressing RGC, or M-cones, respectively. Light exposures lasted 50s, a very short duration from a human circadian biology perspective, in order to gain insight in the relative contributions of the different retinal photoreceptors early on in the establishment of nonvisual responses to light. We also hypothesized that such short exposures would not induce wavelength-specific responses in a large number of brain areas but would mainly affect a few areas involved in early nonvisual responses, presumably subcortical and brainstem areas.

Materials and Methods

Subjects

Participants were healthy, young subjects (N = 15; 8 females; age: 19-27 [median: 22]; BMI: 18.7-27.3 [median: 22.2]). A semi-structured interview established the absence of

medical, traumatic, psychiatric, or sleep disorders. Absence of color blindness was assessed by the 38 plate edition of Ishihara's Test for Color-Blindness (Kanehara Shupman Co., Tokyo, Japan). All participants were non-smokers, moderate caffeine and alcohol consumers, and were not on medication. None had worked on night shifts during the last year or traveled through more than one time zone during the last 2 months. Extreme morning and evening types, as assessed by the Horne-Ostberg Questionnaire (Horne and Ostberg, 1976), were not included. None complained of excessive daytime sleepiness as assessed by the Epworth Sleepiness Scale (Johns, 1991), or of sleep disturbances as determined by the Pittsburgh Sleep Quality Index Questionnaire (Buysse *et al.*, 1989). All participants had normal scores on the 21 item Beck Anxiety Inventory (Beck *et al.*, 1988) and the 21 item Beck Depression Inventory II (Steer *et al.*, 1997). They were right-handed as indicated by the Edinburgh Inventory (Oldfield, 1971). Participants gave their written informed consent and received a financial compensation for their participation. The study was approved by the Ethics Committee of the Faculty of Medicine of the University of Liège.

Volunteers followed a regular sleep schedule during the 7-day period preceding the laboratory segment of the experiment. Compliance to the schedule was assessed using wrist actigraphy (Actiwatch, Cambridge Neuroscience, UK) and sleep diaries. In order to record 2 volunteers on the same day at approximately the same circadian time, volunteers were requested to follow one of 2 sleep schedules differing by 1.5h (2300h - 0700h +/- 30min, or 0030h - 0830h +/- 30min). Volunteers were requested to refrain from all caffeine and alcohol-containing beverages and intense physical activity for 3 days before participating in the study.

Protocol

Subjects were first maintained in dim light (<5 lux) for 2h and then scanned during three consecutive 20 min sessions (Figure 7.1a). Three drops of tropicamidum 0.5% (Tropicol®) were administered in the eyes 20 min before entering the scanner to inhibit pupillary constriction. In each session, subjects were alternatively exposed to monochromatic 50s light exposures separated by 5-to-14s periods of darkness (<0.01 lux) (Figure 7.1b). Monochromatic light was violet (430nm), blue (473nm), or green (527nm) and aimed at S-cones, melanopsin expressing RGC, and M-cones respectively.

In each session two wavelengths were presented and alternated. Each color was presented ten times per session.

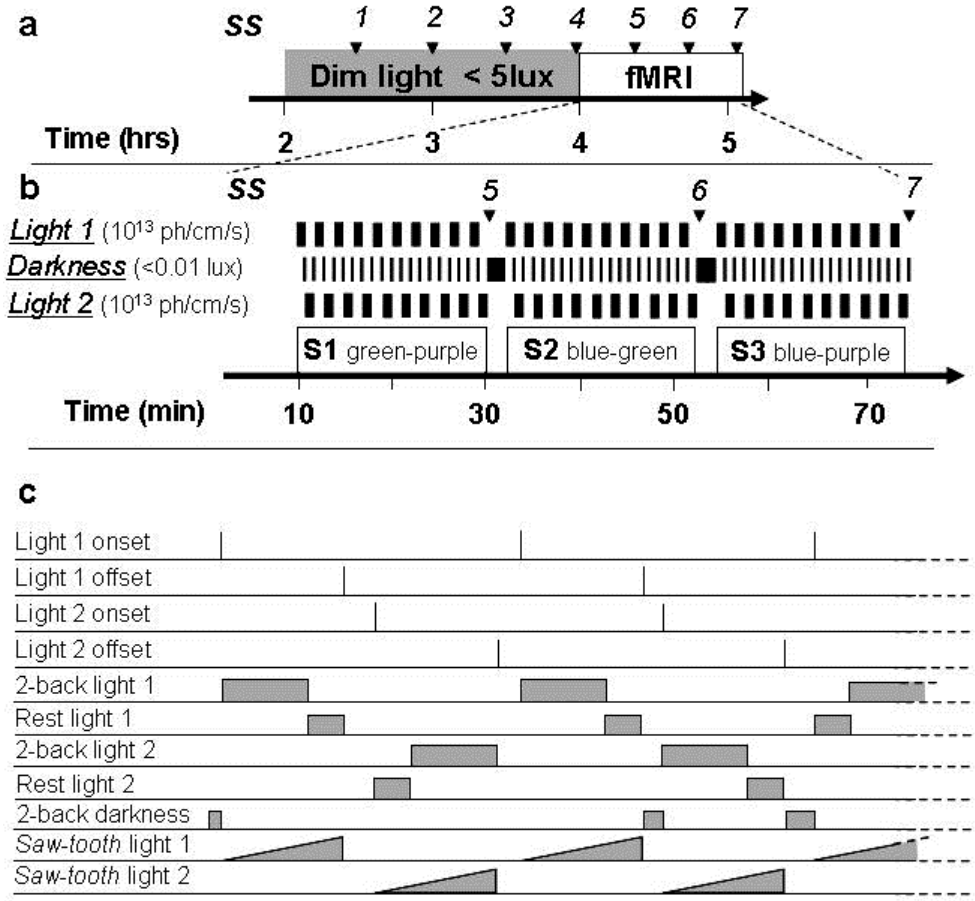

Figure 7.1: Experimental design.

a. General timeline. Time relative to scheduled wake time (hrs). ***Arrows***: subjective sleepiness assessment (*SS 1-7)*.

b. Timeline of the fMRI period and light condition organization.

Black bars indicate occurrences of the different conditions. Note that the combination of light 1 and 2 changes from session to the other. ***S1-3***: sessions 1 to 3 during which 3 combinations of light are employed (combination order is given as example). Time in minutes after entering the scanner. ***Arrows***: subjective sleepiness assessment (*SS 5-7)*.

c. Functions used to model brain activity. See methods for details.

Subjects were exposed to the three possible combinations of wavelengths over the three sessions. The order of the combinations and the wavelength of the first light exposure in each combination, were counter-balanced over subjects. In accordance with other protocols in this research area, the photon densities of all light exposures were

identical to allow the assessment of the relative contribution of the photoreceptors most sensitive to each wavelength. Photon density was set at 10^{13} photons/cm²/s because, at this level, nonvisual responses at night and during the day, depend on the wavelength of the light exposure (Brainard *et al.*, 2001a; Cajochen *et al.*, 2006a; Cajochen *et al.*, 2005; Gamlin *et al.*, 2007; Lockley *et al.*, 2003; Lockley *et al.*, 2006; Munch *et al.*, 2006; Thapan *et al.*, 2001; Vandewalle *et al.*, 2007b). This photon density was equivalent to an illumination level of 4, 7.5, and 24.5 photopic lux for violet, blue and green light exposure, respectively. The first light exposure occurred approximately 4h after habitual wake up time, i.e. during the biological day when melatonin secretion is low (Dijk and Lockley, 2002). During each session, participants performed an auditory *2-back* working memory task (Braver *et al.*, 2001), which is reliably executed by a majority of subjects and does not explicitly depend on visual input. Subjective alertness scores, as assessed by the KSS (Akerstedt and Gillberg, 1990), were collected every 30 minutes during the 2h preparatory period and between each session while in the scanner.

During the data acquisition period, all subjects interacted with the same investigator who used a standardized set of sentences between each session. This protocol was implemented in order to minimize variation in motivational state due to social interactions [*e.g.* encouragement by an investigator which may modify brain responses (Grandjean *et al.*, 2005)]. No feedback was given on performance. Volunteers were trained on a shortened version of the protocol and habituated to the experimental conditions at least a week before the experiment. Subjects had to reach 75% of correct responses on the 2-back task at the end of training to participate to the experiment.

2-back task

Stimuli consisted of nine French monosyllabic consonants that were phonologically different so that they could easily be identified. Stimuli were 500ms long and the inter-stimulus-interval was 2500ms. For each consonant, volunteers were requested to state whether or not it was identical to the consonant presented 2 stimuli earlier, by pressing a button on a keypad for “yes”, and another one for “no”. Series of stimuli were constructed with ~30% positive answers. Fourteen consonants were presented in each illumination period for a total of 35s, and 2 to 5 consonants were presented in half of the darkness periods, for a total of 5 to 12.5s. Series could therefore be 33 consonant long if

a darkness period with the task was placed between 2 consecutive illumination periods where the task was performed. Series were presented only once and were randomly assigned to one of the scanning sessions. Rest periods could last up to 44s if a rest period in darkness was placed between two consecutive illumination rest periods. Stimuli were produced using COGENT 2000 (http://www.vislab.ucl.ac.uk/Cogent/) implemented in MATLAB (Mathworks Inc., MA) on a 2.8 GHz XEON DELL personal computer (Round Rock, TX) and were transmitted to the subjects using MR CONTROL amplifier and headphones (MR Confon, Germany). The first session was preceded by a short session during which volunteers had to set the volume level to ensure an optimal auditory perception during scanning.

Light exposures

Narrow interference band-pass filters (FWHM: 10nm; Edmund Optic, UK) were used to produce the three monochromatic illuminations. A filter wheel (AB301-T, Spectral Products, NM) was computer controlled to switch band-pass filters and thereby change light wavelength. The light was transmitted by a metal-free optic fiber from a source (PL900, Dolan-Jenner Industries, MA) to two small diffusers placed in front of the subjects' eyes. The diffusers were designed for the purpose of this study and ensured a uniform illumination over the entire visual field. Light was administered through a 4 x 5.5 cm frame placed 3 cm away from the eye. Spectra of each monochromatic light were checked at the level of the diffusers (AvaSpec-2048, Avantes, The Netherlands), and the 430nm, 480nm and 532nm band-pass filters used produced light with a maximum radiance at respectively 430.3nm, 472.8nm and 527.3nm. Irradiance could not be measured directly in the magnet, but the light source was calibrated and irradiance estimated to be 10^{13} photons/cm²/s (840-C power meter, Newport, Irvine, CA) after prereceptoral lens absorption for the different wavelengths was taken into account (Stockman and Sharpe, 2000). The total amount of blue light received during the experiment was well below the blue-light hazard threshold (ICNIRP, 1997).

In order to un-correlate task and light onsets, the auditory task was performed during 35s of the 50s illumination periods. Half of the illuminations started with 15s of rest, the other half terminated with 15s rest periods. In addition, a 0-to-1s jitter was implemented between light onset/offset and task onset/offset when they occurred

simultaneously in order to further un-correlate them. Darkness periods (< 0.01 lux) separated all 50s illuminations. The auditory task was performed during half of the darkness periods, the duration of which were then 5 to 12.5s. Rest was requested during the other half; in which case darkness was lasting 9 to 14s. Illuminations with one color were always followed by darkness periods and then by illuminations in the other color of the session.

Behavioral data analysis

Accuracy scores were always very high, so we computed d-prime and criterion values following the signal detection theory (Green and Swets, 1966) in order to identify possible changes in behavior not reflected in overall accuracy. Repeated measure ANOVA with light condition and session as within subject factors were carried out separately on d-prime, criterion and reaction times. Repeated measure ANOVA with repetition as within subject factor were computed on subjective sleepiness scores. All behavioral analyses were computed with Statistica 6.1 (StatSoft France, France).

Functional MRI data acquisition

Functional MRI time series were acquired using a 3T MR scanner (Allegra, Siemens, Germany). Multislice T2*-weighted fMRI images were obtained with a gradient echo-planar sequence using axial slice orientation (32 slices; voxel size: 3.4x3.4x3 mm^3; matrix size 64x64x32; repetition time = 2130ms; echo time = 40ms; flip angle = 90°). The four initial scans were discarded to allow for magnetic saturation effects. There was little variation in the number of scans per session (blue-green sessions: 563.3 ± 5.9 (mean ± SD); violet-blue sessions: 563.4 ± 6.2; green-violet sessions: 563.3 ± 7.5). Head movements were minimized using a vacuum cushion. A structural T1-weigthed 3D MP-RAGE sequence (TR 1960ms, TE 4.43ms, TI 1100 ms, FOV 230 x 173 cm^2, matrix size 256 x 256 x 176, voxel size: 0.9x0.9x0.9mm) was also acquired in all subjects.

Functional MRI data analysis

Functional volumes were analyzed using Statistical Parametric Mapping 5 (SPM5 - http://www.fil.ion.ucl.ac.uk/spm) implemented in MATLAB. They were corrected for head motion, spatially normalized (standard SPM5 parameters) to an echo planar

imaging template conforming to the Montréal Neurological Institute (MNI) space, and spatially smoothed with a Gaussian Kernel of 8mm FWHM. The analysis of fMRI data, based on a mixed effects model, was conducted in two serial steps, accounting respectively for fixed and random effects. For each subject, changes in brain regional responses were estimated using a general linear model in which the different parts of the experimental design were modeled using either boxcar or stick functions, convolved with a canonical haemodynamic response function (Figure 7.1c). Boxcar functions modeled the 15s rest illumination periods, the 35s illumination periods including the 2-back task, and the darkness periods during which the task was performed. Stick functions modeled light onsets and light offsets. Because the melanopsin photoreception system is viewed as a "photon counter" integrating irradiance information over long periods of time (Berson *et al.*, 2002), we hypothesized that some brain areas might see their activity build up during the 50s of illumination, irrespective of whether the task is performed or not, to return to a baseline level once light is turned off. We therefore added "sawtooth-like functions" that modeled this build-up effect (Figure 7.1c). Melanopsin expressing RGC do not cease firing at light offset (Dacey *et al.*, 2005), so brain responses to light offsets are unlikely to represent a nonvisual response to light. Further, rest periods during the illuminations were short as compared to the task periods and were contaminated by the performance of the task. The regressors modeling offsets and rest periods were therefore considered as covariates of no interest together with movement parameters derived from realignment of the functional volumes. High-pass filtering was implemented in the matrix design using a cut-off period of 256 seconds to remove low frequency drifts from the time series. Serial correlations in the fMRI signal were estimated using an autoregressive (order 1) plus white noise model and a restricted maximum likelihood algorithm. The effects of interest were then tested by linear contrasts, generating statistical parametric maps. The summary statistic images resulting from these different contrasts were then further smoothed (6mm FWHM Gaussian Kernel) and entered in a second-level analysis. This second step accounts for inter-subject variance in the main effects of light condition (random effects model) and corresponds to a one-sample *t*-test for brain responses to the 2-back series, light onsets, or sawtooth-like regressors. The resulting set of voxel values for each contrast constituted maps of the t statistics thresholded at $p_{uncorrected} = 0.001$. Statistical inferences

were performed after correction for multiple comparisons on small spherical volumes (svc; 10 mm radius) at a threshold of $p_{svc} = 0.05$, around *a priori* locations of activation. Activations were expected in structures involved the *n-back* tasks, arousal regulation, and showing nonvisual responses to light in our own fMRI and PET work. Brain areas to which the melanopsin expressing RGC project or functionally linked to the SCN, were also considered as *a priori* locations of activation. Standard stereotactic coordinates of previously published *a priori* locations, used for svc, are as follow: amygdala: 22 -6 -15 (Sander *et al.*, 2005); hippocampus: -30 -30 -2 (Vandewalle *et al.*, 2006); lateral geniculate nucleus: -23 -21 -3 (Kastner *et al.*, 2004); locus coeruleus: 2 -32 -20 (Sterpenich *et al.*, 2006); thalamus: -14 -14 -16 (Vandewalle *et al.*, 2007b).

Results

Behavior

In order to rule out a placebo effect, we debriefed the participants about their color preference. Five subjects preferred the green illumination, six the blue light condition, four preferred the violet light. Therefore, differences in expectation or placebo effects are unlikely explanations for the results of this experiment.

Subjects were instructed to be as accurate as possible and that at least 75% of correct responses were requested. Mean accuracy was high for all sessions and light conditions (>93%) indicating that the task was easily performed throughout the protocol. Subject could however sometime execute the task differently and still reach high performance. We computed *d-prime* and *criterion* values to test this hypothesis. Sessions could not be directly compared because conditions changed from one session to the other. We therefore tested whether subjects' behavior in one light condition changed from one session to the other and if light conditions were similar.

Repeated measures ANOVA on d-prime values (Figure 7.2a) with light condition and session as within-subject factors revealed no main effects of light condition (F-value = 0.18; df = 2, 28; p-value = 0.84) and of session (F-value = 0.04; df = 1, 14; p-value = 0.84), and no light condition by session interaction (F-value = 0.18; df = 2,28; p-value =0.83). Repeated measures ANOVA on criterion values (Figure 7.2b)

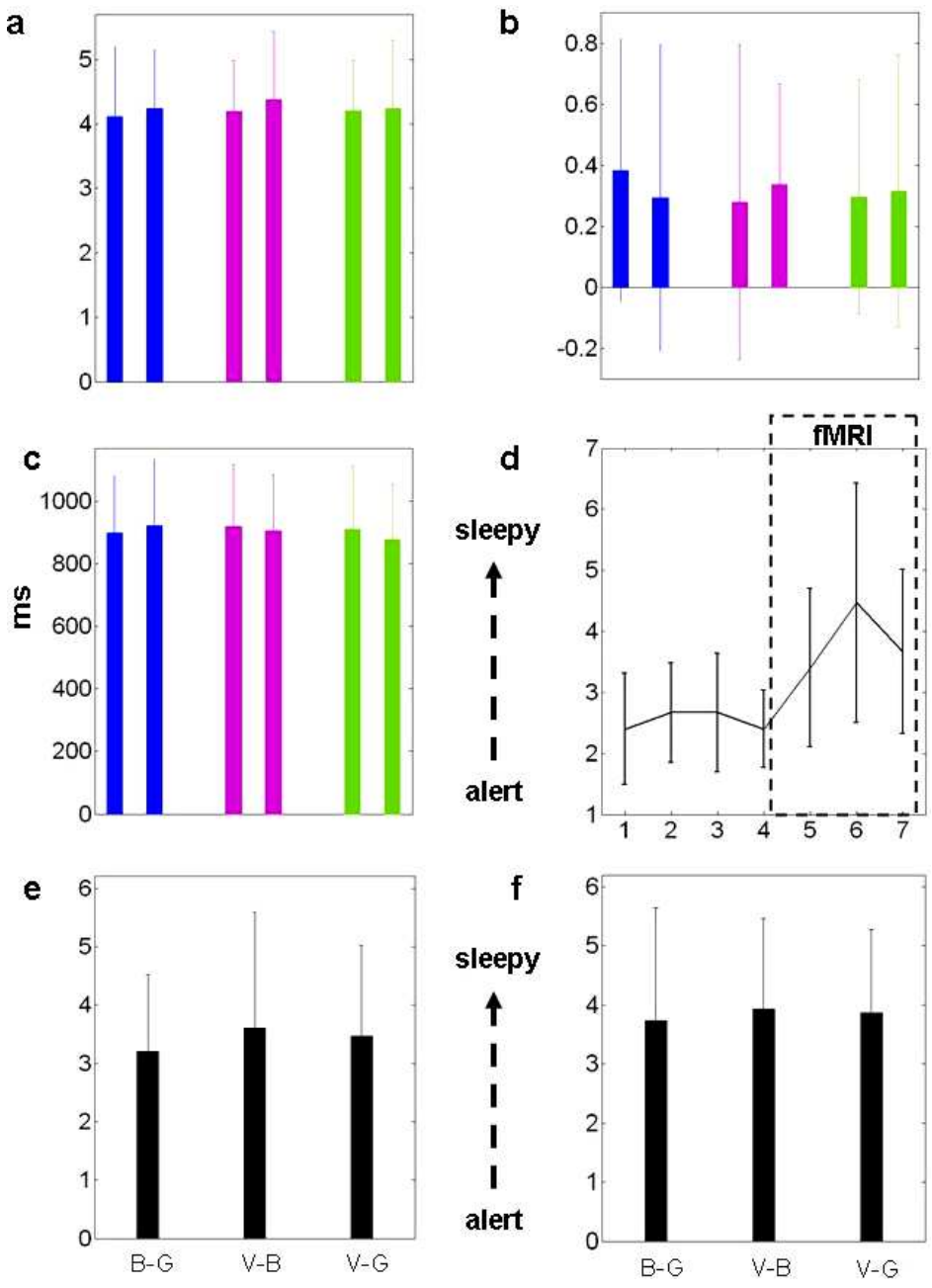

Figure 7.2: Behavioral results.

Mean values ± SEM are plotted. The color of the light corresponds to the bar color.

a. d-prime values in the different light conditions (2 sessions per condition);

b. Criterion values in the different light conditions (2 sessions per condition);

c. Reaction times in the different light conditions (2 sessions per condition);

d. Sleepiness scores evolution across the protocol;

e. Sleepiness collected *before* each session type;

f. Sleepiness collected *after* each session type

with light condition and session as within-subject factors revealed no main effects of light condition (F-value = 0.02; df = 2, 28; p-value = 0.98) and of session (F-value = 0.34; df = 1, 14; p-value = 0.57), and no light condition by session interaction (F -value = 0.24; df = 2, 28; p-value = 0.79).

Subjects were instructed to respond as fast as possible but to avoid anticipation errors, i.e. to prefer accuracy over speed. Mean reaction times for each light condition in each session were nevertheless analyzed for completeness of the results (Figure 7.2c). Again, sessions could not be directly compared because conditions changed from one session to the other. We therefore tested whether subjects' reaction time in one light condition changed from one session to the other and if reaction times were similar across light conditions. Repeated measures ANOVA on mean reaction times with light condition and session as within-subject factors revealed no main effects of light condition (F-value = 0.72; df = 2, 28; p-value = 0.49) and of session (F-value = 0.28; df = 1, 14; p-value = 0.61), and no light condition by session interaction (F -value = 1.19 ; df = 2, 28; p-value = 0.32).

Repeated measure ANOVA on KSS scores (Figure S7.2d) with repetition as within-subject factors revealed main effects of repetition (F-value = 10.22; df = 6, 84; p-value < 10-6). Planned comparisons showed significant differences in KSS scores collected before and after entering the scanner (F-value = 20.67; df = 1, 14; p-value = 0.0005) and between the KSS collected after the second session and those collected after the first and the third session (F-value = 6.67; df = 1, 14; p-value = 0.022). Therefore, entering the scanner and the associated change in posture, significantly increased sleepiness. However, the randomization of session type order prevented this time effect on sleepiness from biasing our data. Indeed, repeated measure ANOVA with KSS score collected before each session (Figure 7.2e) as within subject factor did not show significant differences (F-value = 0.28; df = 2, 28; p-value = 0.76), nor did the repeated measure ANOVA with KSS score collected after each session (Figure 7.3f) as within subject factor (F-value = 0.10; df = 2, 28; p-value = 0.91).

FMRI data

Sustained effects

The analysis of fMRI data first focused on the brain responses recorded during the blocks of 2-back task. The effects described below are therefore sustained because they describe differences between light conditions that maintained during the entire blocks. Significant differences between violet and blue light exposures were detected in the left MFG and in the left thalamus, a few mm away from the location for which we previously found a wavelength dependent effect of light (Vandewalle *et al.*, 2007b), as well as in two areas of the brainstem. Spatial resolution of the fMRI technique does not allow a precise identification of the brainstem nuclei included in the activated areas, but the location of the activations is compatible with several pontine nuclei involved in arousal regulation, and in particular with the LC bilaterally (Figure 7.3; Table 7.1) (Jones, 2003). Activity estimates show (Figure 7.3; right panels) that, compared to the violet light condition, responses were greater under the blue exposure in the four brain areas. No significant differences between blue and green light exposures, and between violet and green light exposures we detected during task periods (Appendix 3: supplemental tables S7.1). The regressors modeling a progressive build-up of the response during the 50s illuminations did not reveal significant differences between light conditions (Appendix 3: supplemental tables S7.2).

Table 7.1. Light condition effects during the performance of the 3-back task.

Brain areas	***xyz***	***Z***	***p***
Blue light > violet light			
Left middle frontal gyrus	-44 42 30	3.45	0.020
Left thalamus	-18 -24 10	3.32	0.028
Left brainstem	-6 -38 -20	3.22	0.035
Right brainstem	6 -30 -16	3.17	0.040

Coordinates (xyz) in the standard MNI space. No other significant light condition effects were found during the performance of the task.

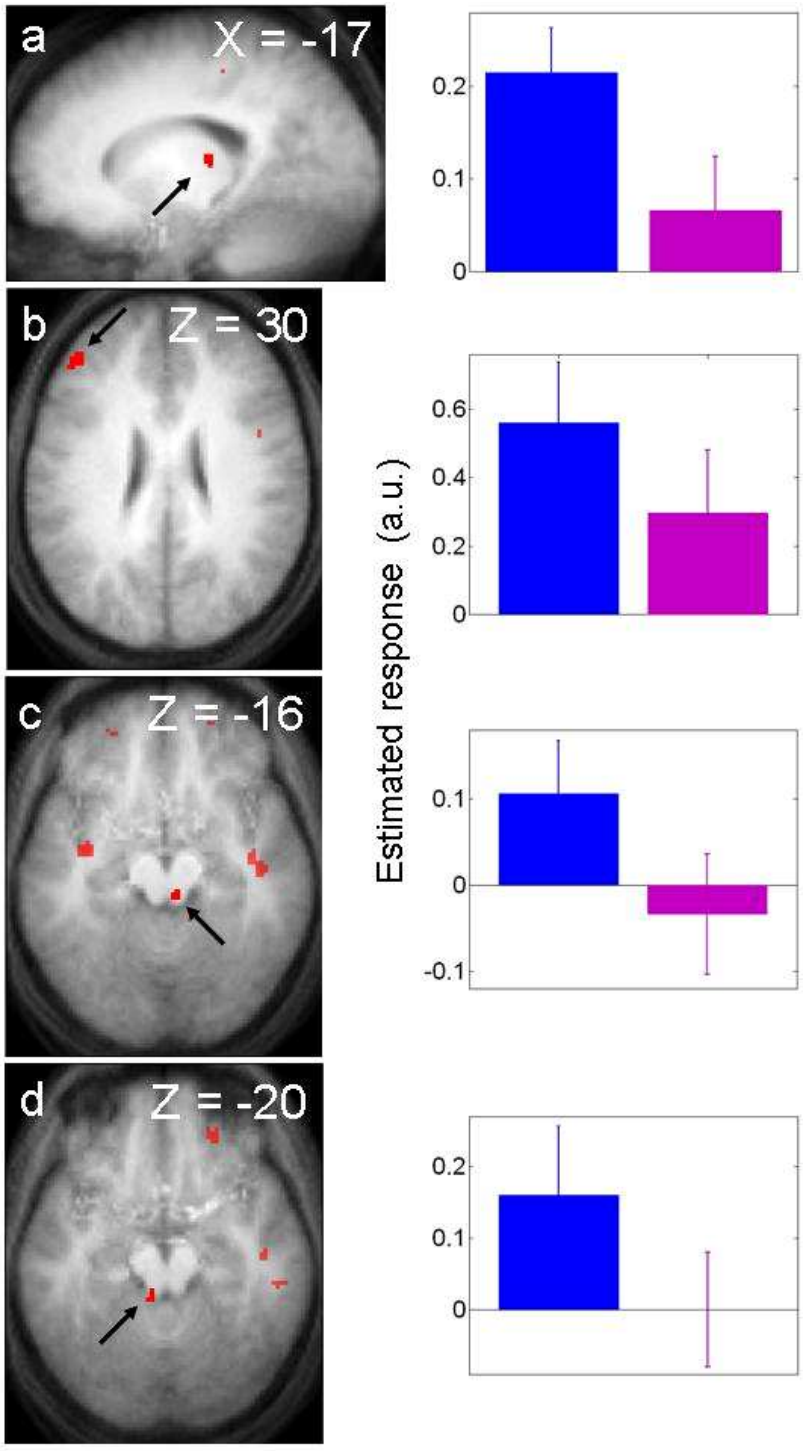

Figure 2: Significant differences between the blue and violet light conditions during the performance of the 2-back task.

Left panels: statistical results overlaid to the population mean structural image ($p_{uncorrected} < 0.001$). ***Right panels:*** Mean parameter estimates of the blue and green light conditions at light onset (a.u. ± SEM). ***a.*** left MFG – ***b.*** left thalamus – ***c.*** right brainstem – ***d.*** left brainstem.

Transient effects

The analysis of fMRI data then focused on the transient brain responses triggered by the onsets of the different exposures. Significant differences between responses to blue and green light onsets were observed in two limbic areas, the left hippocampus and right amygdala, and in the left thalamus, in the same location as during the task (Figure 7.4; Table 7.2). Activity estimates (Figure 7.4, right panels) show that these three brain areas strongly responded to blue light onsets while their activity was barely affected by green light onsets.

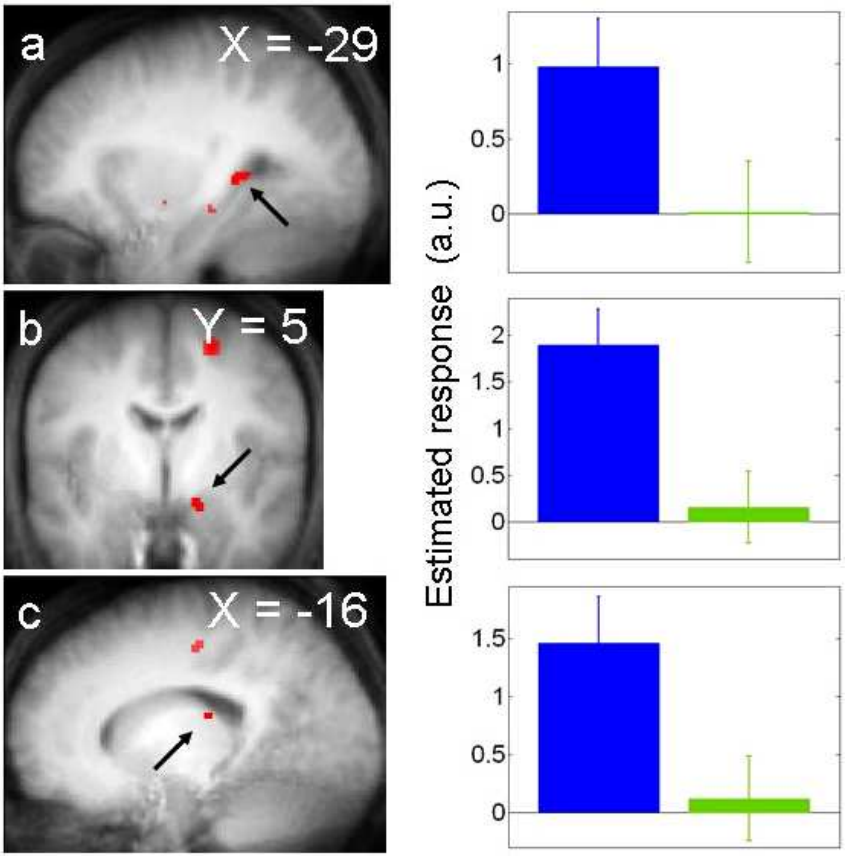

Figure 3: Significant differences between blue and green light conditions at light onset.

Left panels: statistical results overlaid to the population mean structural image ($p_{uncorrected}$ <0.001). ***Right panels.*** Mean parameter estimates of the blue and green light conditions at light onset (a.u. ± SEM). ***a.*** left hippocampus – ***b.*** right amygdala – ***c.*** left thalamus.

No significant differences were found between violet and blue light onsets, while violet light onsets increase left LGN activity significantly more than green light onsets (Table 7.2; Figure 7.5; Appendix 3: supplemental tables S7.3).

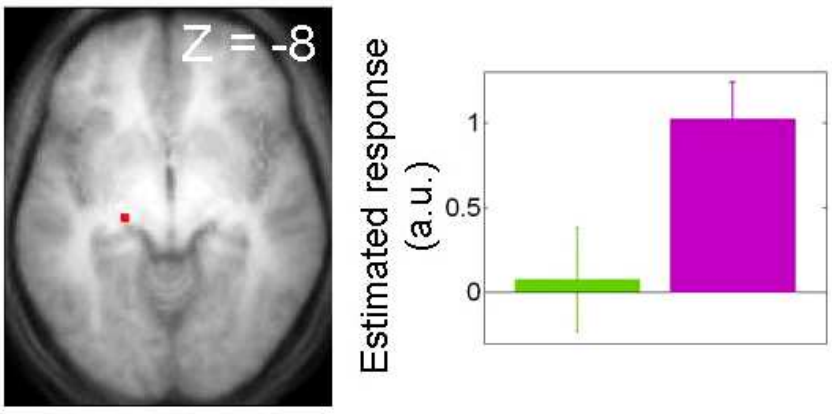

Figure 7.5: Significant differences between green and violet light conditions at light onset in the left LGN.
Left panels: statistical results overlaid to the population mean structural image ($p_{uncorrected}$ < 0.001). ***Right panels.*** Mean parameter estimates of the green and violet light conditions at light onset (a.u. ± SEM).

Table 7.2. Light condition effects at light onset.

Brain areas	***xyz***	***Z***	***p***
Blue light > green light			
Left hippocampus	-28 -38 2	3.57	0.019
Left thalamus	-16 -24 18	3.37	0.034
Right amygdala	16 -4 -18	3.31	0.039
Violet light > green light			
Left LGN	-22 -22 -10	3.43	0.029

Coordinates (xyz) in the standard MNI space. No other significant light condition effects were found at light onset.

Discussion

This study compared the brain responses elicited by violet, blue and green monochromatic light exposures of short duration (50s) and equal irradiance (10^{13} ph/cm^2/s), presented in pairs in 3 separate sessions as alternating blocks of 50 seconds. The results are consistent with two of our predictions. First, these short light exposures produce detectable wavelength-sensitive modulation of the regional brain responses evoked by a working memory task. In particular, blue light is superior to violet light in eliciting this response modulation. These results cannot be accounted for by any measurable difference in alertness or performance, nor by any order or expectation effect. Second, these modulations are considered as sustained because the brain activity is continuously enhanced during the 50s blue light blocks and consistently so during the whole blue/violet fMRI session. Although sustained, these light-induced responses still represent early nonvisual responses as compared to those we reported after 18min of blue monochromatic light exposures (Vandewalle *et al.*, 2007b). They primarily involve subcortical areas related to arousal (brainstem and thalami). At the cortical level, the responses are enhanced in a single area, the MFG. This result contrasts with the enhanced responses in widespread cortical regions elicited by longer exposures (Vandewalle *et al.*, 2007b) and suggest that the functional recruitment of the cortex require longer exposures, and possibly the activating influence of subcortical structures.

In addition, another, unexpected, finding concerned transient responses triggered at the onset of light exposures in two limbic areas, *i.e.* the amygdala and the hippocampus, and the left thalamus, irrespective of whether the subject was engaged in the working memory task. These results are remarkable because blue light was superior to green light in eliciting these brain responses, even though illuminance was about 5 times higher for the green light.

Collectively, these sustained and transient responses show the efficacy of short wavelength (473nm) light in modulating brain activity, and indirectly suggest the involvement of melanopsin expressing RGC, which are the photoreceptors most sensitive to this wavelength.

Sustained effects

A sustained enhancement of responses to the working memory task was observed during the exposures to blue, rather than violet light, in the brainstem, the thalamus, and the left MFG. No difference in response was observed when contrasting blue to green lights, or violet to green lights. These results suggest that the sustained response modulation by monochromatic light is most sensitive to blue light and least sensitive to violet light. The status of green light can not be precisely estimated but is consistent with an intermediate sensitivity. By inference, these results suggest that melanopsin RGC contribute most to these sustained nonvisual responses whereas the contribution of S-cones is the weakest and the involvement of M-cones is intermediate. Accordingly, melanopsin RGC and M-cones (Dkhissi-Benyahya *et al.*, 2007) seem to contribute greatly to nonvisual responses to light during the first minutes of the exposure in rodents.

The early recruitment of the brainstem by blue light is tentatively located in the LC. This result is important because it is the first time a brainstem structure is shown to respond to light in human. The LC appears as a key structure in our design. First, it could receive nonvisual information from the SCN, with which it is functionally connected in rodents (Aston-Jones *et al.*, 2001). Second, as the major source of brain norepinephrine, it is in position to modify the level of arousal (Aston-Jones and Cohen, 2005; Saper *et al.*, 2005a). Third, the LC is also involved in cognition and in executive processes in particular (Aston-Jones and Cohen, 2005).

Thalamic nuclei appear as the structures most consistently recruited in humans by nonvisual responses to light [polychromatic white light exposure (Vandewalle *et al.*, 2006); monochromatic 470nm blue light exposure (Vandewalle *et al.*, 2007b)]. Like the brainstem, the thalamus is a key structure involved in the interaction between alertness and cognition in humans (Foucher *et al.*, 2004) and it is recruited by working memory tasks (Cabeza and Nyberg, 2000). In addition the thalamus might receive irradiance information through a two step pathway linking melanopsin RGC to the superior colliculus which in turn projects to the pulvinar (Morris *et al.*, 1999).

Cortical responses were enhanced after recurring 50s periods of blue (relative to violet) monochromatic light exposure only in the left MFG, an area implicated in working memory (Cabeza and Nyberg, 2000). This limited recruitment of cortical areas

contrasts with our previous experiments, which used longer light exposures. Exposures to white light for about 21 minutes enhanced cortical responses to an auditory attention task in widespread cortical areas [dorso-lateral prefrontal cortex, intraparietal sulcus, superior parietal lobe, insula, precuneus, anterior and posterior cingulate cortices and superior temporal gyrus (Vandewalle *et al.*, 2006)]. Likewise, 18 min exposure to monochromatic blue (470nm) light (as compared to green (550nm) light) increased the responses induced by a working memory task in the left IPS, SMG, MFG, and right insula (Vandewalle *et al.*, 2007b). Collectively, these findings suggest that nonvisual responses require some time to build-up in the cortex. The assessment of this time course will require further studies characterizing the relations between photon density, duration of light exposure, and regional brain responses. Such studies will benefit from the methodological advance presented in this paper, namely within-session assessment of light-induced brain responses, which provide a fast, reliable technique to characterize light-induced brain responses.

As there are no direct connections between nonvisual system and the cortex, we surmise that the light-induced enhancement of cortical responses follows indirect pathways involving activating subcortical structures.

Transient effects

An unexpected result was the responses in left hippocampus, left thalamus, and right amygdala at light onsets of blue, relative to green light. Such differential response was not observed between blue and violet lights or between violet and green lights. These results are surprising for several reasons. Because the visual system is most sensitive to green (555nm) light (Buck, 2003), and since light onset is a typical visual stimulus, we expected green light to induce the greatest responses at onsets. In addition, M- and L-cones signals were reported to elicit activating ON responses in melanopsin RGC whereas S-cones were reported to mediate inhibiting OFF responses (Dacey *et al.*, 2005). Green light should therefore increase activity in these melanopsin expressing RGC while violet light should decrease their activity. Brain responses mediated by melanopsin RGC should therefore be least sensitive to violet light. Taken together, these elements suggest that melanopsin expressing RGC contribute most to these

transient limbic and thalamic nonvisual responses, whereas the contribution of M-cones is the weakest and the involvement of S-cones is intermediate.

Due to its anatomical connectivity, the amygdala is in good position to quickly receive irradiance information. The medial amygdala receives direct connections from melanopsin expressing RGC in rodents (Hattar *et al.*, 2006). In addition, a functional pathway linking the retina to the amygdala and bypassing the visual cortex through the superior colliculus and thalamus has been proposed in humans (Morris *et al.*, 1999). The hippocampus is connected to the amygdala (Aggleton, 1992), and both structures receives numerous afferents from the LC (Castle *et al.*, 2005), a (potential) key component of nonvisual response system receiving indirect retinal projections (Aston-Jones *et al.*, 2001).

At present, the functional significance of the limbic responses are unclear. However, it is tempting to suggest that blue light can modulate emotional processing by the amygdala. In this perspective, recent data demonstrated that long term light exposure regime employed in light therapy can overcome seasonal affective disorder symptoms as well as those of other psychiatric disorders (Wirz-Justice *et al.*, 2004). Direct assessment of the influence of light on emotional processing should address this question.

Our protocol is very different from those used in vision neuroscience, because color vision investigations use isoluminant stimuli to account for luminance and brightness brain processing (*e.g.* Landisman and Ts'o, 2002; Tootell *et al.*, 2004). The significant difference in left LGN activity between violet and green light onset is therefore difficult to interpret. It is unlikely that it is related the melanopsin RGC projections to the LGN found in Macaques (Dacey *et al.*, 2005), since it was not found in the session involving blue light.

Conclusion

This study is part of a series of investigations of light processing in the entire human brain (Perrin *et al.*, 2004; Vandewalle *et al.*, 2006; Vandewalle *et al.*, 2007b). We demonstrate that a few tens of seconds of light induce immediate and significant changes in brain activity and that melanopsin expressing RGC seem to provide the most important contribution to these changes. Our results also suggest that specific pathways

recruiting melanopsin but also maybe non-melanopsin expressing RGC, relay light information from the retina to different brain areas. Our data further suggest that light can indirectly enhance cortical responses by recruiting activating structures in the brainstem and thalamus.

Acknowledgements

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8. General discussion

This thesis investigated the brain responses to light from a chronobiology point of view. Human brain light processing has been study extensively in vision neuroscience (Gegenfurtner, 2003; Solomon and Lennie, 2007; Ungerleider and Pasternak, 2003; Wassle, 2004). The neural bases of visual processing are well established and are still under intense investigation. In contrast, little was known about the brain mechanisms involved in nonvisual responses to light in humans at the time this thesis was initiated. The PET study of Perrin *et al.* (2004) demonstrated that functional neuroimaging could be used to investigate these responses, even in small structures such as the hypothalamus and paved the way for the three fMRI experiments presented here.

Visual vs. nonvisual responses

The terms "*circadian photoreception system*" could be employed to describe the longer term effects of light on circadian rhythms, but since acute responses to light, such as those we describe, do not necessarily affect circadian rhythms, it does not seem appropriate. The terms "*nonvisual*" or "*non-image forming*" were introduced to describe the novel aspect of light processing that did not directly relate to vision (Foster, 2005; Gooley *et al.*, 2003). However the visual system recruits an important part of the brain (cf. Figure 2.2) (Ungerleider, 1995) and many brain functions can be indirectly related to vision. For example, both the ventral and dorsal visual streams reach the hippocampus (Ungerleider and Pasternak, 2003) and the thalamic pulvinar is in close relation with the occipital cortex (Casanova, 2003). One cannot exclude that part of the nonvisual effects of light we detected in these areas are mediated by the visual occipital cortex.

In addition, data are now accumulating to show overlaps between nonvisual and visual photoreceptions. Rods and cones are necessary to induce full nonvisual responses to light (Dkhissi-Benyahya *et al.*, 2007; Hattar *et al.*, 2003), while melanopsin expressing RGC project to structures typically involved in vision, such as the LGN, both in rodents and primates (Dacey *et al.*, 2005; Gooley *et al.*, 2003; Hattar *et al.*, 2006). Furthermore, melanopsin expressing RGC appear to regulate visual processing

both in rodents and in humans (Barnard *et al.*, 2006; Hankins and Lucas, 2002), and non-expressing melanopsin RGC project to nonvisual brain structures (Gooley *et al.*, 2003; Sollars *et al.*, 2003), while a class of cone of unknown function exclusively express melanopsin (Dkhissi-Benyahya *et al.*, 2006). Finally, in the third study of this thesis, we reported differential brain responses to various monochromatic light stimuli that appeared at light onset. It is unclear whether at this time scale it is appropriate to consider these as nonvisual responses

Although, the term *nonvisual* did not appear completely appropriate, we chose to use it in this thesis because this is the current consensus term in the scientific community. If overlaps between both systems continue to accumulate, one might speak of photoreception as a whole and maybe should not try to distinguish what might not be distinguishable.

Methodological developments underlying our research strategy

Each of the fMRI studies reported in this thesis built on the experience gathered over the years and explored novel aspects of nonvisual responses to light. We were the first to assess nonvisual brain responses to light using fMRI. Whereas PET describes enduring functional states of the brain, event related fMRI characterizes transient cerebral responses to a cognitive challenge (Josephs and Henson, 1999). It implies that, whereas PET indiscriminately detected any light-induced modification of rCBF, the reported responses essentially characterized the interaction between the nonvisual effects of light and human cognition. It was not certain that, although light exposure changes brain activity state, it would also change the specific processes elicited by cognitive challenges. In our view, fMRI was a necessary step forward because of its much better temporal and spatial resolutions.

A second critical choice we initially had to make was to run daytime instead of night time studies. Chronobiology originally considered light as an environmental factor exerting only a weak influence on human physiology (Aschoff *et al.*, 1971; Waterhouse and DeCoursey, 2004). Potent effects of night time light exposures were then established (Czeisler *et al.*, 1986; Lewy *et al.*, 1980), but only recently were daytime effects of light exposure acknowledged (Hashimoto *et al.*, 1997; Jewett *et al.*, 1997). These influences were typically weaker than those of night time exposures. Only a few

studies actually demonstrated that these influences could induce acute responses in partially sleep deprived subjects (Phipps-Nelson *et al.*, 2003; Ruger *et al.*, 2005a). The use of daytime bright light exposure to detect nonvisual brain responses to light was therefore unusual in the chronobiology field and it remained quite challenging to detect these responses in well rested subjects.

Having shown that a 20 min daytime bright white light exposure was an efficient brain activity modulator, we then aimed at showing wavelength dependent effects of nonvisual brain responses. It was however not certain that the short (18min) exposures of light as those used in our first fMRI study, would elicit any detectable nonvisual responses. Indeed, all studies that had demonstrated nonvisual effects in humans had used night time exposure (Brainard *et al.*, 2001a; Cajochen *et al.*, 2006b; Cajochen *et al.*, 2006a; Lockley *et al.*, 2003; Lockley *et al.*, 2006; Munch *et al.*, 2006; Thapan *et al.*, 2001) and effects on brain function assessed by EEG (Lockley *et al.*, 2006; Munch *et al.*, 2006) and on behavior (Lockley *et al.*, 2006) were detected only after or during prolonged light exposures. In addition, only one experiment showed wavelength dependent improvement in performance (Lockley *et al.*, 2006).

The final, and maybe most important, innovation reported in this thesis is the use of very short light exposure of 50s (short from a human chronobiology point of view). One study recently used 10s monochromatic illumination, presumably during the day, to compute action spectra of human pupillary constriction (Gamlin *et al.*, 2007). However it did not deal with nonvisual brain responses to light related to a cognitive function. This within-session manipulation of light condition was a very important step in our research strategy because it opened multiple possibilities to assess various aspects of nonvisual brain responses to light (see *perspectives* below).

What are the brain mechanisms involved nonvisual responses to light in human?

Although the light-induced changes we observed were not always related to behavioral changes, they support the view that light affects physiology and behavior at a time it is naturally surrounding most people, and that its spectral quality and intensity are important factors to take into account. We detected effects that suggest that light exposure quickly modulates brain activity in structures involved in alertness and

attention regulation. The light-induced increases in activity in the thalamus and in brainstem neurons, possibly of the locus coeruleus, are maybe the strongest indicators of a rapid and important influence of light on alertness.

This thesis does not provide a comprehensive characterization of the mechanisms underlying the nonvisual effects of light but it provides some new information. Based on this fragmentary information, we propose the following scenario.

1) Exposure durations and cascades of nonvisual events

We demonstrated that daytime light exposure can acutely affect brain functions related to two distinct higher cognitive processes, *i.e.* attention and working memory. Bright white light and blue light either maintained or increased activity in several brain areas involved in these processes. We suspect that these brain responses, and especially those of the third experiment, which employed 50s exposures, are involved in the establishment of nonvisual brain responses to light. They are likely to precede the recruitment of cortical areas and any observable change in behavior or physiology (Brainard *et al.*, 2001a; Cajochen *et al.*, 2006b; Cajochen *et al.*, 2006a; Lockley *et al.*, 2003; Lockley *et al.*, 2006; Munch *et al.*, 2006; Thapan *et al.*, 2001). In this view, light would induce a cascade of functional events. The recruitment of activating subcortical structures would lead to the activation of cortical task-dependent areas and eventually to changes in alertness, behavior and performance. However, future experiments using similar tasks and wavelength exposures as in our fMRI experiments but more intense or longer exposures should specifically address this question.

2) The involvement of the brainstem

The early recruitment of activating brainstem structures by blue light exposure which we detected in the brainstem could result in widespread nonvisual effects of light at the cortical level. Many nuclei of the ascending arousal system lay in the brainstem and several are compatible with the pontine location of the effect detected, such as the laterodorsal tegmental nucleus (LDT), the dorsal raphe (DR), or the LC (Jones, 2003; Saper *et al.*, 2005a). A recruitment of the LC by blue light is supported by the functional link with the SCN reported in rodent (Aston-Jones *et al.*, 2001). Our results would then imply that there is also a functional link between the SCN and LC in human. The LC, being the major source of the arousal promoting norepinephrine and projecting to most of the brain, could be responsible for all the effect of light exposure we observed in

other brain areas in all three experiments, including in the thalamus (Aston-Jones and Cohen, 2005). However, we cannot exclude that other nuclei may be involved. The DR and LDT also receive indirect projections from the SCN through the DMH (Deurveilher and Semba, 2005), but their functionality has not been demonstrated yet. The LDT contain cholinergic neurons, that project to the thalamus (Saper *et al.*, 2005a). They could modulate its activity and be responsible for thalamic activation in our experiment. The thalamus would then in turn modulate activity in other brain regions. Neurons of the DR are serotonergic and send numerous ascending projections to the forebrain and cortex (Jones, 2003). Their involvement could also result in widespread modulation in cortical.

There are several possible reason why we did not observe activation of brainstem nuclei in the first two experiments. First, between-session comparisons are far less sensitive than within-session contrasts, because of inter-session variability of the data. It is likely that future experiments will also benefit from within-session designs to detect small brain structures involved in nonvisual responses to light. Second, brainstem nuclei might stop firing as soon as the light exposure ceases and would therefore not be observed in the following minutes of darkness during which we assessed brain response in the first experiment. Third, habituation processes may also occur in these nuclei and differential activation between prolonged blue and green light exposure may not reach significance during 18 min. Fourth, in the last experiment we could show that the effects induced by violet and blue light exposures were more different than the effect induced by blue and green light exposures. In our second experiment we only contrasted blue and green light and this may be why the brainstem was not identified in this experiment.

3) <u>The recruitment of the thalamus</u>

The thalamus is the only structure which was affected by light condition in all three experiments. The pulvinar mediated the interaction between light exposure and alertness is the first experiment, while a location which we tentatively identified as the dorsal part of the pulvinar was affected by the monochromatic light exposures. The pulvinar is implicated in arousal regulation, and the thalamus in general in executive functions such as working memory (Cabeza and Nyberg, 2000; Coull *et al.*, 2004; Foucher *et al.*, 2004; Portas *et al.*, 1998). This might explain the slightly different

locations of activation between the attentional and working memory paradigm used in the first experiment and in the last two, respectively. However the thalamus relays most information to the cortex, either from the retina to visual cortex (LGN), or between cortical areas (Pulvinar) (Sherman, 2005; Shipp, 2004). It can therefore regulate information flow in the brain, and an effect of light on such a brain structure is likely to result in widespread cortical effects.

4) Early limbic responses

It is likely that the thalamus and brainstem modulations we observed in the last experiment represent the early nonvisual effects of light. However, in the same experiment fast cortical responses in the amygdala and hippocampus were detected at light onset. These fast responses are probably explained in part by the anatomical connectivity of these limbic structures. The amygdala receives direct inputs from the melanopsin expressing RGC (Hattar *et al.*, 2006). The amygdala in turn directly project to the hippocampus (Aggleton, 1992), which is also receiving activating inputs form the brainstem (Castle *et al.*, 2005).

5) Late neocortical responses

The effects on cortical activity probably represent a second or third step in the pathway mediating nonvisual effects of light. The first experiment of this thesis demonstrated that the multiple cortical brain areas affected by light exposure presented different response dynamics. Responses to monochromatic light also showed that the MFG activity was modulated within the first seconds of a light exposure and remained affected for 18 min, while the influence of light on the insula, SMG and IPS could only be detected using a 18 min illumination. The pathways mediating different light responses involve different SCN projections notably to the DMH, SPZ, PVH (Saper *et al.*, 2005b). It is therefore possible that these pathways affect different brain areas with various dynamics. Accordingly, nonvisual responses to light presenting different dynamics have been reported for other physiological measures. For example, significant differences between the effects of blue and green monochromatic lights were detected after 30 min of illumination for melatonin suppression and subjective sleepiness, 45 min for CBT, and 90 min for heart rate (Cajochen *et al.*, 2005). The effects persisted for 30 to 120 min after cessation of the light stimuli for these parameters (Cajochen *et al.*, 2005; Munch *et al.*, 2006).

What are the retinal photoreceptors involved nonvisual responses to light in human?

We did not have direct access to the retinal photoreceptors involved in the effects we detected. We can only make inferences based on the wavelengths of the exposures we used. Based on the literature, it is very likely that all cones and melanopsin expressing RGC contributed to our results (Dkhissi-Benyahya *et al.*, 2007; Hattar *et al.*, 2006; Panda *et al.*, 2002). Because irradiance levels were above of the scotopic range (approximately from below 10^7 ph/cm^2/s to 10^{11} ph/cm^2/s) (Dacey *et al.*, 2005), rods were saturated by the light intensities we used and are unlikely to be greatly involved. Both the second and third experiments of this thesis support the view that the melanopsin expressing RGC were major contributors to the effects, at light onset and during 50s and 18 min of illumination. Photoreceptors can be excited by photons of a wavelength they are not most sensitive to (Gegenfurtner, 2003) and might therefore have contributed to the response of melanopsin RGC under blue monochromatic light exposure. It is likely then that S-cones would have sent inhibitory inputs, while M-cones sent excitatory ones (Dacey *et al.*, 2005).

Our second experiment revealed an intriguing decrease in activity in the left thalamus, IPS, SMG, MGF, and right insula, under green light exposure followed by a return to approximately baseline level. The explanation for this phenomenon is unclear. Increases in one condition and decreases in the other are expected when carrying out interaction analyses. However, the decrease under green light was important and appeared to drive the interaction in some brain areas. Green light may actively decrease brain activity, either by an action on the photoreceptors most sensitive to it (M- and L-cones), or through an effect of light information on the brain, but no data are available to support either of these views. Independently of the process involved, a decreased activity under green light exposure effect is surprising when considering the recent publication showing that the contribution of M-cones to nonvisual brain responses to light is significant at least during the first minutes of illumination (Dkhissi-Benyahya *et al.*, 2007). The results of our third experiment also support a non-negligible contribution from M-cones to nonvisual brain responses during the first 50s of illumination. Differences between both experiments may be related to the fact that green light would induce different responses when immediately preceded by a blue light exposure or by

prolonged darkness. The cumulative effect of several periods of 50s of green light could also modify the responses. More investigations are required to characterize the time course of the relative contributions of the different retinal photoreceptors.

Contrary to what had been suggested based on subjective sleepiness data (Revell *et al.*, 2006), data of our third experiment showed that S-cones do not appear to mediate the effects observed during the first 50s of an illumination. Whether this would be the case using longer illumination is unknown, but seems unlikely given the attenuation in firing observed in cones under constant illumination (Dacey *et al.*, 2005). S-cones do seem, however, to contribute to brain responses to light at onset. The literature suggests that this contribution would have to be mediated by non-expressing-melanopsin RGC that were shown to project to nonvisual brain areas, or by a distinct melanopsin RGC population (Dacey *et al.*, 2005; Gooley *et al.*, 2003; Hattar *et al.*, 2006; Sollars *et al.*, 2003; Wong *et al.*, 2007).

Possible implications

Daytime light exposure could be a potent tool to counter daytime sleepiness. Attempts to design light exposures to prevent lapses of alertness in populations such as older people, sleep disorder patients, or professionals at risk (*e.g.* aircraft crew, lorry and tube drivers, healthcare professionals, military, police), should take into account the fast dynamics of the effects of light on brain function and the wavelength specificity demonstrated in this thesis.

Performance of a working memory task is partly regulated by the circadian system (van Eekelen and Kerkhof, 2003) and light exposure influenced working memory-related brain activity. Implicit sequence learning or short-term explicit word pair recall tasks also receive circadian influences (Cajochen *et al.*, 2004; Wyatt *et al.*, 1999). In two experiments of this thesis, we found light-induced activity modulation in the hippocampus, which is strongly implicated in declarative memory (Squire and Zola-Morgan, 1991). Although we did not assess effects of light on this brain function, our results could indicate that hippocampal-dependent memory processes are influenced by light irradiance. From this perspective, both short-term (working memory) and longer term memory could benefit from appropriate light environment.

The light-induced modulations of activity in limbic and paralimbic structures such as the amygdala and right insula, could also be related to attention modulation or, for the latter, to working memory. Interestingly, these two structures are also implicated in emotion regulation (Critchley, 2005; Sterpenich *et al.*, 2006). Light exposure could therefore affect emotional cognition. Light therapy is already used to treat mood disorders such as seasonal affective disorders (Wirz-Justice, 2006). The biological mechanism involved in the action of light on these disorders is unknown. The acute effects of light exposure on structures involved in emotion regulation, offer a hint to explain them.

The literature reviewed in chapter 1 and 2, and the results of this thesis suggests that bright white light or blue light at around 460-480nm should be used to increase alertness, performance, mood, etc. Caution should however be taken when deciding to use light treatment, or particular lighting systems. Prolonged bright white or blue light exposure could indeed have detrimental effects. For instance, bright light sources can cause photoretinitis or *retinal burn*. This phenomenon is also referred to as *blue light retinal injury* because it appears to be mainly caused by the shorter wavelengths of the visible spectrum (ICNIRP, 1997). However, only very bright light sources such as the sun or welding arcs seem to be hazardous. Furthermore, bright light exposure influences circadian phase (Czeisler *et al.*, 1986) and could induce sleep disturbances if administered at the wrong time. Light exposure also acutely affects thermoregulation or sleep EEG (Cajochen *et al.*, 2005; Dijk *et al.*, 1991; Munch *et al.*, 2006) which could also lead to physiological disturbances. In brief, the detrimental influences of repeated prolonged blue or bright white light exposures have not been completely assessed, and light level should not be increased, or the spectral composition modified, without considering these possible negative influences.

Other considerations

Light history seems to play an important role in mediating the impact of a given light exposure (Hebert *et al.*, 2002; Jasser *et al.*, 2006; Wong *et al.*, 2005). We therefore placed a period of dim light (< 5 lux) before the fMRI recording to “wash out” the previous effects of light and to increase the sensitivity of the protocol. This preparatory period was reduced from 3 to 2h between the first and third experiments of this thesis.

Given the low light level of the 50s illuminations (maximum 25 lux for green light), little light adaptation occurred in the course of this last experiment. Nevertheless it did induce some adaptation which reduced the impact of the preparatory period and suggest that shorter periods of dim light can be used and still maintain sensitivity to different wavelengths. This preparatory period should be reduced as much as possible to investigate nonvisual light effects in more natural or realistic conditions, easier to transpose to real life situations.

Perspectives and future directions

A significant finding of this thesis is that within-session design using very short light exposures enables the detection of nonvisual responses. Effects located in small structures and early in the light exposure will therefore be more likely to be detected using within-session protocols. In natural conditions, the light levels we are exposed to change continuously. This creates a series of ever changing light exposures that may recruit the photoreceptors and brain areas involved in early nonvisual brain responses. It may therefore be very important to know these early effects.

In our view, several further lines of investigation can be envisaged. We summarize those most directly related to this thesis .

1) Dose-response curves and action spectra

Action spectrum and dose-response studies of brain responses to light could now be carried out by alternating brief monochromatic light exposures of different wavelengths and/or by varying their irradiance levels. It would be of primary interest first to know whether responses of all brain areas show the same dose-response to wavelength, or if they vary according to the brain region. An interesting way of carrying out action spectra and dose-response studies simultaneously would be to constantly modulate the wavelength composition and intensity of a continuous long duration illumination. This could be achieved by two or three light sources of varying wavelengths pooled at the diffuser level. This procedure would diminish subject movements in reaction to light onsets, allow high intensity lights, and permit the use of many wavelengths in a single session.

2) Nonvisual effects of light on other cognitive processes

We demonstrated nonvisual effects of light on attention and an executive function. One could attempt now to generalize these effects of light to other brain functions, such as long-term memory or emotion regulation. We showed that light affects brain areas sustaining these functions, but we did not specifically assessed the effect of light on these functions. Effect of light on memory could be tested at encoding or retrieval and effects at retrieval could be studied at different post-encoding delays.

Preliminary results of a recent study we carried out at the Cyclotron Research Center indicate that light indeed affects emotion regulation. Analyses completed so far suggest that activity in areas involved in voice recognition (bilateral superior temporal sulcus), and maybe in the hippocampus, is increased by light exposure in a wavelength dependent manner. Forty seconds of monochromatic blue (473nm) light exposure appear to increase activity more that 40s of green (527nm) monochromatic exposure in these areas (Figure 8.1). These exciting preliminary results could constitute the first direct evidence for a biological effect of light on emotion regulation.

3) Circadian variations of light-induced brain responses

Long term effect of light on circadian phase and acute light induced changes vary during the day (Czeisler *et al.*, 1989; Jewett *et al.*, 1991; Jewett *et al.*, 1997; Khalsa *et al.*, 2003; Minors *et al.*, 1991; Ruger *et al.*, 2005a; Van Cauter *et al.*, 1994). It would be of interest to determine how time of day affects brain responses to light. It could affect the amplitude of the effects but it could also change their locations.

4) Life-span variations of light induced brain responses

Middle aged or older people could be tested in order to determine how the reduction in lens transmittance and pupil size (Brainard *et al.*, 1997; Charman, 2003) observed with age affects nonvisual brain responses. Results could maybe be related to changes in circadian and sleep physiology reported with age (Buysse *et al.*, 2005; Gaudreau *et al.*, 2001).

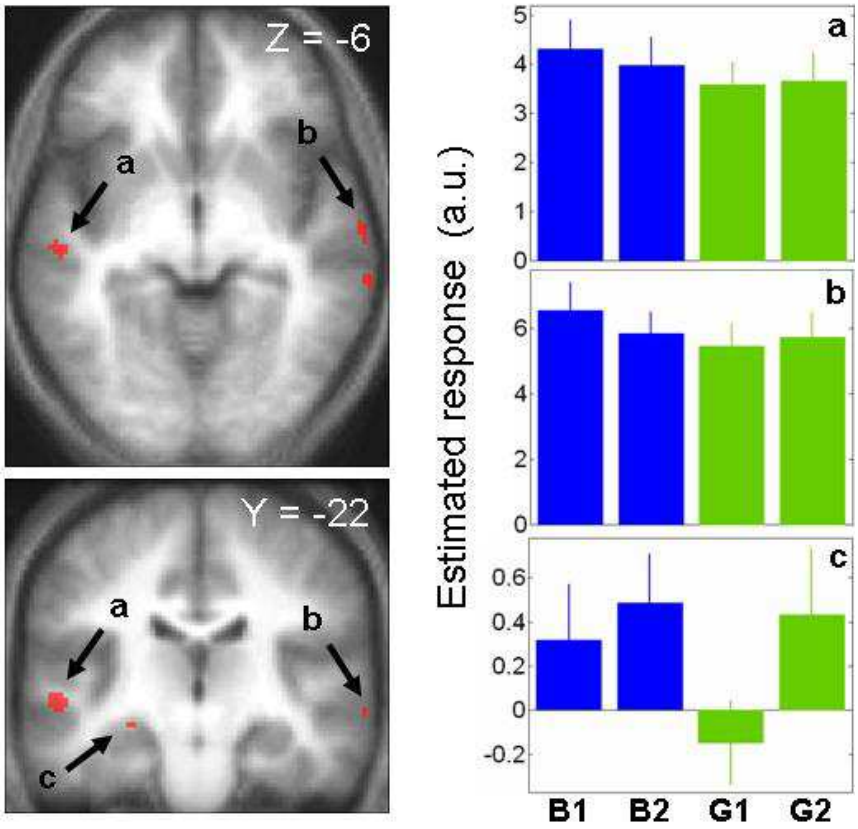

Figure 8.1: Preliminary results of an investigation of the effects of blue and green monochromatic light exposures on emotion processing.

Protocol overview: Subjects (N = 17) were maintained in dim-light (< 5 lux) for 90 min before performing an auditory task inspired from Grandjean *et al.* (2005) in fMRI. The task consisted in a stream of work-like sounds pronounced either by men or women. Subjects were required to state the gender of the person pronouncing each word-like sound. Untold to the subjects was that half of the stimuli was said in a neutral manner, while the other half was negative. Subjects were exposed to 12 alternations of blue (473nm) and green (527nm) monochromatic light exposures of 40s separated by 15 to 25s of darkness. Two photon densities were used per monochromatic light: irradiance was equal to 7 x 10^{12} ph/cm^2/s in half of the exposures, while it was equal to 3 x 10^{13} ph/cm^2/s in the other half.

Preliminary results: *Left panels:* significant differences in the processing of negative word-like sounds between blue and green monochromatic light exposures (both irradiance levels are pooled together). ***a.*** left superior temporal sulcus (-56 -24 -4; Z = 3.55; p_{svc} = 0.015); ***b.*** right superior temporal sulcus (68 -28 4; Z = 3.47; p_{svc} = 0.019); ***c.*** left hippocampus (-26 -24 -14; Z = 3.33; p_{svc} = 0.027). Statistical results are overlaid to the population mean structural image ($p_{uncorrected}$ < 0.001).

5) Selected populations

Issues about photoreceptor contributions to nonvisual responses in humans are difficult to address because we can only rely on natural mutants, or on injuries to access photoreceptors directly. Color blind as well as completely blind individuals could be studied to quantify the contribution of classical photoreceptors to nonvisual effects. It is however very often M- and L-cones that are affected in color-blindness, rarely S-cones. Investigations in blind individuals would certainly provide a strong demonstration of the effect of light on brain activity through nonvisual means. The role of melanopsin could be assessed directly in individuals who having intact eye-brain connections and no visual confound could possibly bias the results. By using monochromatic lights of different wavelengths, melanopsin sensitivity to the different parts of the visible spectrum could also be assessed in blind individuals. Melanopsin polymorphisms, if identified in human, would also be of great interest both if the mutation decreases its efficiency as a photopigment or as a photoisomerase, but also if it renders the nonvisual system blind to blue light.

Effects of polymorphism of different clock genes could also be investigated. Preliminary results of a large study which we have just completed suggests that the variable-number tandem-repeat polymorphism in the coding region of the circadian clock gene *PER3* reported to affect sleep and performance (Viola *et al.*, 2007) also influences the brain responsiveness to blue (473nm) [and to green (527nm)] light exposures (Figure 8.2). This effect depends on the sleep pressure participants were subjected to. Again this result is the first demonstration of the impact of a clock gene on human cognition and its impact on light elicited brain responses during the execution of

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Right panels: activity estimates (a.u. ± SEM) in these regions, all showing a greater response to blue light exposure as compared to green light exposure. *B1:* bleu light condition – low irradiance; *B2:* bleu light condition – high irradiance; *G1:* green light condition – low irradiance; *G2:* green light condition – high irradiance.

NB: Both temporal region activations survived controls ensuring that these effects were specifically related to emotion, while the hippocampus activation did not survive all controls. No significant differences ($p_{uncorrected} < 0.001$) were detected when the processing of neutral word-like sounds were compared between light conditions.

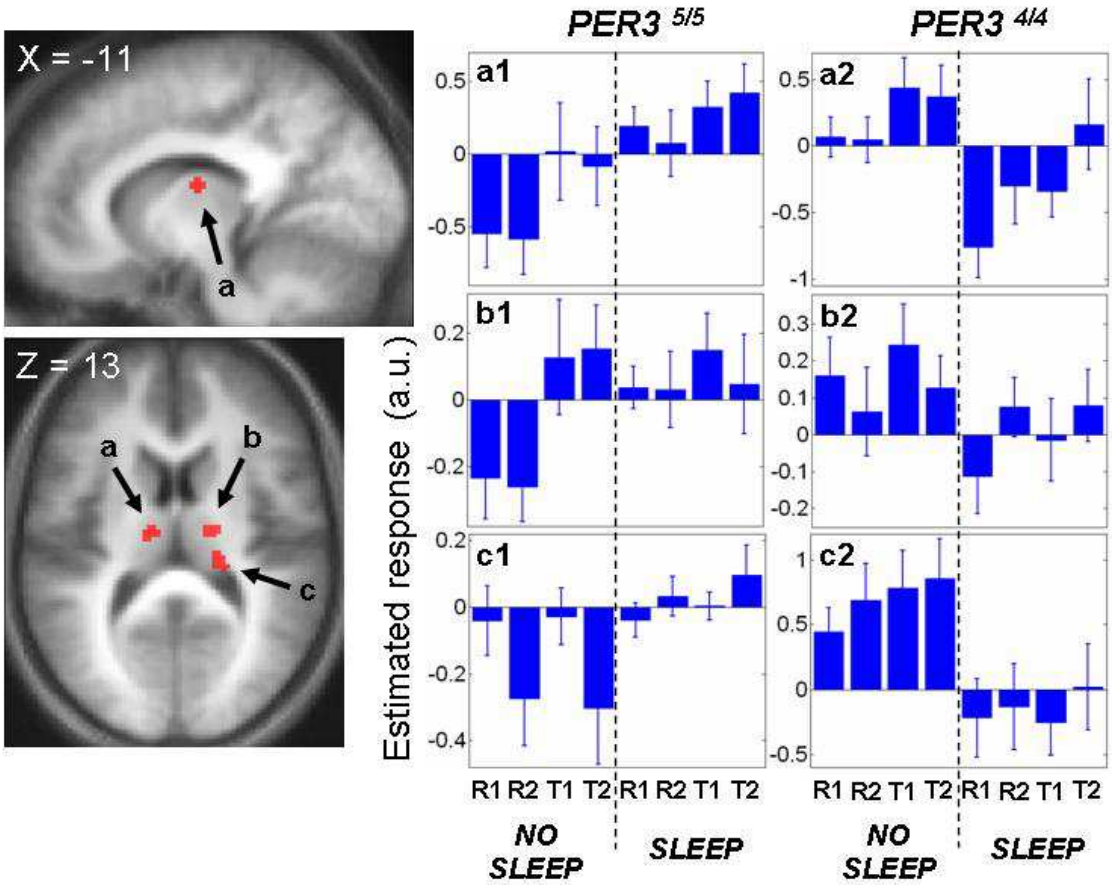

Figure 8.2: Preliminary results of an investigation of the influence of the 4 or 5 tandem-repeat polymorphism in *PER3* (*PER3* $^{4/4}$ and *PER3* $^{5/5}$) on the brain responses elicited by blue monochromatic light exposures under two sleep pressure conditions.

Protocol overview: Thirteen *PER* $^{4/4}$ and eleven *PER3* $^{5/5}$ subjects came to the laboratory on two separate visits. For both visits, they arrived in the evening and performed an fMRI session the following morning. On one occasion they were sleep deprived until the fMRI recording, while on the other they were allowed to sleep. On both occasions, they were first maintained in dim-light (< 5 lux) and required to minimize their movements for 90 min before performing an auditory *3-back* task (Cohen *et al.*, 1997) in fMRI (they were maintained in dim-light all night during the sleep deprivation protocol). Subjects were exposed to 6 alternations of blue (473 nm) and green (527nm) monochromatic light exposures of 60s separated by 5 to 14s of darkness. Two photon densities were used per monochromatic light: irradiance was equal to 9 x 10^{12} ph/cm^2/s in half of the exposures, while it was equal to 3 x 10^{13} ph/cm^2/s in the other half. During 30s of the 60s illumination periods, they were "at rest", while they performed the task during the remaining 30s.

a cognitive task. It will hopefully set the stage for other genetics investigations. In addition, this experiment compared light-induced effects at night (~2100h) and in the morning (~0800h) and could provide interesting insights on the time of day variations in brain responses to light, independent of the genotypes of the participants.

6) Patient populations

Characterizing the brain sensitivity to light of patients suffering from depressive illnesses or degenerative diseases could help determining the role of light therapy in the symptom improvement observed in some patients after light therapy treatments (Wirz-Justice, 2006).

7) Pharmacological studies

Pharmacological agent could be administered to participants in order to demonstrate that specific brain areas or a specific system mediates nonvisual effects of light. Clonidine activates a2-adrenoreceptors in the brain and suppresses release of norepinephrine (Aston-Jones and Cohen, 2005). Since the LC is almost the unique source of this neurotransmitter, its involvement in nonvisual response to light could determined using clonidine in placebo-controlled design.

8) Animal studies

Animal models could also be used in combination with neuroimaging techniques. For example, primates could be recorded in fMRI after the administration of the compounds blocking cone and rod signals which have been used to determine

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Preliminary results: *Left panels:* significant differences between both genotypes in the differences in brain response elicited by blue light exposure (both irradiance levels are pooled together), detected after a night of sleep or of the sleep deprivation. ***a.*** left thalamus (-10 -12 16; Z = 3.57; p_{svc} = 0.01); ***b.*** right thalamus (18 -10 10; Z = 3.33; p_{svc} = 0.019); ***c.*** right pulvinar (18 -26 16; Z = 3.57; p_{svc} = 0.01). Statistical results are overlaid to the population mean structural image ($p_{uncorrected}$ < 0.001). *Middle and right panels:* activity estimates (a.u. ± SEM) in these regions in the *PER3* $^{5/5}$ *(a1, b1, c1)* and in the *PER3* $^{4/4}$ *(a2, b2, c2)* populations. Although results require to be analyzed in details, they suggest that the significant differences in the effects of blue light exposure between both genotypes arise mainly from differences after the sleep deprivation. *R1:* rest periods – low irradiance; *R2:* rest periods – high irradiance; *T1:* 3-back task – low irradiance; *T2:* 3-back task – high irradiance.

NB: Differences ($p_{uncorrected}$ < 0.001) were detected in response to green light exposure but have not been analyzed yet.

melanopsin contribution to pupillary constriction in Macaques (Gamlin *et al.*, 2007). Quantifying brain function in small animals such as rats or cats could also be considered.

Neuroimaging results could be used as localizers to point to particular brain areas of interest for further investigations at a lower level. Single or multiunit recordings could be carried out in animals to detect LC or pulvinar light responses for instance. Specific lesions of these structures or use of pharmaceutical agent directed to a specific brain system (such as clonidine) could also be considered.

Conclusion

It is fascinating to see that, even if the eye and photoreception have been extensively studied for more than a century, novel cells and circuitry can still be identified. Given the intense attention it has been subjected to, it is also surprising that a entire new aspect of photoreception has been discovered as recently as about ten years ago. Study of the nonvisual effects of light constitute a field of research that has only started to reveal the broad influence of light. For this reason alone, nonvisual effects of light is an exciting area of research. But when the possible (and sometime very speculative) benefits from light therapy are also considered (*e.g.* improving conditions related to shift-work, jet-lag, daytime sleepiness, psychiatric and sleep disorders, improvement of memory and mood), or when the research on nonvisual responses to light is connected to other research field (*e.g.* genetics, psychiatry), it becomes a truly thrilling research area.

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Perrin F, Peigneux P, Fuchs S, Verhaeghe S, Laureys S, Middleton B, Degueldre C, Del Fiore G, **Vandewalle G**, Balteau E, Poirrier R, Moreau V, Luxen A, Maquet P, and Dijk DJ (2004) Nonvisual responses to light exposure in the human brain during the circadian night. *Curr Biol* **14**:1842-1846.

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Appendix 1: Chapter 5 supplemental tables

Supplemental table S5.1

Day (light > no-light) by session (post 1 > baseline) interaction ($p_{uncorrected} < 0.001$). These responses were not considered significant because they did not survive the correction for multiple comparisons either on the whole brain volume (no prior) or on a volume of interest centered on published coordinates (priors available).

Brain areas	***x***	***y***	***z***	***Z***
Left superior temporal sulcus	-34	-66	18	3.89
Left frontopolar cortex	-24	56	-4	3.75
Right superior frontal gyrus	14	-4	78	3.41
Right insula	42	-22	20	3.31
Right striate cortex	6	-72	4	3.30
Right precentral gyrus	30	-34	70	3.25
Right parahippocampus	12	-38	-10	3.25

Supplemental table S5.2

Day (light > no-light) by session (post 1 > post 2) interaction ($p_{uncorrected} < 0.001$). These responses were not considered significant because they did not survive the correction for multiple comparisons either on the whole brain volume (no prior) or on a volume of interest centered on published coordinates (priors available).

Brain areas	***x***	***y***	***z***	***Z***
Right caudate	26	-38	14	3.85
Left caudate	-18	-24	22	3.82
Right insula	44	-14	26	3.25

Supplemental table S5.3

Other day (light > no-light) by session interactions computed ($p_{uncorrected} < 0.001$) indicating that there was no significant increase in response in the 2nd post-exposure session and no decrease in brain response were elicited by light exposure.

These responses were not considered significant because they did not survive the correction for multiple comparisons either on the whole brain volume (no prior) or on a volume of interest centered on published coordinates (priors available).

Brain areas	***x***	***y***	***z***	***Z***
Day (light > no-light) by session (baseline > post 1) interaction				
No significant results at p<0.001 uncorrected				
Day (light > no-light) by session (post 2 > baseline) interaction				
Right orbito-frontal cortex	6	46	-24	3.74
Day (light > no-light) by session (baseline > post 2) interaction				
Left inferior frontal sulcus	-48	28	22	3.78
Day (light > no-light) by session (post 2 > post 1) interaction				
No significant results at p<0.001 uncorrected				

Supplemental table S5.4

Day (light > no-light) by session (post 1 > baseline) interaction computed on the brain responses modulated by time ($p_{uncorrected} < 0.001$).

These responses were not considered significant either because they did not survive the correction for multiple comparisons either on the whole brain volume (no prior) or on a volume of interest centered on published coordinates (priors available).

Brain areas	***x***	***y***	***z***	***Z***
Right insula	36	0	-18	3.92
Left subgenual anterior cingulated	-4	22	-8	3.78
Left middle temporal gyrus	-54	-42	-18	3.65
Left middle temporal gyrus	-58	-62	-12	3.65
Right middle frontal gyrus	48	6	46	3.65
Right striate cortex	-12	-84	-4	3.54
Right fusiform gyrus	46	-56	-22	3.45
Right putamen	32	-14	-10	3.39
Left precuneus	-2	-62	58	3.35
Middle occipital gyrus	50	-80	4	3.34
Striate cortex	0	-84	18	3.32
Right middle temporal gyrus	50	-54	-2	3.20
Left superior parietal gyrus	18	-66	64	3.26

Supplemental table S5.5

Day (light > no-light) by session (post 2 > baseline) interaction computed on the brain responses modulated by time ($p_{uncorrected}$ <0.001).

These responses were not considered significant either because they did not survive the correction for multiple comparisons either on the whole brain volume (no prior) or on a volume of interest centered on published coordinates (priors available).

Brain areas	***x***	***y***	***z***	***Z***
Right medial frontal cortex	8	18	50	4.10
Right insula	38	2	-18	3.57
Left superior temporal gyrus	-50	10	-14	3.50
Left putamen	-24	14	16	3.46
Right temporal pole	36	10	-30	3.20

Supplemental table S5.6

Multiple regression analysis performed on the day by session (post 1 > baseline) interaction using the 1st eigenvector over subjects of the singular value decomposition of the 18 KSS scores collected in 16 subjects (responders and non-responders).

These responses were not considered significant because they did not survive the correction for multiple comparisons either on the whole brain volume (no prior) or on a volume of interest centered on published coordinates (priors available).

Brain areas	***x***	***y***	***z***	***Z***
Left cerebellum	-10	-86	-38	4.46
Right cerebellum	16	-90	36	4.34
Left cerebellum	-48	-64	-42	3.89
Right frontopolar cortex	14	56	-6	3.64
Tail of right caudate nucleus	20	-22	20	3.61
Right superior frontal gyrus	0	22	56	3.60
Right cerebellum	34	-78	36	3.57
Left superior frontal gyrus	-50	4	50	3.51
Tail of left caudate nucleus	-16	-28	18	3.43
Left ventral globus pallidus	-22	-10	-12	3.35
Head of the right caudate nucleus	10	22	14	3.35
Right orbitofrontal cortex	18	34	-10	3.33
Left supramarginal gyrus	-48	-54	38	3.28
Left cerebellum	-48	-62	-28	3.27
Right superior frontal gyrus	12	42	58	3.18

Appendix 2: Chapter 6 supplemental tables

Supplemental table S6.1

Day (blue > green) by session (2 > 1) interaction ($p < 0.001$ *uncorrected*).
All voxels remained after use of an exclusive mask for baseline differences (session 1 green > session 1 blue; $p = 0.05$ *uncorrected*) ruling out possible confounds arising from these differences.
These responses were not considered significant because they did not survive the correction for multiple comparisons either on the whole brain volume (no prior) or on a volume of interest centered on published coordinates (priors available) or because they were not observed in other interactions.
Z-scores are reported for the interaction masked by tendency for activation during the 2-back task. Only clusters of at least 5 voxels are reported.

Brain areas	***x***	***y***	***z***	***Z***
Right superior frontal sulcus	16	14	60	3.28
Right superior temporal sulcus	54	-24	-14	3.25
Right precentral sulcus	28	-6	60	3.17
Left superior temporal sulcus	-62	-38	0	3.17

Supplemental table S6.2

Day (blue > green) by session (2 > 3) interaction ($p < 0.001$ *uncorrected*).

All voxels remained after use of a mask excluding differences between days at the end of the protocol (session 3 green > session 3 blue; $p = 0.05$ *uncorrected*), ruling out possible confounds arising from these differences.

These responses were not considered significant because they did not survive the correction for multiple comparisons either on the whole brain volume (no prior) or on a volume of interest centered on published coordinates (priors available), or because they were not observed in other interactions.

Z-scores are reported for the interaction masked by tendency for activation during the 2-back task. Only clusters of at least 5 voxels are reported.

Brain areas	***x***	***y***	***z***	***Z***
Right temporo-parietal junction	46	-38	34	4.48
Right middle frontal gyrus	44	10	56	4.13
Left superior precentral sulcus	-26	-20	54	4.04
Right thalamus	16	2	12	3.96
Left cerebellum	-8	-48	-28	3.89
Right cingulated sulcus	6	24	38	3.69
Right middle frontal sulcus	42	38	20	3.68
Right middle frontal sulcus	30	22	26	3.67
Right intraparietal sulcus	30	-54	38	3.47
Right superior temporal gyrus	58	10	-14	3.33
Left inferior frontal sulcus	-28	10	34	3.35

Supplemental table S6.3

Remaining day by session interaction were computed ($p < 0.001$ *uncorrected*).

These responses were not considered significant because they did not survive the correction for multiple comparisons either on the whole brain volume (no prior) or on a volume of interest centered on published coordinates (priors available).

Z-scores are reported for the interaction masked by tendency for activation during the 2-back task.

Only clusters of at least 5 voxels are reported.

Brain areas	***x***	***y***	***z***	***Z***
Day (blue > green) by session (3 > 1) interaction				
No significant results at p<0.001 uncorrected				
Day (blue > green) by session (1 > 3) interaction				
Left cerebellum	-24	-60	-46	3.69
Right cerebellum	12	-76	-40	3.44
Day (blue > green) by session (1 > 2) interaction				
No significant results at p<0.001 uncorrected				
Day (blue > green) by session (3 > 2) interaction				
No significant results at p<0.001 uncorrected				

Appendix 3: Chapter 7 supplemental tables

Supplemental tables S7.1

Light condition effects during the *2-back task* ($p_{uncorrected} < 0.001$). These responses were not considered significant because they did not survive the correction for multiple comparisons either on the whole brain volume (no prior) or on a volume of interest centered on published coordinates (priors available).

BLUE LIGHT > GREEN LIGHT

No significant voxel at p=0.001 *uncorrected.*

GREEN LIGHT > BLUE LIGHT

No significant voxel at p=0.001 *uncorrected.*

VIOLET LIGHT > BLUE LIGHT

Brain areas	*xyz*	*Z*
Right hippocampus	28 -24 -12	3.33

BLUE LIGHT > VIOLET LIGHT

Brain areas	***xyz***	***Z***
Left precentral sulcus	-38 -22 52	4.36
Right insula	36 -28 16	4.18
Left superior frontal gyrus	-8 0 72	4.00
Right superior frontal sulcus	24 20 66	3.72
Right inferior frontal gyrus	56 22 4 38 48 40	3.68 3.52
Left insula	-30 -28 14	3.53
Right middle occipital gyrus	32 -96 -8	3.49
Right superior precentral sulcus	44 12 34	3.32
Right superior temporal sulcus	48 -20 -16	3.32
Left lateral fissure	-56 -22 14	3.18
Right lateral fissure	54 -24 10	3.16

GREEN LIGHT > VIOLET LIGHT

No significant voxel at p=0.001 *uncorrected.*

VIOLET LIGHT > GREEN LIGHT

Brain areas	***xyz***	***Z***
Right middle occipital gyrus	56 -68 0	3.34
Left middle occipital gyrus	-50 -82 9	3.69

Supplemental tables S7.2

Light condition effects modeled by *saw-tooth-like regressors* ($p_{uncorrected} < 0.001$). These regressors model brain activity that show progressive build up during the whole 50s illumination. These responses were not considered significant because they did not survive the correction for multiple comparisons either on the whole brain volume (no prior) or on a volume of interest centered on published coordinates (priors available).

BLUE LIGHT VS. GREEN LIGHT

Brain areas	***xyz***	***Z***
Right cuneus	16 -92 6	3.48

GREEN LIGHT VS. BLUE LIGHT

No significant voxel at p=0.001 *uncorrected.*

VIOLET LIGHT VS. BLUE LIGHT

Brain areas	***xyz***	***Z***
Left superior frontal gyrus	-6 16 54	3.62
Right inferior frontal gyrus	42 24 20	3.40
Right lateral fissure	54 -22 8	3.41
Left insula	-32 16 -8	3.37
Right parieto-occipital fissure	12 -66 36	3.30
Right superior temporal sulcus	42 -16 -14	3.29
Left lateral fissure	-56 -24 14	3.25
Right cingulate sulcus	2 -46 46	3.18

BLUE LIGHT VS. VIOLET LIGHT

No significant voxel at p=0.001 *uncorrected.*

GREEN LIGHT VS. VIOLET LIGHT

Brain areas	***xyz***	***Z***
Right central sulcus	64 -34 44	3.92
Right inferior frontal gyrus	52 40 4	3.34
Left middle occipital gyrus	-52 -74 2	3.28

VIOLET LIGHT VS. GREEN LIGHT

Brain areas	***xyz***	***Z***
Left cuneus	-8 -104 10	3.36

Supplemental tables S7.3

Light condition effects at *light onsets*. These responses were not considered significant because they did not survive the correction for multiple comparisons either on the whole brain volume (no prior) or on a volume of interest centered on published coordinates (priors available).

BLUE LIGHT > GREEN LIGHT

Brain areas	*xyz*	*Z*
Right superior frontal sulcus	24 -4 60 18 2 64	4.34 4.11
Left insula	-38 -10 16	4.19
Left middle occipital gyrus	-20 -88 6	3.54
Left hippocampus	-28 -24 -14	3.35
Left superior frontal gyrus	-6 -10 60	3.22

GREEN LIGHT > BLUE LIGHT

No significant voxel at p=0.001 *uncorrected.*

VIOLET LIGHT > BLUE LIGHT

Brain areas	*xyz*	*Z*
Left cerebellum	-18 -82 -26	3.81
Right superior temporal sulcus	66 -34 -10	3.66
Left inferior frontal sulcus	-28 42 14	3.64
Left superior frontal sulcus	-28 -8 52	3.59
Left hippocampus	-34 -10 -32 -24 -28 -10	3.58 3.17
Right posterior cingulate cortex	2 -42 44	3.50
Right angular gyrus	42 -64 30	3.39
Right parieto-occipital fissure	26 -60 6	3.37

BLUE LIGHT > VIOLET LIGHT

No significant voxel at p=0.001 *uncorrected.*

VIOLET LIGHT > GREEN LIGHT

Brain areas	***xyz***	***Z***
Right hippocampus	28 -38 4	3.30

GREEN LIGHT > VIOLET LIGHT

Brain areas	***xyz***	***Z***
Right parahippocampus	26 -40 -14	3.92
Left precuneus	-2 -42 58	3.82
Left anterior cingulate cortex	-6 52 4	3.81
Right inferior frontal gyrus	62 22 2	3.38
Left posterior cingulate cortex	-6 -24 42	3.18