8/29/00

PROV

JC918 U.S. PTO 08/28/00

jc866 U.S. PTO 60/228493 08/28/00

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PTO/SB/16 (2-98)
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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
George C.	BRAINARD	Haddonfield, New Jersey

☐ *Additional inventors are being named on the ___ separately numbered sheets attached hereto*

TITLE OF THE INVENTION (280 characters max)

A NEW PHOTORECEPTOR SYSTEM FOR MELATONIN REGULATION

CORRESPONDENCE ADDRESS

Direct all correspondence to:

☐ Customer Number 24358 → *Type Customer Number here*

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OR

☐ Firm *or* Individual Name	Clifford Kent Weber				
Address					
Address					
City		State		ZIP	
Country		Telephone	(215) 503-0757	Fax	

ENCLOSED APPLICATION PARTS *(check all that apply)*

- [X] Specification *Number of Pages* 4
- [X] Small Entity Statement
- [X] Drawing(s) *Number of Sheets* 12
- [X] Other (specify) Express Mail Letter

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT *(check one)*

- ☐ A check or money order is enclosed to cover the filing fees
- [X] The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 50-0491

FILING FEE AMOUNT ($): $150.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☐ No.

[X] Yes, the name of the U.S. Government agency and the Government contract number are: NIH RO1NS36590 and NSF IBN9809916

Respectfully submitted,

SIGNATURE Clifford K. Weber

TYPED or PRINTED NAME Clifford K. Weber

TELEPHONE (215) 503-0757

Date 08/28/00

REGISTRATION NO. *(if appropriate)* 42,215

Docket Number: BRA01-P0003

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is used by the public to file (and by the PTO to process) a provisional application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the complete provisional application to the PTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, Washington, D.C., 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Box Provisional Application, Assistant Commissioner for Patents, Washington, D.C., 20231.

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Patent fees are subject to annual revision.
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TOTAL AMOUNT OF PAYMENT ($) $75.00

Complete if Known	
Application Number	
Filing Date	
First Named Inventor	BRAINARD, George
Examiner Name	
Group / Art Unit	
Attorney Docket No.	BRA01-P0003

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1. [X] The Commissioner is hereby authorized to charge indicated fees and credit any overpayments to:

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Deposit Account Name: Thomas Jefferson University

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FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
101	690	201	345	Utility filing fee	
106	310	206	155	Design filing fee	
107	480	207	240	Plant filing fee	
108	690	208	345	Reissue filing fee	
114	150	214	75	Provisional filing fee	$75.00

SUBTOTAL (1) ($) 75.00

2. EXTRA CLAIM FEES

	Extra Claims		Fee from below	Fee Paid
Total Claims	-20** =	X		=
Independent Claims	- 3** =	X		=
Multiple Dependent				=

**or number previously paid, if greater; For Reissues, see below

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
103	18	203	9	Claims in excess of 20
102	78	202	39	Independent claims in excess of 3
104	260	204	130	Multiple dependent claim, if not paid
109	78	209	39	** Reissue independent claims over original patent
110	18	210	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($)

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
105	130	205	65	Surcharge - late filing fee or oath	
127	50	227	25	Surcharge - late provisional filing fee or cover sheet.	
139	130	139	130	Non-English specification	
147	2,520	147	2,520	For filing a request for reexamination	
112	920*	112	920*	Requesting publication of SIR prior to Examiner action	
113	1,840*	113	1,840*	Requesting publication of SIR after Examiner action	
115	110	215	55	Extension for reply within first month	
116	380	216	190	Extension for reply within second month	
117	870	217	435	Extension for reply within third month	
118	1,360	218	680	Extension for reply within fourth month	
128	1,850	228	925	Extension for reply within fifth month	
119	300	219	150	Notice of Appeal	
120	300	220	150	Filing a brief in support of an appeal	
121	260	221	130	Request for oral hearing	
138	1,510	138	1,510	Petition to institute a public use proceeding	
140	110	240	55	Petition to revive - unavoidable	
141	1,210	241	605	Petition to revive - unintentional	
142	1,210	242	605	Utility issue fee (or reissue)	
143	430	243	215	Design issue fee	
144	580	244	290	Plant issue fee	
122	130	122	130	Petitions to the Commissioner	
123	50	123	50	Petitions related to provisional applications	
126	240	126	240	Submission of Information Disclosure Stmt	
581	40	581	40	Recording each patent assignment per property (times number of properties)	
146	690	246	345	Filing a submission after final rejection (37 CFR § 1.129(a))	
149	690	249	345	For each additional invention to be examined (37 CFR § 1.129(b))	

Other fee (specify) _____

Other fee (specify) _____

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SUBTOTAL (3) ($)

SUBMITTED BY				Complete (if applicable)	
Name (Print/Type)	Clifford Kent Weber	Registration No. (Attorney/Agent)	42,215	Telephone	(215) 503-0757
Signature	Clifford Kent Weber			Date	08/28/2000

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TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Application Number	Not Known
Filing Date	Not Known
First Named Inventor	BRAINARD, George C.
Group Art Unit	
Examiner Name	
Total Number of Pages in This Submission: 22	Attorney Docket Number: BRA01-P0003

ENCLOSURES *(check all that apply)*

- [X] Fee Transmittal Form
  - [ ] Fee Attached
- [ ] Amendment / Response
  - [ ] After Final
  - [ ] Affidavits/declaration(s)
- [ ] Extension of Time Request
- [ ] Express Abandonment Request
- [ ] Information Disclosure Statement
- [ ] Certified Copy of Priority Document(s)
- [ ] Response to Missing Parts/ Incomplete Application
  - [ ] Response to Missing Parts under 37 CFR 1.52 or 1.53
- [ ] Assignment Papers *(for an Application)*
- [X] Drawing(s)
- [ ] Licensing-related Papers
- [ ] Petition Routing Slip (PTO/SB/69) and Accompanying Petition
- [ ] To Convert a Provisional Application
- [ ] Power of Attorney, Revocation Change of Correspondence Address
- [ ] Terminal Disclaimer
- [X] Small Entity Statement
- [ ] Request for Refund
- [ ] After Allowance Communication to Group
- [ ] Appeal Communication to Board of Appeals and Interferences
- [ ] Appeal Communication to Group *(Appeal Notice, Brief, Reply Brief)*
- [ ] Proprietary Information
- [ ] Status Letter
- [X] Additional Enclosure(s) *(please identify below)*:
  - Provisional Application Cover Sheet and Provisional Application, including specification

Remarks

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm or Individual name	Clifford Kent Weber
Signature	Clifford Kent Weber
Date	August 28, 2000

CERTIFICATE OF MAILING

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

ATTORNEY DOCKET NO.: **BRA01-P0003**

TITLE: **A NEW PHOTORECEPTOR SYSTEM FOR MELATONIN REGULATION**

INVENTORS: **BRAINARD, George**

"Express Mail" Label No. **EE 388 354 145 US**
Date of Deposit – **August 28, 2000**
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By Clifford Kent Weber

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Box Provisional Patent Application
Commissioner of Patents & Trademarks
Washington, DC 20231

Sir:

PROVISIONAL APPLICATION TRANSMITTAL LETTER

Transmitted herewith for filing, please find the following:

(XX) Specification containing 4 pages.

(XX) Transmittal Form

(XX) Provisional Application Cover Sheet

(XX) Fee Transmittal

(XX) Small Entity Statement

Respectfully submitted,

Clifford Kent Weber

Clifford Kent Weber, Esq.
Registration No. 42,215

PTO/SB/11 (12-97)
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STATEMENT CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) & 1.27(d))--NONPROFIT ORGANIZATION

Docket Number (Optional) BRA01-P0003

Applicant, Patentee, or Identifier: Thomas Jefferson University
Application or Patent No.: Not Known
Filed or Issued: Not Known
Title: A NEW PHOTORECEPTOR SYSTEM FOR MELATONIN REGULATION

I hereby state that I am an official empowered to act on behalf of the nonprofit organization identified below:
NAME OF NONPROFIT ORGANIZATION Thomas Jefferson University
ADDRESS OF NONPROFIT ORGANIZATION 1020 Walnut St. Suite 620
Philadelphia, PA 19107

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☒ the specification filed herewith with title as listed above.
☐ the application identified above.
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Each person, concern, or organization having any rights in the invention is listed below:

☐ no such person, concern, or organization exists.
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NAME OF PERSON SIGNING Alan B. Kelly
TITLE IN ORGANIZATION OF PERSON SIGNING University Counsel
ADDRESS OF PERSON SIGNING 1020 Walnut Street Suite 630, Philadelphia, PA 19103
SIGNATURE _____ DATE August 28, 2000

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Human Melatonin Regulation is Not Mediated by the Three Cone Photopic Visual System

ABSTRACT The aim of this study was to test if the three cone photopic visual system is the primary ocular photoreceptor input for human circadian regulation by determining the effects of different wavelengths on light-induced melatonin suppression. Healthy subjects with stable sleeping patterns (wake-up time 7:30 AM ± 12 min) and normal color vision were exposed at night to full-field 505 nm or 555 nm monochromatic stimuli or darkness for 90 min. Plasma collected before and after exposures was quantified for melatonin. Subjects exposed to 10 irradiances at 505 nm showed no significant differences across mean pre-exposure melatonin values ($F=0.505$). A sigmoidal fluence-response curve fitted to the melatonin suppression data ($R^2=0.97$) indicated that 9.34×10^{12} photons/cm^2/sec induced a half-saturation response (ED_{50}) while 6.84×10^{13} photons/cm^2/sec induced a saturation melatonin suppression response. Further, a dose of 4.19×10^{13} photon/cm^2/sec at 505 nm was significantly stronger ($P<0.01$) than an equal photon dose at 555 nm for melatonin suppression. These data demonstrate that the cone system that mediates human photopic vision is not the primary photoreceptor system to transduce light stimuli for melatonin regulation.

Introduction

The underlying neuroanatomy and neurophysiology which mediate vision have been studied extensively over the past two centuries. More recently, the retinohypothalamic tract (RHT), a distinct neural pathway which supports circadian regulation by environmental light, has been shown to project from the retina to the suprachiasmatic nuclei (SCN) in the hypothalamus (1, 2). A well-defined multisynaptic neural pathway extends from the SCN to the pineal gland which transmits information about light and circadian time for entraining the rhythmic production and secretion of the hormone melatonin (1-4). In addition to synchronizing pineal indolamine circadian rhythms, ocular exposure to light during the night can acutely suppress melatonin synthesis and secretion (5, 6). Light-induced melatonin suppression is a well-defined, broadly used marker for photic input to the RHT and SCN (2, 4, 7, 8).

Currently, it is not known what photoreceptors transduce light stimuli for circadian regulation. Studies on animals with hereditary or light-induced retinal degeneration have raised the possibility that neither the rods nor the cones used for vision participate in light-induced melatonin suppression, circadian locomotor phase-shifts, or photoperiodic responses (8-12). Furthermore, bilateral removal of the eyes from rod-less, cone-less transgenic mice abolished light-induced circadian phase-shifting and acute melatonin suppression (8, 12). Recently, light-induced melatonin suppression and circadian entrainment have been demonstrated in humans with complete visual blindness (13) and with specific color vision deficiencies (14). The study on humans with color vision deficiencies showed that protanopic and deuteranopic subjects who lacked functioning long wavelength-sensitive cones (red, or L cones), and middle wavelength cone photoreceptors (green, or M cones), exhibited normal light-induced melatonin suppression and entrainment of the melatonin rhythm (14). Thus, by themselves, neither the red nor green cone system could be the primary input for melatonin regulation, at least in humans with color vision deficiencies. Together, the results from human and animal circadian studies on different forms of visual blindness suggest that melatonin regulation by light is controlled, at least in part, by photoreceptors which differ from the photoreceptors that mediate vision.

Recent studies with various vertebrate species have identified several new molecules which may serve as circadian photopigments. These putative photopigments include both opsin-based molecules, such as vertebrate ancient (VA) opsin and melanopsin, as well as non-opsin molecules like the cryptochromes (15-17). Among these new photopigments, only melanopsin has been specifically localized to the human retina (18). The molecular identification of these candidate photopigments and their localization in the retina and/or neural components of the circadian system make them well-suited to act as circadian phototransducers. Functional data confirming their direct role in circadian photoreception, however, are lacking.

The aim of this study was to test the hypothesis that the three cone system which supports photopic (daytime) vision is the primary input for pineal melatonin suppression in humans with normal, healthy eyes. The peak wavelength sensitivity of the photopic visual system is generally believed to be near 555 nm (19). If melatonin regulation is mediated primarily by the three cone photopic visual system, then 555 nm light would be the most potent wavelength for regulating melatonin secretion. Our data show that 505 nm is approximately four times stronger than 555 nm in suppressing melatonin. These results demonstrate that the ocular photoreceptor primarily responsible for pineal melatonin regulation in humans, is not the cone system that is believed to mediate photopic vision. This study is the first test of a specific photoreceptor system for melatonin regulation in humans with healthy, intact eyes.

Subjects, Materials and Methods

Subjects. The healthy females (N=6) and males (N=10) in this study had a mean ± SEM age of 25.7 ± 0.8 yrs, demonstrated normal color vision as measured by the Ishihara and Farnsworth

Munsell D-100 tests (mean FM score: 64.2 ± 11.5), had a stable sleeping pattern (mean wake-up time 7:30 AM ± 12 min), and signed an approved IRB consent document before participating.

Light exposure protocol. Each experiment began at midnight when subjects entered a dimly lit room (10 lux). One drop of 0.5% Cyclopentolate HCl was placed in each eye to dilate the pupils, and blindfolds were placed over subjects' eyes. Subjects remained sitting upright in darkness for 120 min. While still blindfolded and just prior to 2:00 AM, a blood sample was taken by venipuncture. During light exposure, each subject's head rested in an ophthalmologic head holder facing a Ganzfeld apparatus that provided a concave, patternless, white reflecting surface encompassing the subject's entire visual field. The subjects were exposed to the light stimulus from 2:00 to 3:30 AM. During this 90 min exposure, subjects sat quietly, kept their eyes open and gazed at a fixed target dot in the center of the Ganzfeld dome. Subject compliance for keeping their eyes open and the subjects' pupil size were monitored by a miniature video camera. At 3:30 AM, a second blood sample was taken. Each subject was exposed to complete darkness from 2:00 to 3:30 AM on their control night and was tested with at least 6 days between each nighttime exposure. Plasma samples were assayed for melatonin by RIA (20). The minimum detection limit of the assay was 0.5 - 2.0 pg/mL. Control samples had 8% and 14% intra-assay coefficients of variation.

Light production and measurement. Experimental light stimuli were produced by xenon arc lamps (Photon Technology Int'l , Inc., Princeton, NJ) enclosed in a light-proof chamber and cooled by high-speed fans and water circulation. An exit beam of light from each source was directed by a parabolic reflector, and excess heat in this beam was reduced by a water filter. Monochromatic wavelengths (10-11 nm half-peak bandwidth) were produced by a grating monochromator and light irradiance was controlled by a manual diaphragm. The resulting light beam was directed into the top area of a Ganzfeld dome and reflected evenly off the walls into volunteers' eyes. The entire reflecting surface of the dome was coated with a white surface with a 95-99% reflectance efficiency over the 400 to 760 nm range. Routine measurement of the light irradiance ($\mu W/cm^2$) was done with a J16 Meter with a J6512 irradiance probe (Tektronix, Beaverton, OR). Experimental light stimuli reflected from the Ganzfeld domes were measured at volunteers' eye level immediately before and after the 90 min exposure. Additional measures were taken each half hour of the exposure to insure stimulus stability and enable intensity readjustment. Subjects in the 505 nm series were exposed to intensities ranging from 0.011 to 97 $\mu W/cm^2$ (a 3 log unit photon density range of 10^{10} to 10^{13} photons/cm^2). Subjects exposed to 555 nm received control or a 15 $\mu W/cm^2$ (4.2×10^{13} photons/cm^2) exposure.

Statistics. Two-tailed, Students' t tests were used to assess significance of raw melatonin change from 2:00 to 3:30 AM. These data were then converted to % control-adjusted melatonin change scores as described elsewhere (21). For the 505 nm data, sets of pre-exposure melatonin values and % control-adjusted melatonin change scores were analyzed with one-way, repeated measures ANOVA. Significant differences between groups were assessed with post-hoc Scheffe F-tests; alpha was set at 0.05. For the 505 nm mean % control-adjusted melatonin suppression data, the computer program Origin 6.0 (Microcal, Northampton, MA) was used to fit a fluence-response curve to a 4 parameter model as described elsewhere (22), and to test for goodness-of-fit of the data by coefficient of correlation.

Results

The full 505 nm data complement, from raw melatonin values to a final fluence-response curve, is illustrated in Figures 1-3. Across all nights of testing, there were no significant differences (F=0.85) between sets of pre-exposure melatonin values indicating that plasma levels were consistent across the different study nights. Figure 1 shows the mean ± SEM pre- and post-exposure melatonin values. One-way, repeated measures ANOVA showed a significant effect of light intensity on plasma melatonin % change scores (F=17.17, P<0.0001). Paired t tests demonstrated that compared to the 0 $\mu W/cm^2$ control night, all intensities at or above 5.5 $\mu W/cm^2$ significantly suppressed melatonin (P<0.05 or less). In contrast, irradiances of 2.8, 1.4 and 0.011 $\mu W/cm^2$ did not suppress melatonin compared to the control night (Scheffe F values: 0.97, 0.01 and 0.02, respectively). As illustrated in Figure 2, all melatonin data were converted to control-adjusted % change scores. As with the melatonin % change scores, ANOVA showed a significant effect of light intensity on plasma melatonin % control-adjusted change scores (F=13.59, P<0.0001).

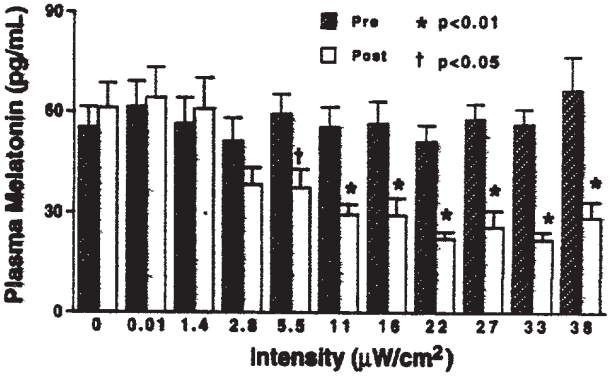

Figure 1: In this graph the bars represent group mean + SEM plasma melatonin values (N=8) before and after monochromatic light exposure at 505 nm. There were no significant variations across mean melatonin pre-light exposure values (F=0.85). Paired, two-tailed Students' t tests demonstrated which light intensities elicited a significant melatonin suppression.

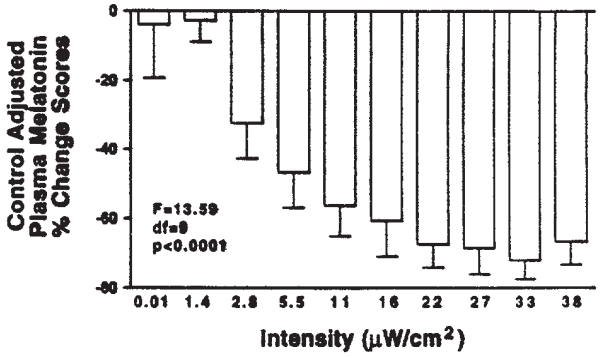

Figure 2: This graph illustrates group mean + SEM % control-adjusted melatonin change values (N=8) at 505 nm monochromatic light exposure. The figure shows that progressively higher light irradiance exposure produces increasingly greater melatonin suppression.

Figure 3 illustrates a best fit, sigmoidal fluence-response curve which plots melatonin % control-adjusted

scores against stimulus photon density. The specific formula for this curve is included in the figure.

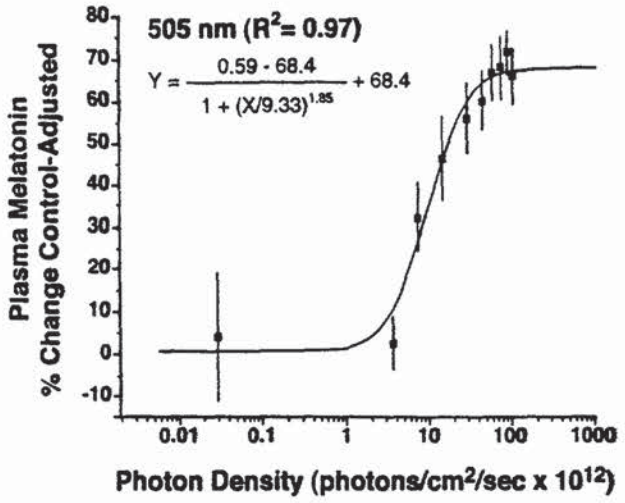

Figure 3: This figure demonstrates the fitted fluence-response curve for photon density and % control-adjusted melatonin suppression (N=8). Each data point represents one group mean ± SEM.

Subjects exposed to 555 nm received both control (0 $\mu W/cm^2$) and 15 $\mu W/cm^2$ (4.2 x 10^{13} photons/cm^2) exposures. For the control and light exposure nights, the mean pre-exposure raw melatonin scores were 64.4 ± 12.5 and 59.6 ± 6.2, while the mean post-exposure scores were 62.6 ± 10.5 and 49.1 ± 6.0, respectively. The modest drop in melatonin over the 90 min 555 nm light exposure period was not statistically significant (t=1.69, df=7, P=0.14). For comparison of responses to 505 nm and 555 nm, Figure 4 illustrates % control-adjusted melatonin suppression relative at equal photon densities across the two wavelengths. These data reveal that 505 nm is significantly stronger than 555 nm in suppressing melatonin (t=-3.04, df=14, P<0.01).

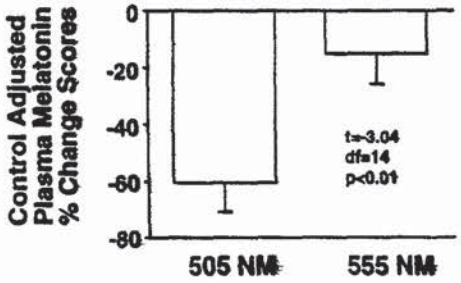

Figure 4: The bars represent group mean + SEM values relative to an equal photon dose of 4.2 x 10^{13} photons/cm^2. These data show that the 505 nm % control-adjusted plasma melatonin suppression is significantly stronger than that for 555 nm.

Discussion

The data presented here demonstrate that: 1) there is a clear fluence-response relationship between graded light intensities of 505 nm light and melatonin suppression, and 2) that 505 nm light is significantly stronger than 555 nm light for suppressing melatonin in healthy, human subjects. Previous studies with animals and humans have illustrated fluence-response relationships for melatonin suppression and circadian phase-shifting with exposure to monochromatic light (23-25) as well as white light (22, 26). A recent study on human subjects suggests that a four parameter curve is optimum for modeling light-induced melatonin suppression and circadian phase shifting (27). That contention matches our own earlier animal data (22) as well as the 505 nm data reported here.

The demonstration that 505 nm light is more potent than 555 nm light for controlling melatonin has important basic science and clinical implications. In humans, it is well-established that higher levels of ocular illumination are required for stimulating the circadian system than for supporting vision (6, 25, 30). Consequently, many investigators have considered the three cone photopic visual system to be responsible for stimulating circadian and neuroendocrine responses since this part of the visual system is responsive to "bright" daytime levels of illumination. Over the past 20 years most of the published literature on human circadian responses to light reports light levels in terms of illuminance (lux, lumens) which is a specific measure based on the traditional sensitivity curve of the photopic visual system. The peak wavelength sensitivity of that curve is 555 nm (19). Indeed, some researchers have argued that their data support the notion that the visual cones are involved in circadian phase-shifting in humans (28). If melatonin regulation is mediated primarily by the three cone photopic visual system, then 555 nm light should be the most potent wavelength for regulating melatonin. Our data do not support this hypothesis. On the contrary, 505 nm is significantly stronger, photon for photon, than 555 nm in suppressing melatonin. The clinical implication of this result is that it is not optimum to use photometric values (lux) for quantifying light used therapeutically in patients with certain sleep disorders or circadian disruption due to shiftwork or intercontinental jet travel as is the current standard practice (29).

Ideally, the best circadian measurement system would match the action spectrum for human circadian regulation. That action spectrum would not only elucidate the relative circadian potencies of different wavelengths, but it should help identify the photoreceptor that initiates signals from the retina to the SCN. Unfortunately, an action spectrum has yet to be established for any circadian response in humans. It remains possible that one of the "classic" visual photopigments such as rhodopsin or the short wavelength sensitive (blue) cone opsin mediates circadian phototransduction. Alternatively, one of the new opsin or non-opsin molecules that have been proposed as circadian photopigments (15-17) may prove to serve this function. Until the action spectrum for circadian regulation in humans has been clarified, it remains reasonable to continue using photometric terminology and measurements for characterizing light that regulates the circadian system.

In summary, monochromatic 505 nm light suppressed melatonin in a fluence-response manner, and is approximately four times stronger than a 555 nm stimulus at an equal photon dose for melatonin suppression. These data demonstrate that the three cone system that is believed to mediate human photopic vision is not the primary

photoreceptor system to transduce light stimuli for melatonin regulation.

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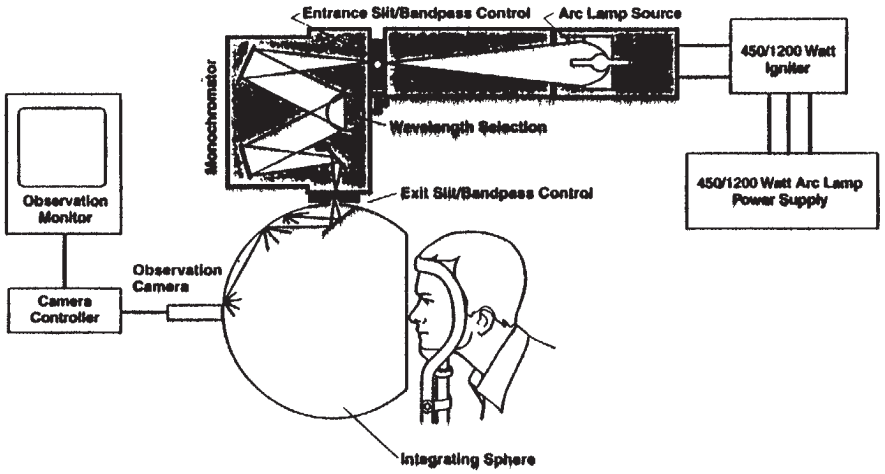
ACTION SPECTRUM METHODS

- Each volunteer studied on separate nights with at least one week between experiments.
- Volunteers exposed to: 1) one night of no light exposure, 2) six or more nights of light exposure from 02:00 to 03:30.
- All light exposures are monochromatic and done in a ganzfeld apparatus.
- Each fluence-response curve uses a within subjects design.
- Blood assayed for melatonin content by RIA.

(1) BASIC PROTOCOL



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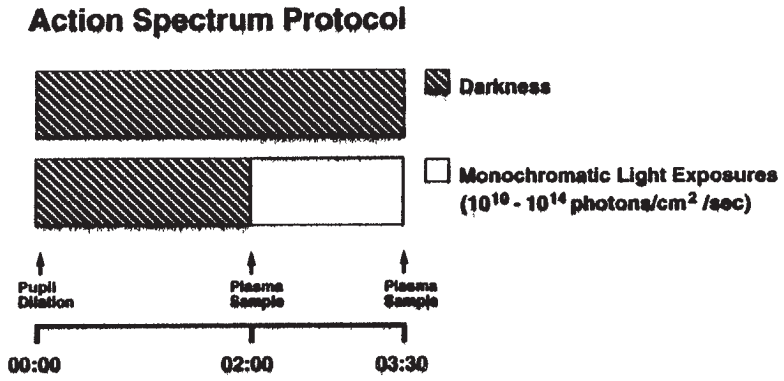

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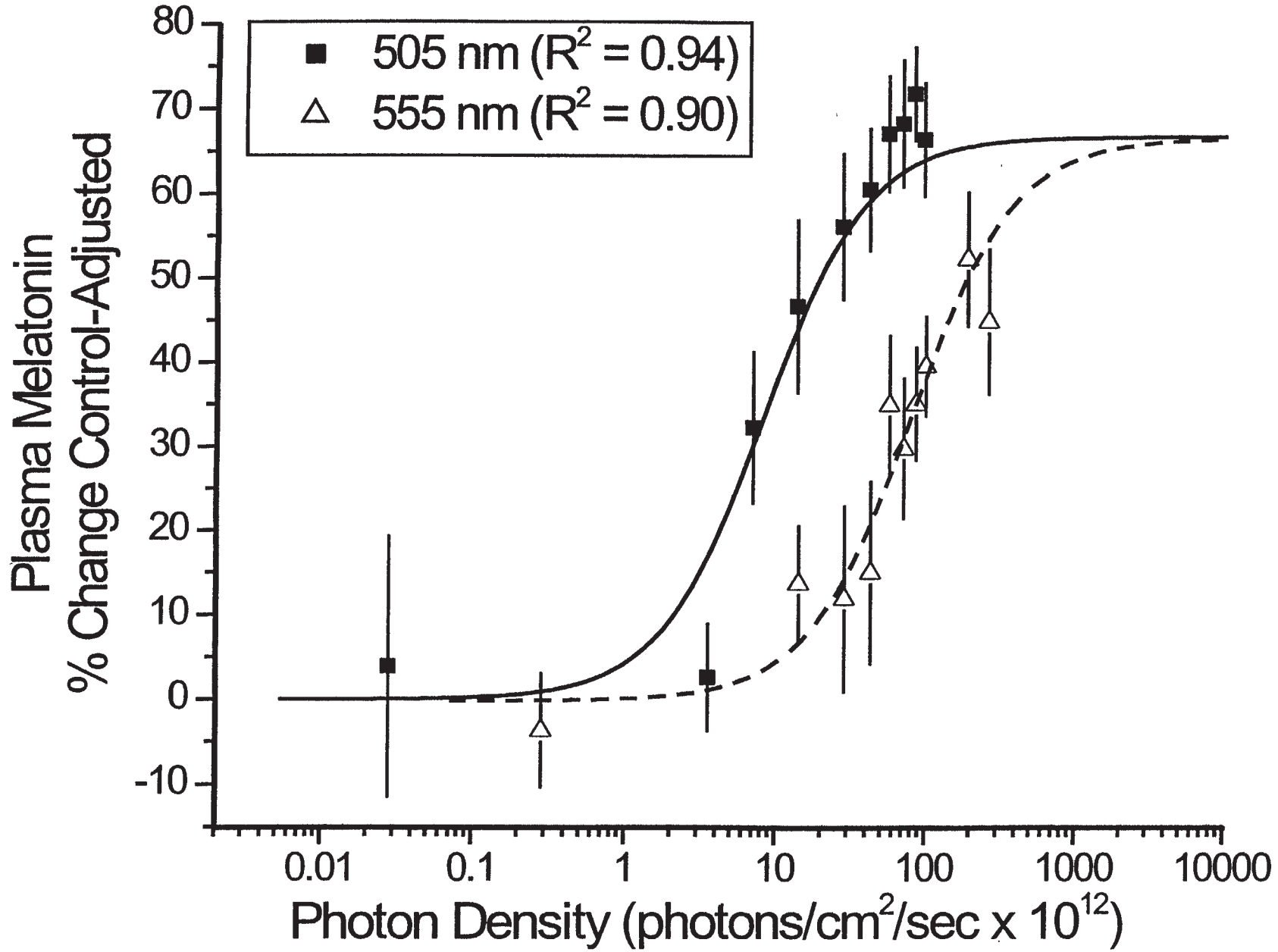
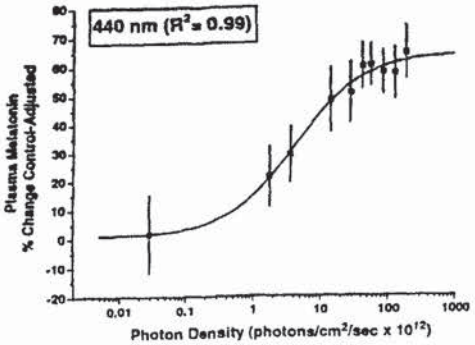
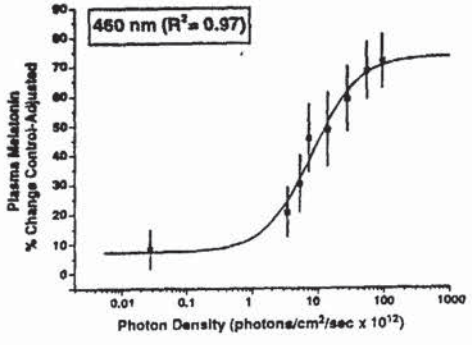
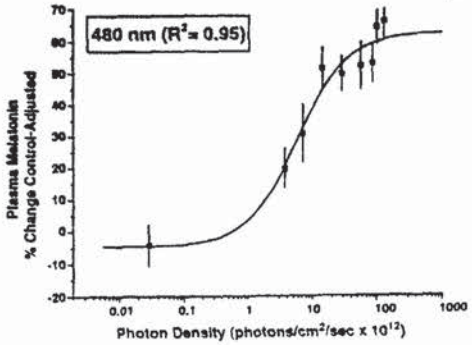


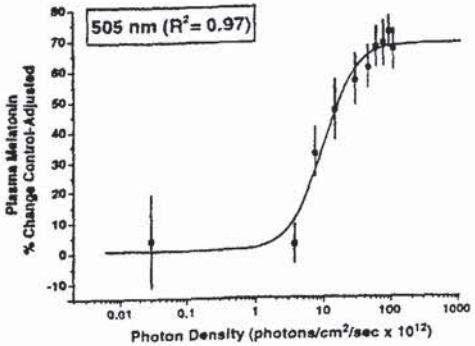
(4.) THIS SHOWS DATA NOT INCLUDED IN JCEM MANUSCRIPT CONFIRMING 3 CONE VISUAL SYSTEM DOESN'T MEDIATE CIRCADIAN SYSTEM.

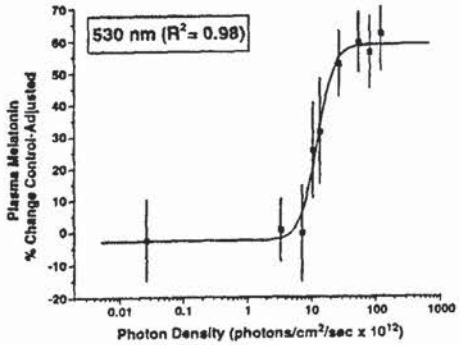
Exposures in Healthy Subjects (N=63)

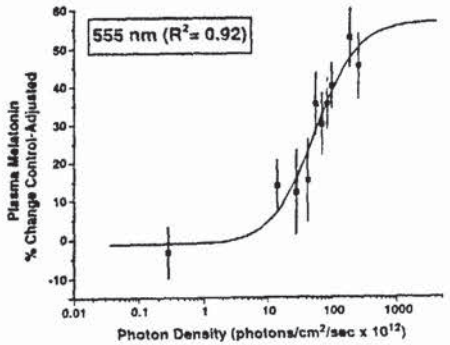


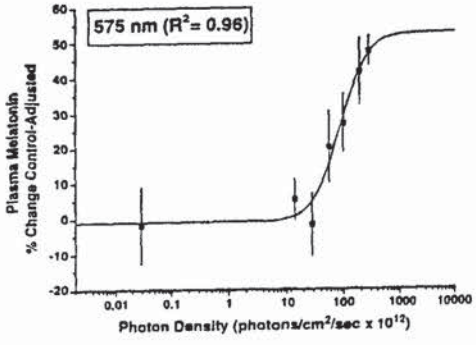


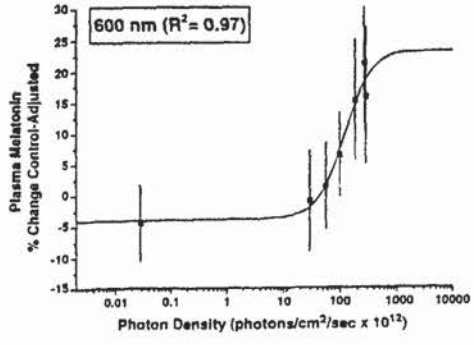






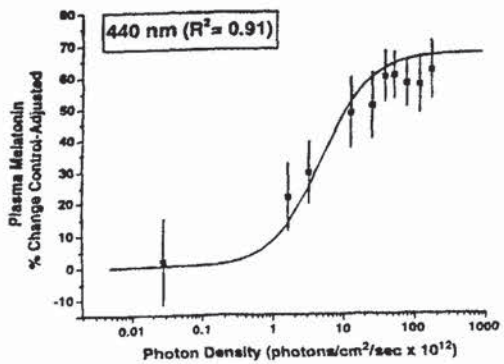


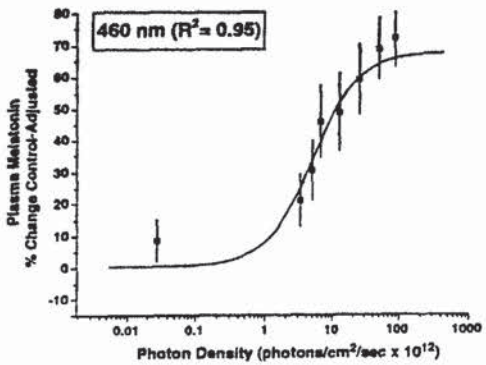


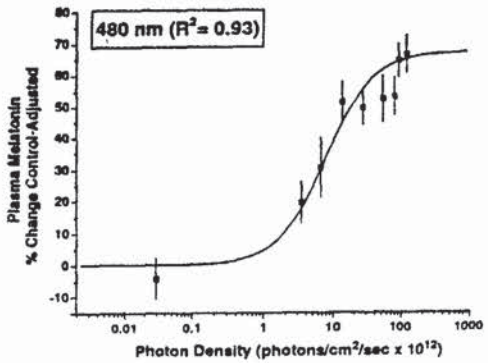


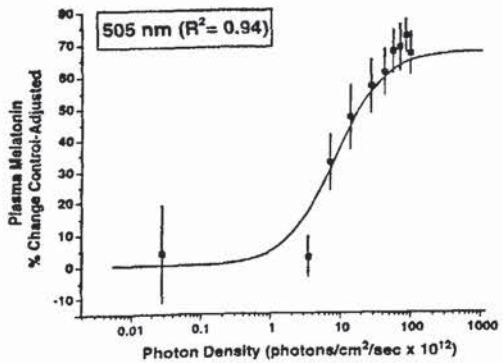
(5.) 8 FLUENCE-RESPONSE CURVES FOR 8 VISIBLE WAVELENGTHS. EACH CURVE IS FROM 7-8 SUBJECTS. THE ARE MATHEMATICALLY UNRESTRAINED.

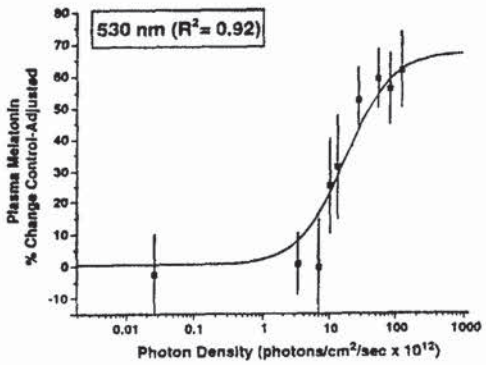
Exposures in Healthy Subjects (N=63)
Fitted to Univariant Curves

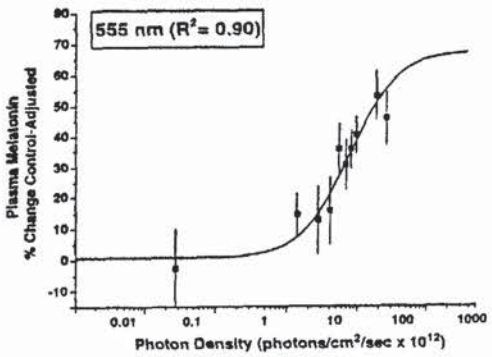


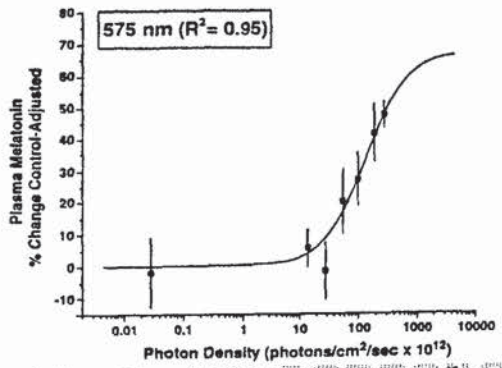


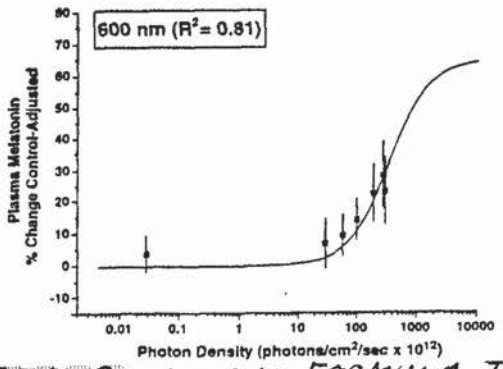




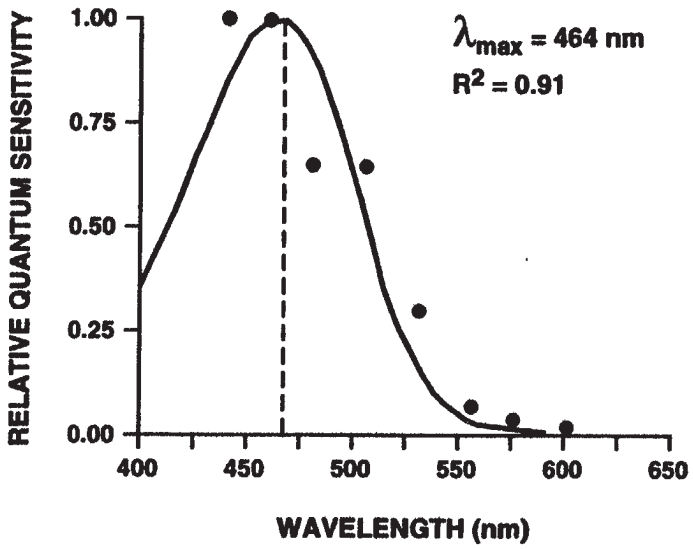




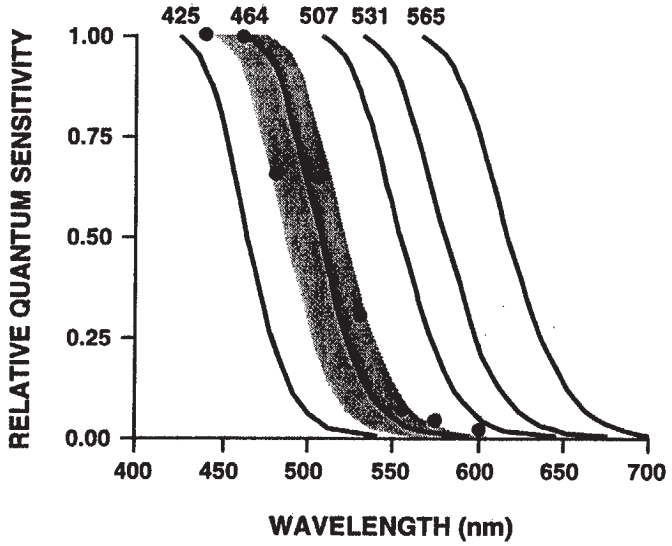




(6.) THESE CURVES ARE CONSTRAINED TO AN IDEALIZED SIGMOIDAL FORMULA TO MAKE THEM UNIVARIANT. THE DATA ARE THE SAME AS FIGURE (5.)



(7.) THE MEAN ED_{50} OF EACH FLUENCE-RESPONSE CURVE IS SHOWN AS A DOT. THEY ARE FITTED TO A VITAMIN A1 OPSIN NOMOGRAM. THE PEAK WAVELENGTH IS 464NM. THE DATA POINTS ARE DERIVED FROM FIGURE (6).

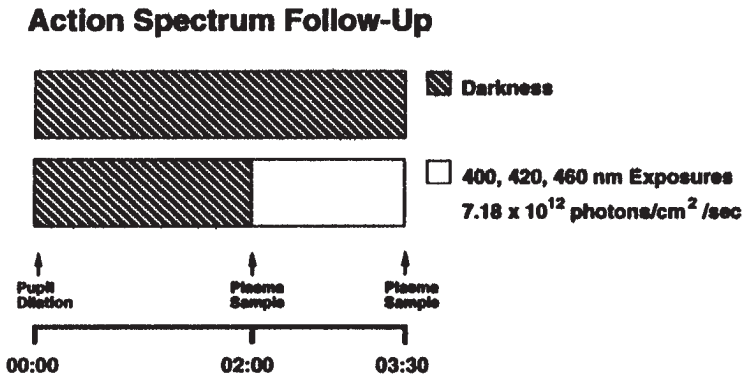


ONE STANDARD DEVIATION AROUND THE PEAK PUTS THE PEAK FROM 446 NM TO 477 NM.

TWO STANDARD DEVIATIONS AROUND THE PEAK (95% CONFIDENCE INTERVAL) PUTS THE PEAK IN THE RANGE OF 428 TO 490 N.M. THIS IS A VERY CONSERVATIVE ESTIMATE OF VARIABILITY

(8) THIS GRAPH SHOWS THE LONG WAVELENGTH LIMB OF THE FOUR KNOW HUMAN RETINAL PHOTOPIGMENTS WHICH ARE VITAMIN A, OPSINS (BLUE CONE, λMAX = 425NM; RHODOPSIN, OR ROD CELL λMAX = 507; GREEN CONE, λMAX = 531 NM; RED CONE, λMAX = 565 NM). DATA FROM FIGURE (7) IS INCLUDED (λMAX = 464 NM). THE SHADED AREA SHOWS +/- ONE STANDARD DEVIATION.

4 copies



(9) FIGURES (9.)-(12.) MAY OR MAY NOT BE PRESENTED AT THE GORDON CONFERENCE. THIS STUDY PROBES THE SHORT WAVELENGTH LIMB OF OUR 464 NM NOMOGRAM. THE PRELIMINARY DATA SUPPORT THE VALIDITY OF A VITAMIN A, OPSIN NOMOGRAM AT 464 PEAK.

(10)

460 nm appears stronger than 400 + 420 nm for suppressing melatonin

(11)

460 NM APPEARS STRONGER FOR MELATONIN SUPPRESSION THAN 420 + 400 NM.

Ya (12)

460 nm APPEARS STRONGER THAN 400 + 420 nm FOR MELATONIN SUPPRESSION.