

8/29/00

PROV

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PTO/SB/16 (2-98)

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jc866 U.S. PTO
60/228493

08/28/00

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					
Direct all correspondence to: CORRESPONDENCE ADDRESS					
☐ Customer Number		24358		Place Customer Number Bar Code Label here	
OR Type Customer Number here					
☐ 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)					
☒ Specification Number of Pages		4		☒ Small Entity Statement	
☒ Drawing(s) Number of Sheets		12		☒ 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				FILING FEE AMOUNT (\$)	
☒ The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 50-0491				\$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.					
☒ 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

Date 08/28/00

TYPED or PRINTED NAME

Clifford K. Weber

REGISTRATION NO.

42,215

(if appropriate)

Docket Number:

TELEPHONE

(215) 503-0757

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.

Verilux Ex. 1020

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IPR2023-00940

Page 00001

FEE TRANSMITTAL for FY 2000

Patent fees are subject to annual revision.
Small Entity payments must be supported by a small entity statement,
otherwise large entity fees must be paid. See Forms PTO/SB/09-12.
See 37 C.F.R. §§ 1.27 and 1.28.

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

METHOD OF PAYMENT (check one)

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

Deposit Account Number 50-0491

Deposit Account Name Thomas Jefferson University

- ☒ Charge Any Additional Fee Required
Under 37 CFR §§ 1.16 and 1.17

2. ☐ Payment Enclosed:
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FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code (\$)	Small Entity Fee Code (\$)	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

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

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

Large Entity Fee Code (\$)	Small Entity Fee Code (\$)	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 (\$)	Small Entity Fee Code (\$)	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) _____

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* Reduced by Basic Filing Fee Paid

SUBTOTAL (3) (\$)

SUBMITTED BY

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

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

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT	
Firm or Individual name	Clifford Kent Weber
Signature	<i>Clifford Kent Weber</i>
Date	August 28, 2000

CERTIFICATE OF MAILING			
I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231 on this date:			
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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

Typed Name: CLIFFORD KENT WEBER

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

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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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(NAME OF STATE _____)
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☐ the application identified above.
☐ the patent 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 $\pm$ 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/cm²/sec induced a half-saturation response (ED_{50}) while 6.84×10^{13} photons/cm²/sec induced a saturation melatonin suppression response. Further, a dose of 4.19×10^{13} photon/cm²/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 deutanopic 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 $\pm$ 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 $\pm$ 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 ($\mu\text{W}/\text{cm}^2$) 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 $\mu\text{W}/\text{cm}^2$ (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 $\mu\text{W}/\text{cm}^2$ (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 $\pm$ 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 $\mu\text{W}/\text{cm}^2$ control night, all intensities at or above 5.5 $\mu\text{W}/\text{cm}^2$ significantly suppressed melatonin ($P<0.05$ or less). In contrast, irradiances of 2.8, 1.4 and 0.011 $\mu\text{W}/\text{cm}^2$ 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 ($F=13.59$, $P<0.0001$).

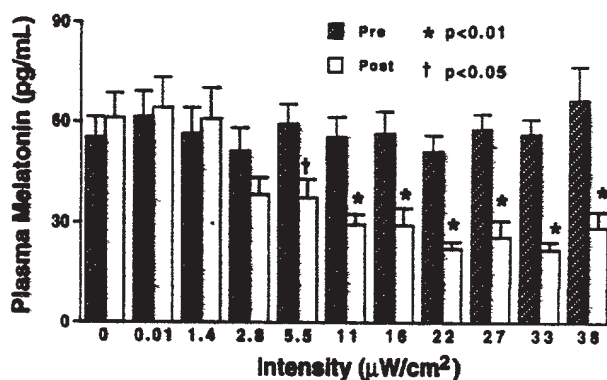


Figure 1: In this graph the bars represent group mean + SEM plasma melatonin values ($N=8$) before and after monochromatic light exposure at 505 nm. There were no significant variations across mean melatonin pre-light exposure values ($F=0.85$). Paired, two-tailed Students' t tests demonstrated which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

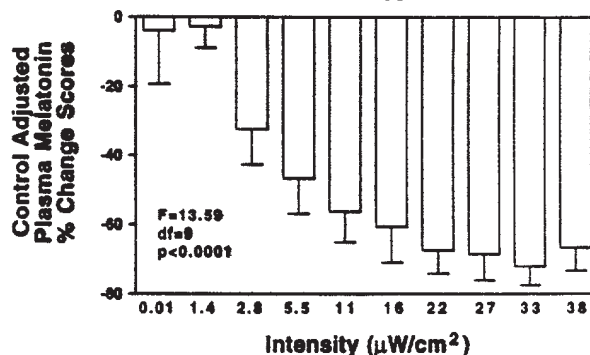


Figure 2: This graph illustrates group mean + SEM % control-adjusted melatonin change values ($N=8$) at 505 nm monochromatic light exposure. The figure shows that progressively higher light irradiance exposure produces increasingly greater melatonin suppression.

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.

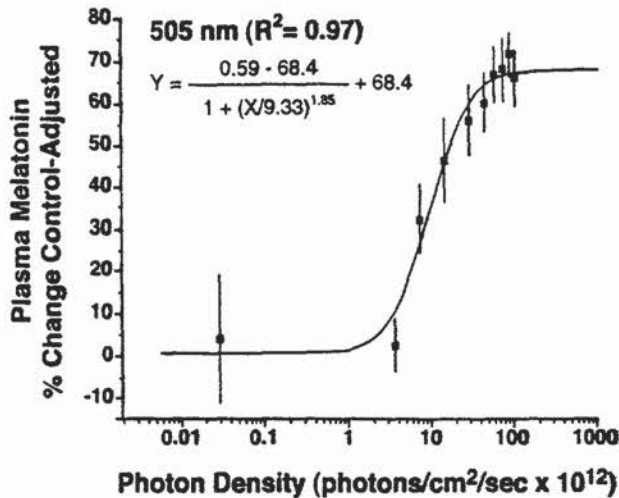


Figure 3: This figure demonstrates the fitted fluence-response curve for photon density and % control-adjusted melatonin suppression (N=8). Each data point represents one group mean $\pm$ SEM.

Subjects exposed to 555 nm received both control (0 $\mu\text{W}/\text{cm}^2$) and 15 $\mu\text{W}/\text{cm}^2$ (4.2×10^{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 ($t=-3.04$, $df=14$, $P<0.01$).

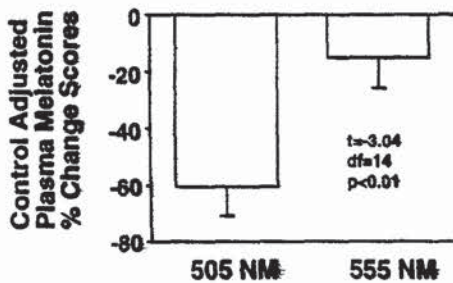


Figure 4: The bars represent group mean $\pm$ SEM values relative to an equal photon dose of 4.2×10^{13} photons/ cm^2 . These data show that the 505 nm % control-adjusted plasma melatonin suppression is significantly stronger than that for 555 nm.

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

450/1200 Watt Arc Lamp Power Supply

450/1200 Watt Igniter

Entrance Slit/Bandpass Control

Arc Lamp Source

Wavelength Selection

Exit Slit/Bandpass Control

Monochromator

Integrating Sphere

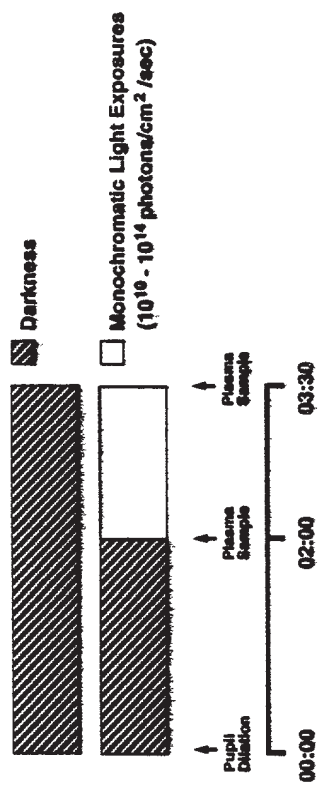
Observation Camera

Camera Controller

Observation Monitor

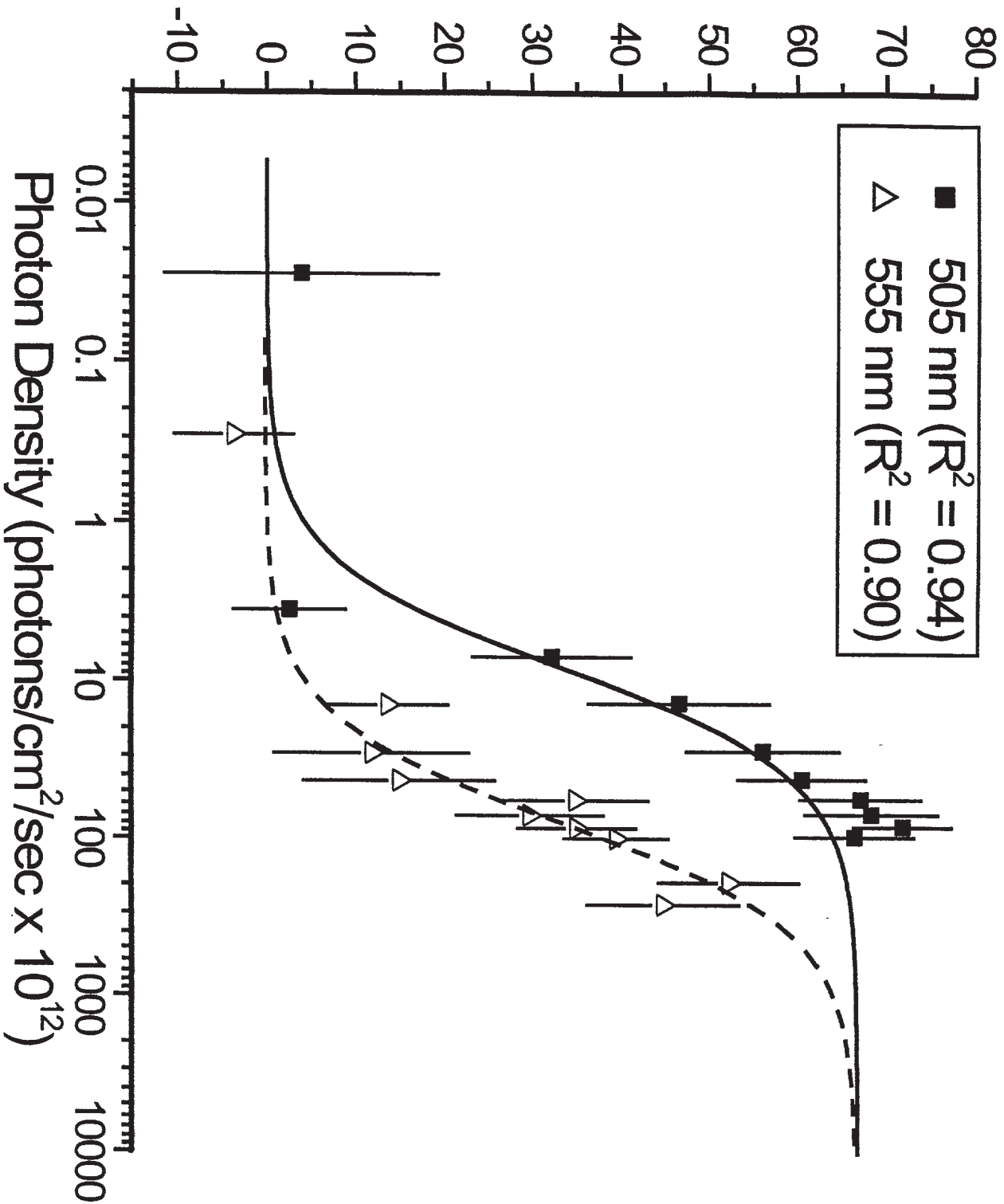
2

Action Spectrum Protocol



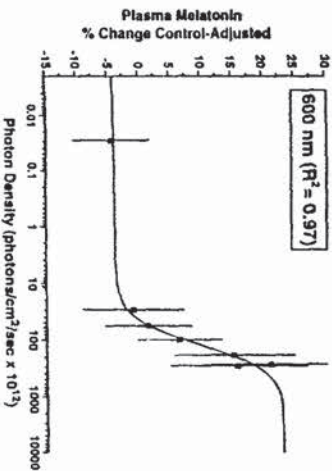
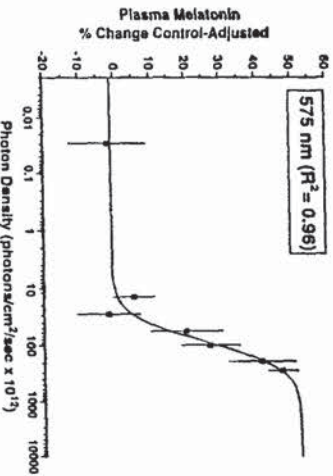
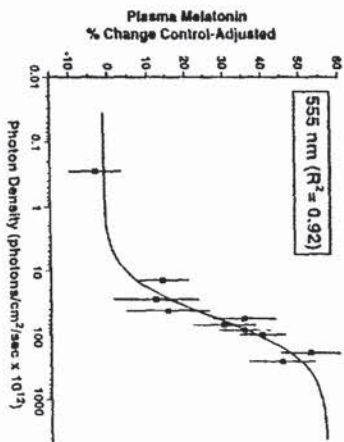
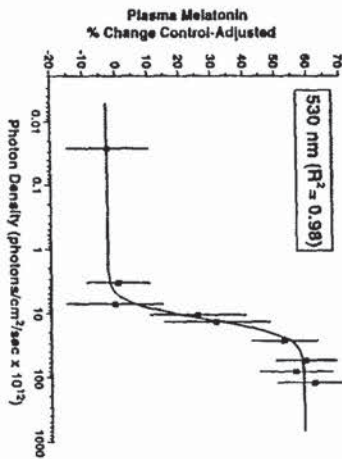
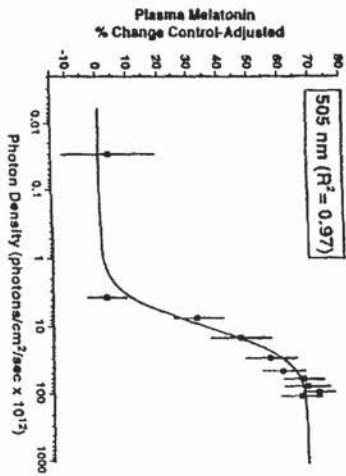
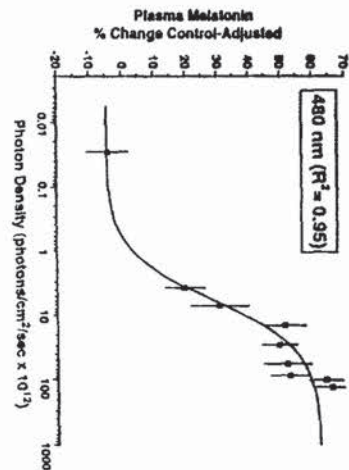
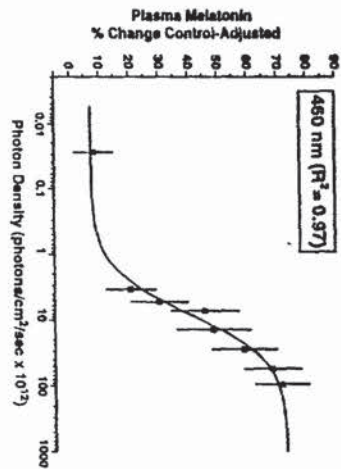
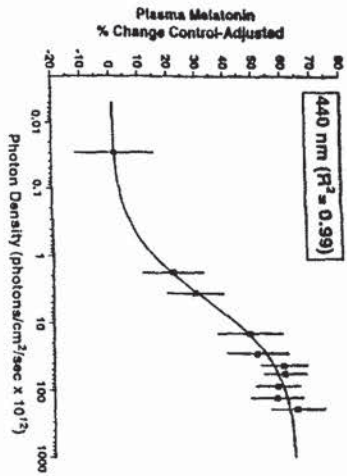
③. SPECIFIC PROTOCOL

Plasma Melatonin % Change Control-Adjusted



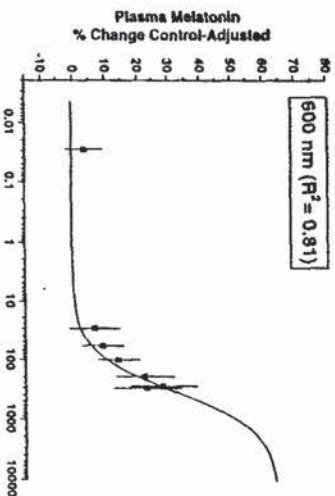
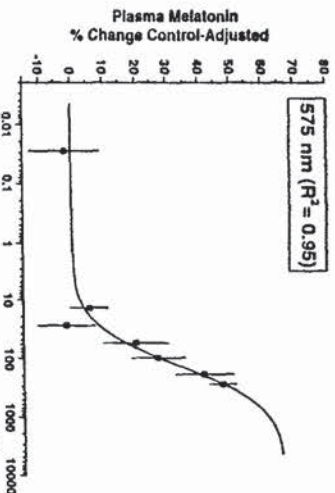
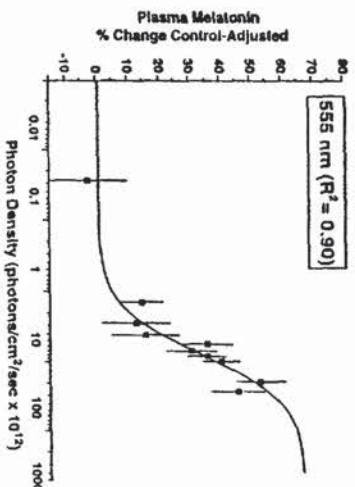
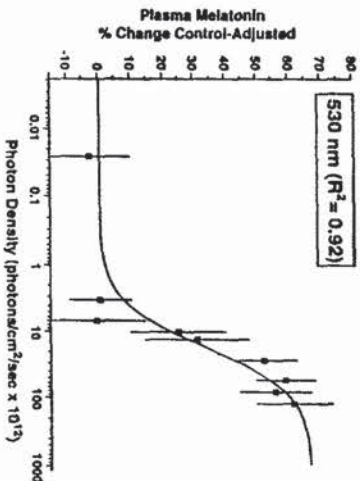
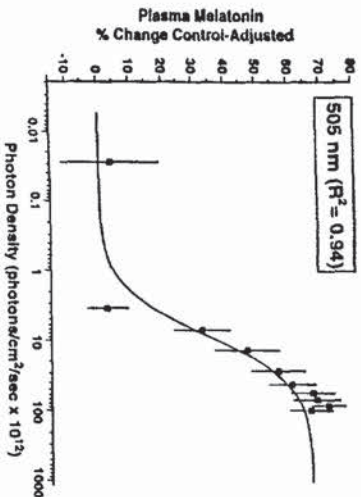
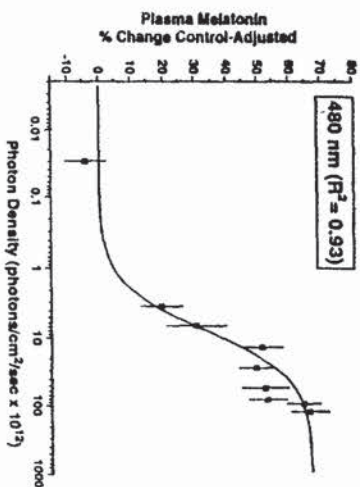
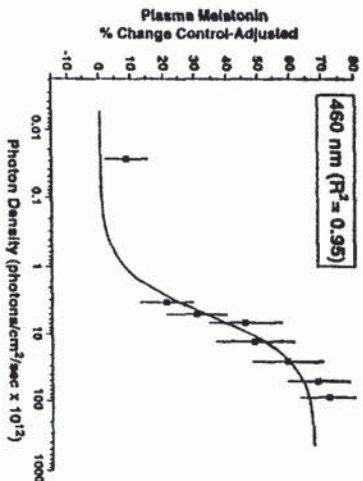
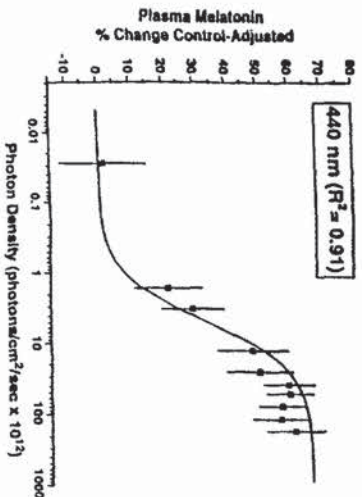
④ THIS SHOWS DATA NOT INCLUDED IN JCEH MANUSCRIPT
CONFIRMING 3 CORE VISUAL SYSTEM DOESN'T MEDIATE CIRCADIAN SYSTEM.

Exposures in Healthy Subjects (N=63)

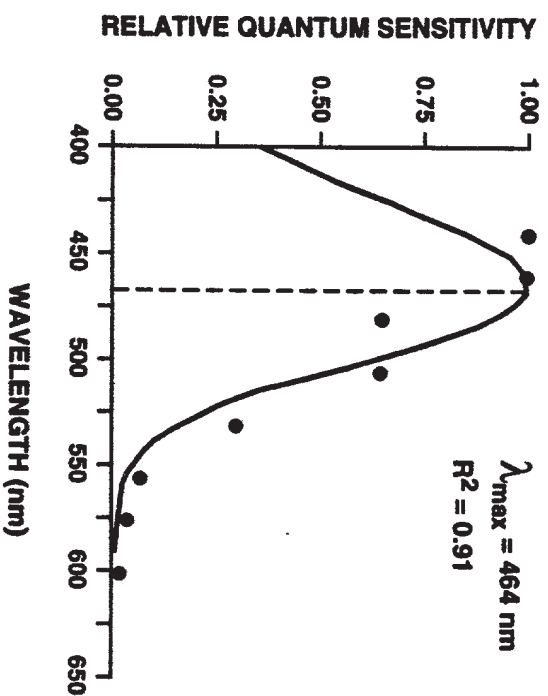


5. FLUENCE-RESPONSE CURVES FOR 8 VISIBLE WAVELENGTHS. EACH CURVE IS FROM 7-8 SUBJECTS. THE ARE MATHEMATICALLY UNRESTRAINED.

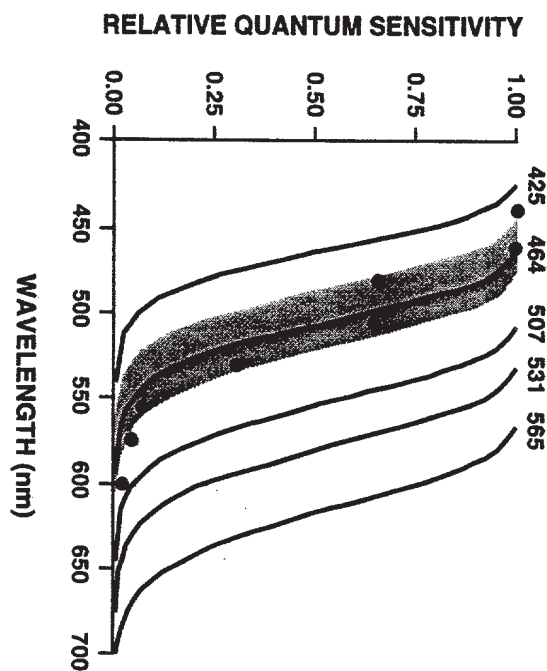
Exposures in Healthy Subjects (N=63) Fitted to Univariate Curves



THESE CURVES ARE CONSTRAINED TO AN IDENTICAL SIGMOIDAL FORMULA TO MAKE THEM UNIVARIANT. THE DATA ARE THE SAME AS FIGURE 5.



7. THE MEAN ED₅₀ OF EACH FLUENCE-RESPONSE CURVE IS SHOWN AS A DOT. THEY ARE FITTED TO A VITAMIN A1 OPSIN NOMOGRAM. THE PEAK WAVELENGTH IS 464 nm. THE DATA POINTS ARE DERIVED FROM FIGURE 6.



⑧

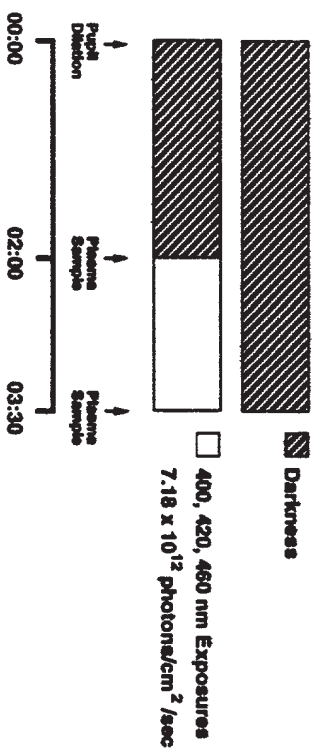
THIS GRAPH SHOWS THE LONG WAVELENGTH LIMB OF THE FOUR KNOW HUMAN RETINAL PHOTOPIGMENTS WHICH ARE VITAMIN A, OPSINUS (BLUE CONE, $\lambda_{MAX} = 425\text{nm}$; RHODOPSIN, OR ROD CELL

$\lambda_{MAX} = 507$; GREEN CONE, $\lambda_{MAX} = 531\text{nm}$; RED CONE, $\lambda_{MAX} = 565\text{nm}$). DATA FROM FIGURE ⑦ IS INCLUDED ($\lambda_{MAX} = 464\text{nm}$). THE SHADED AREA SHOWS $\pm$ ONE STANDARD DEVIATION.

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

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

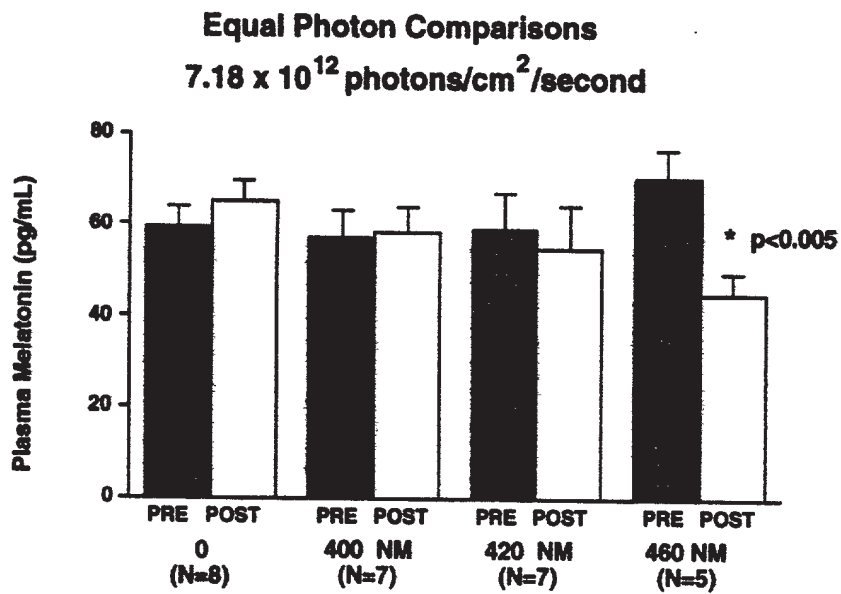
Action Spectrum Follow-Up



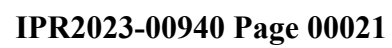
(9) FIGURES (9)-(12) MAY OR MAY NOT BE PRESENTED AT THE GORDON CONFERENCE. THIS STUDY PROBES THE SHORT WAVELENGTH LIMB OF OUR 464 NM NO MOGRAAT. THE PRELIMINARY DATA SUPPORT THE VALIDITY OF A VITAMIN A, OPSIN NO MOGRAAT AT 464 PEAK.

⑬

460 NM APPEARS STRONGER THAN 400 + 420 NM FOR
SUPPRESSING MELATONIN



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



12

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

