

Scientific Symposium
The Health Effects of Shift Work

Toronto, April 12, 2010

NIGHT WORK, NIGHT LIGHT AND CANCER: ANIMAL EVIDENCE

David E. Blask

Laboratory of Chrono-Neuroendocrine Oncology

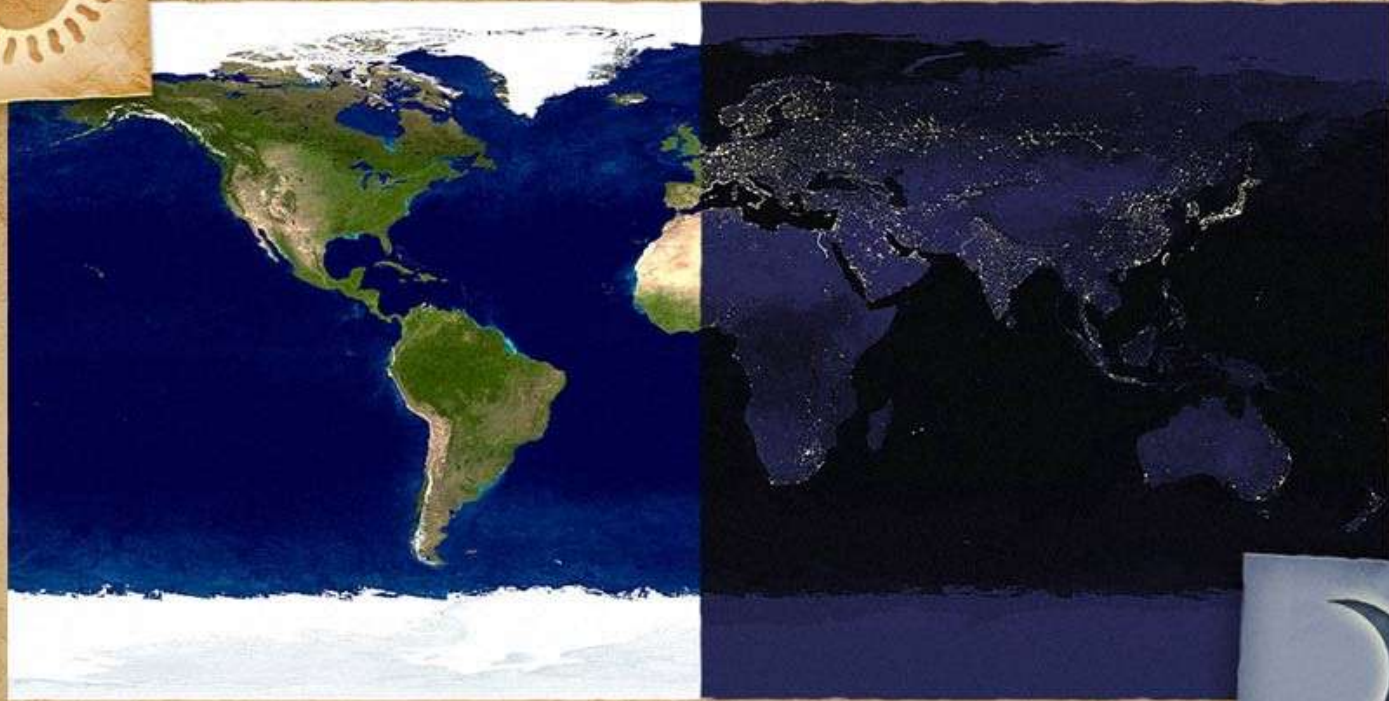
Department of Structural & Cellular Biology

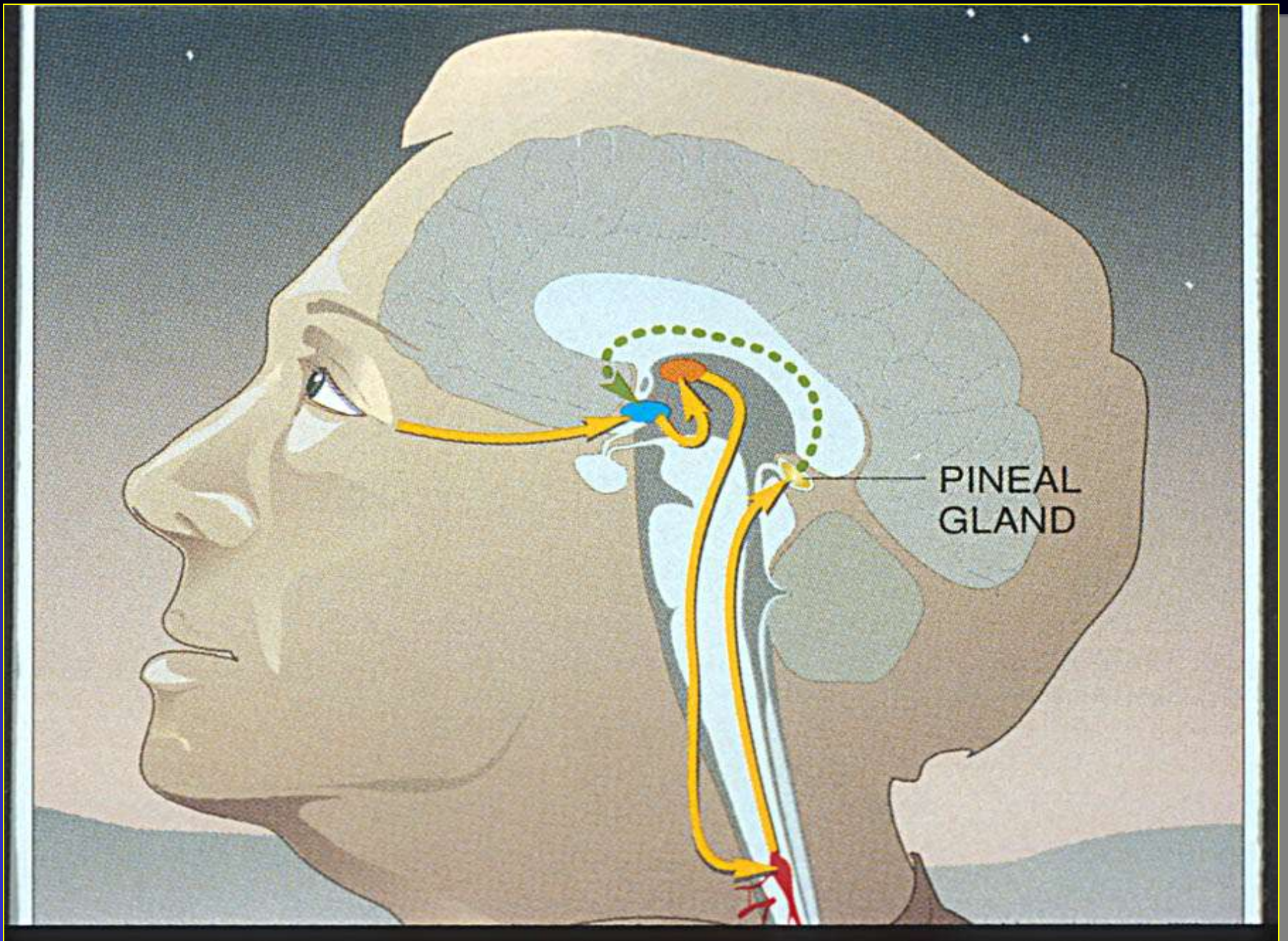
Tulane University School of Medicine & Tulane Cancer Center

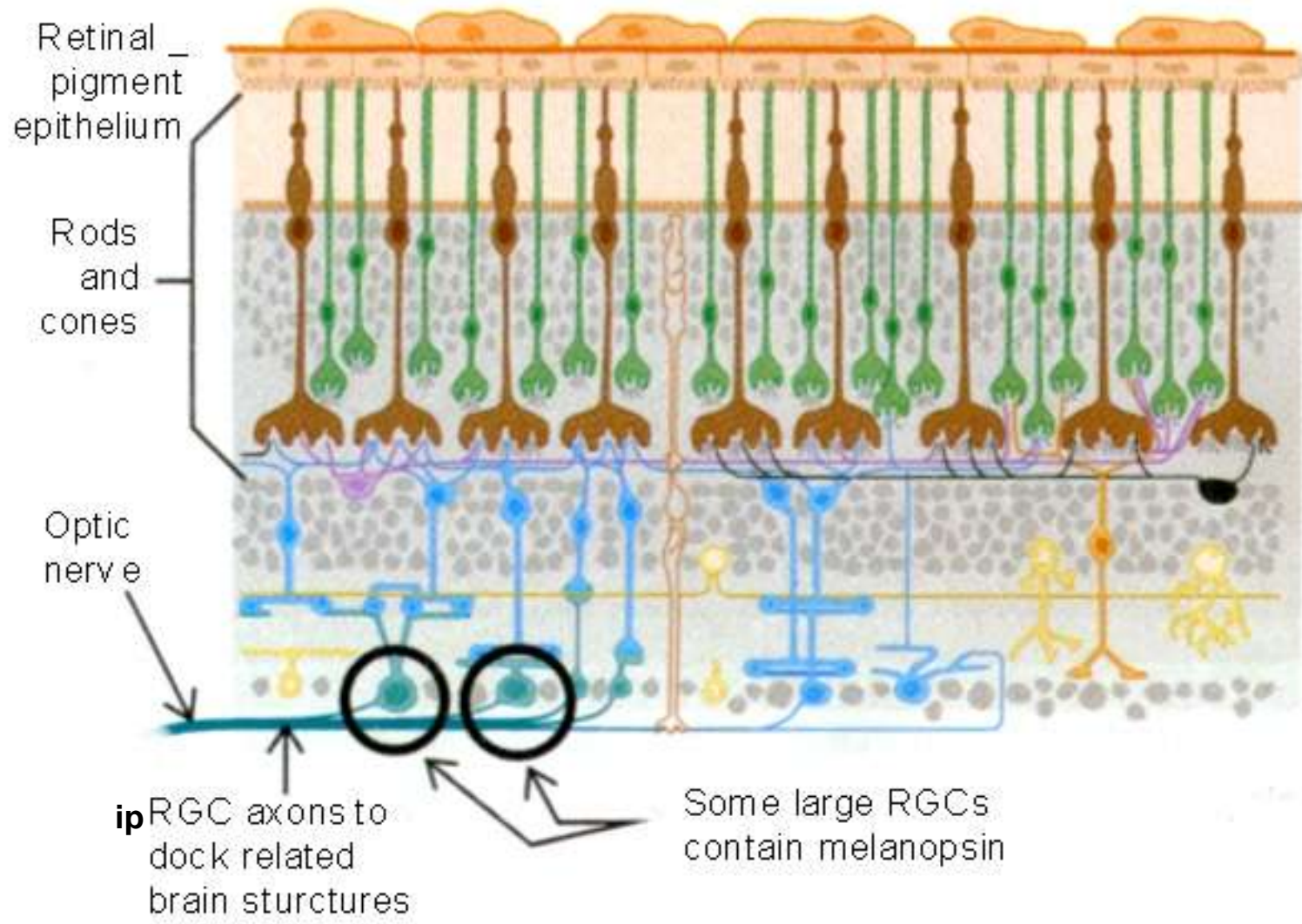
New Orleans, Louisiana USA

LIGHT

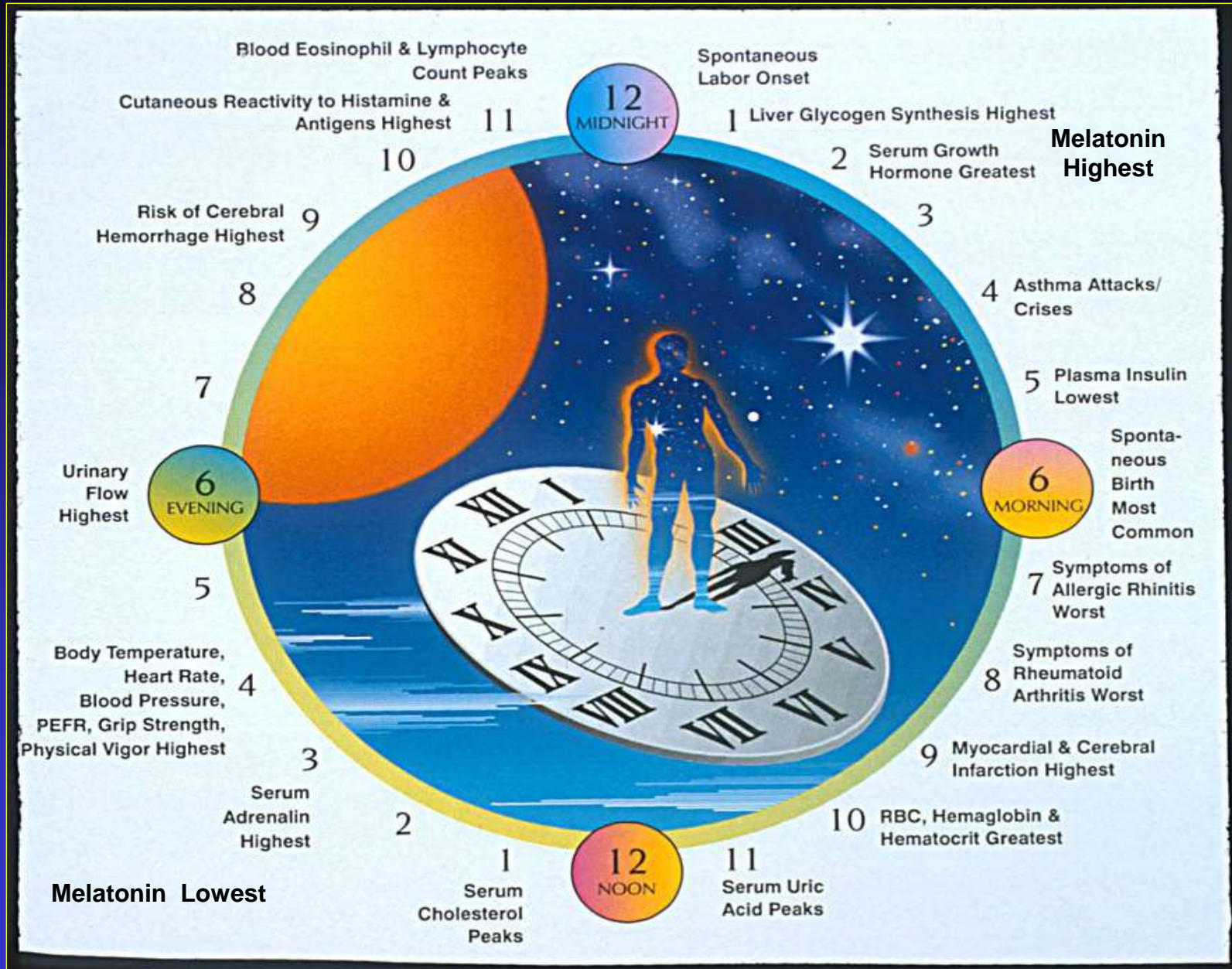
DARK

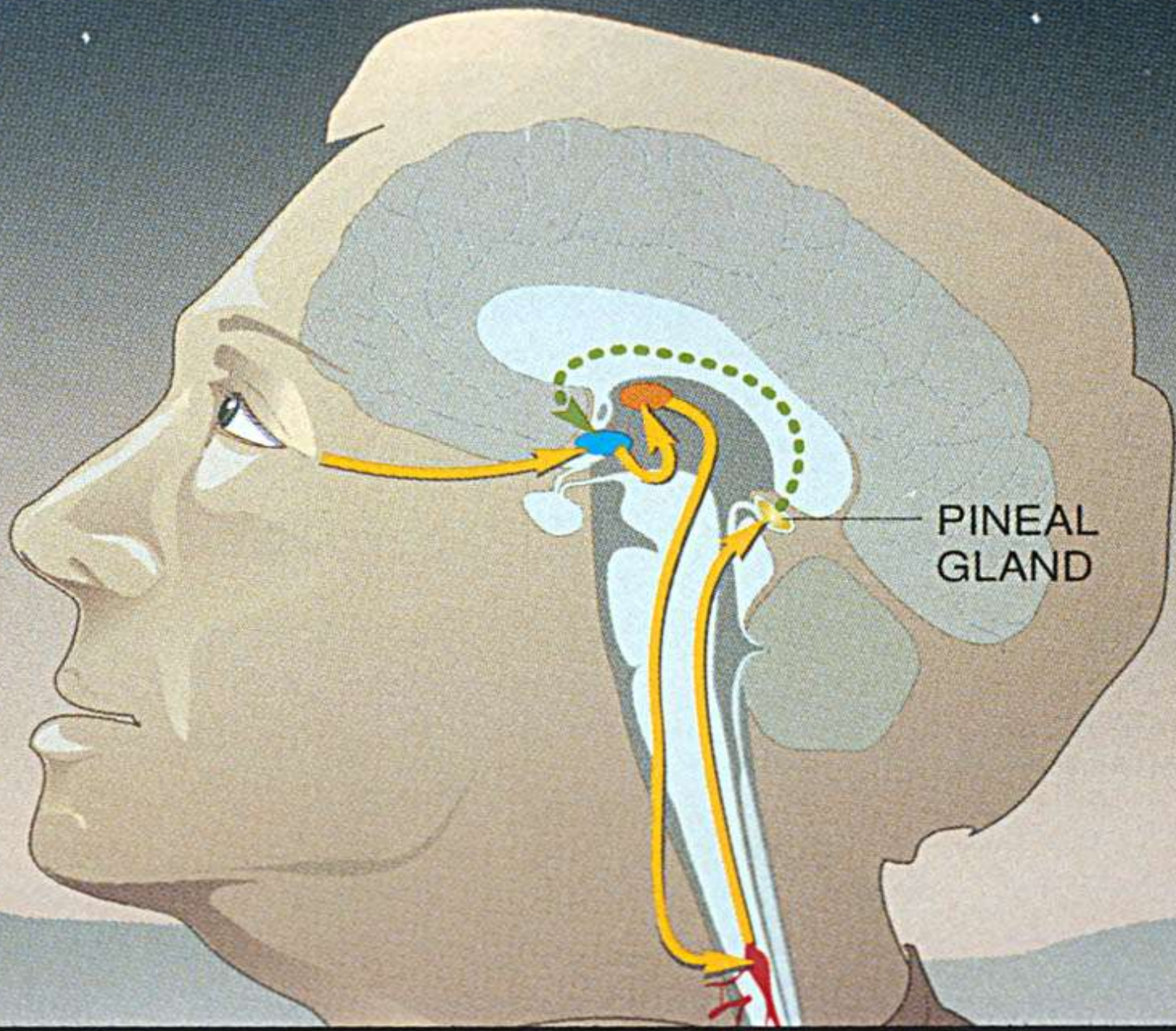






CIRCADIAN RHYTHMS





PINEAL
GLAND

CIRCADIAN RHYTHMS

ENVIRONMENTAL
LIGHT
INTENSITY



PLASMA
MELATONIN
CONCENTRATION



SLEEP-WAKE
CYCLE



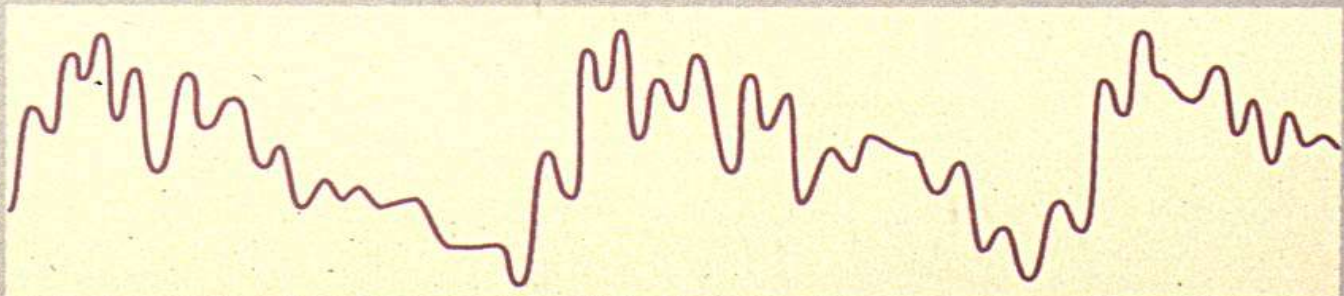
TIME OF DAY

8 16 24 8 16 24 8

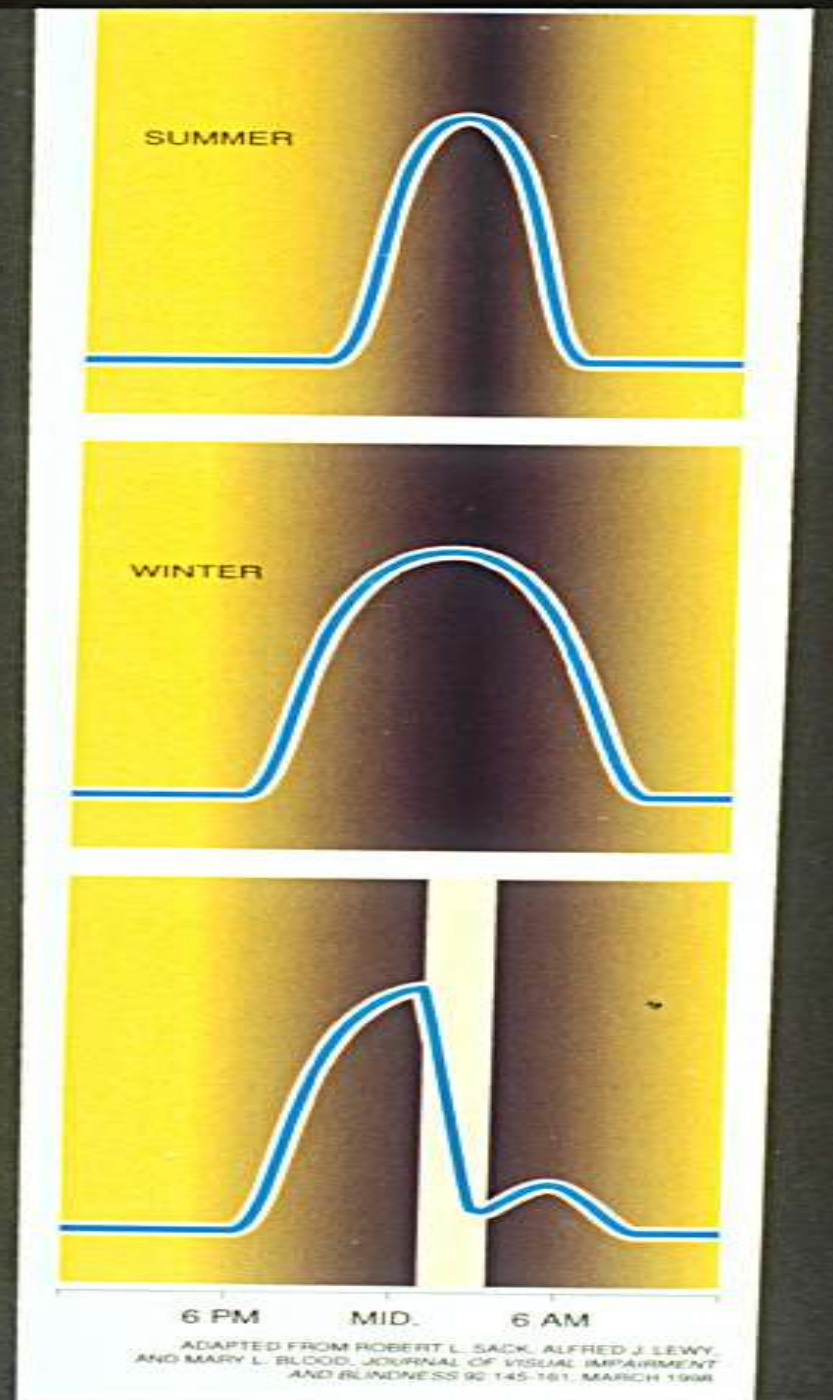
BODY
TEMPERATURE

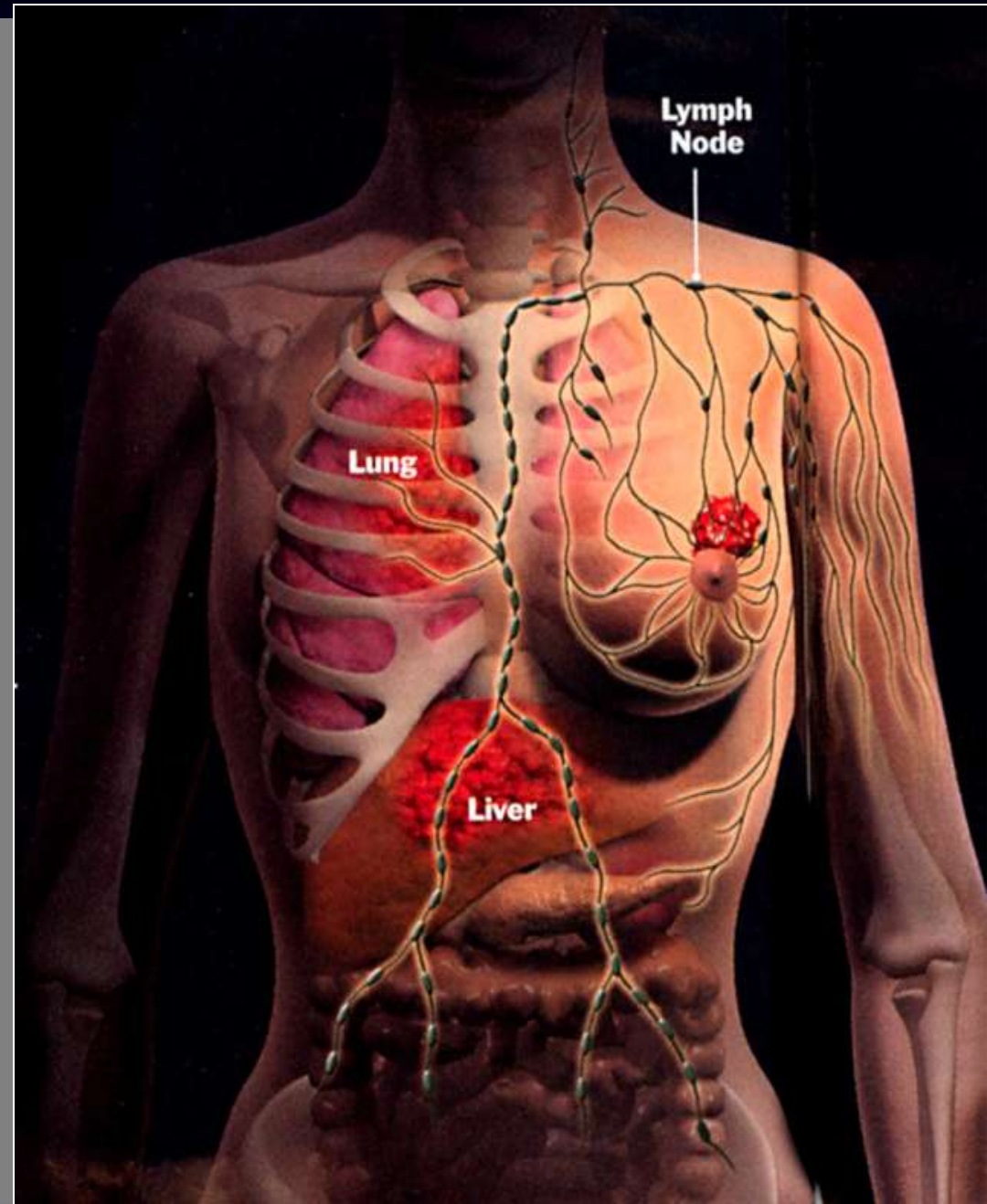


PLASMA
CORTISOL
CONCENTRATION



IMPACT OF PHOTOPERIODIC DAYLENGTH ON THE DURATION OF THE NOCTURNAL MELATONIN SIGNAL





Lymph Node

Lung

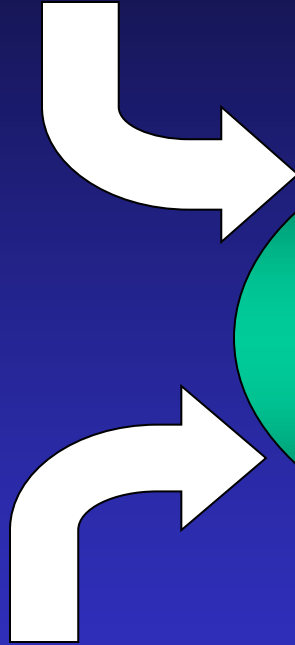
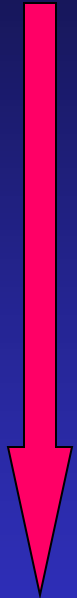
Liver

Constant Light and Light During Darkness: Effects Experimental Breast Tumorigenesis

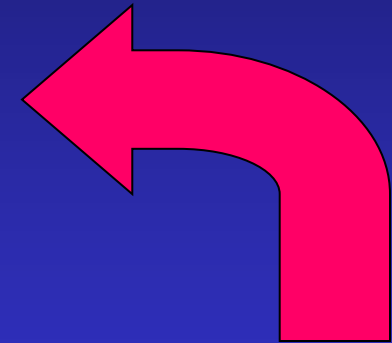
- Spontaneous breast cancer development on C3H mice - increased tumorigenesis
- Spontaneous breast cancer development in C3H –HeJ mice (retinal degeneration) - decreased tumorigenesis
- Carcinogen (DMBA and NMU)-induced breast cancer development in rats- increased tumorigenesis in 8 studies; no effect in 3 studies; decreased tumorigenesis in 1 study
- Carcinogen (DMBA)-induced breast cancer growth in rats- increased growth
- Human breast cancer xenograft growth in nude rats - increased growth

**CIRCADIAN
SYSTEM
(BIOLOGICAL
CLOCK)**

**NOCTURNAL
LIGHT
EXPOSURE**



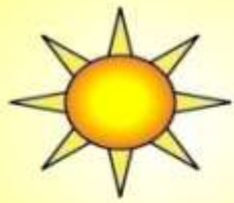
**BREAST
CANCER**



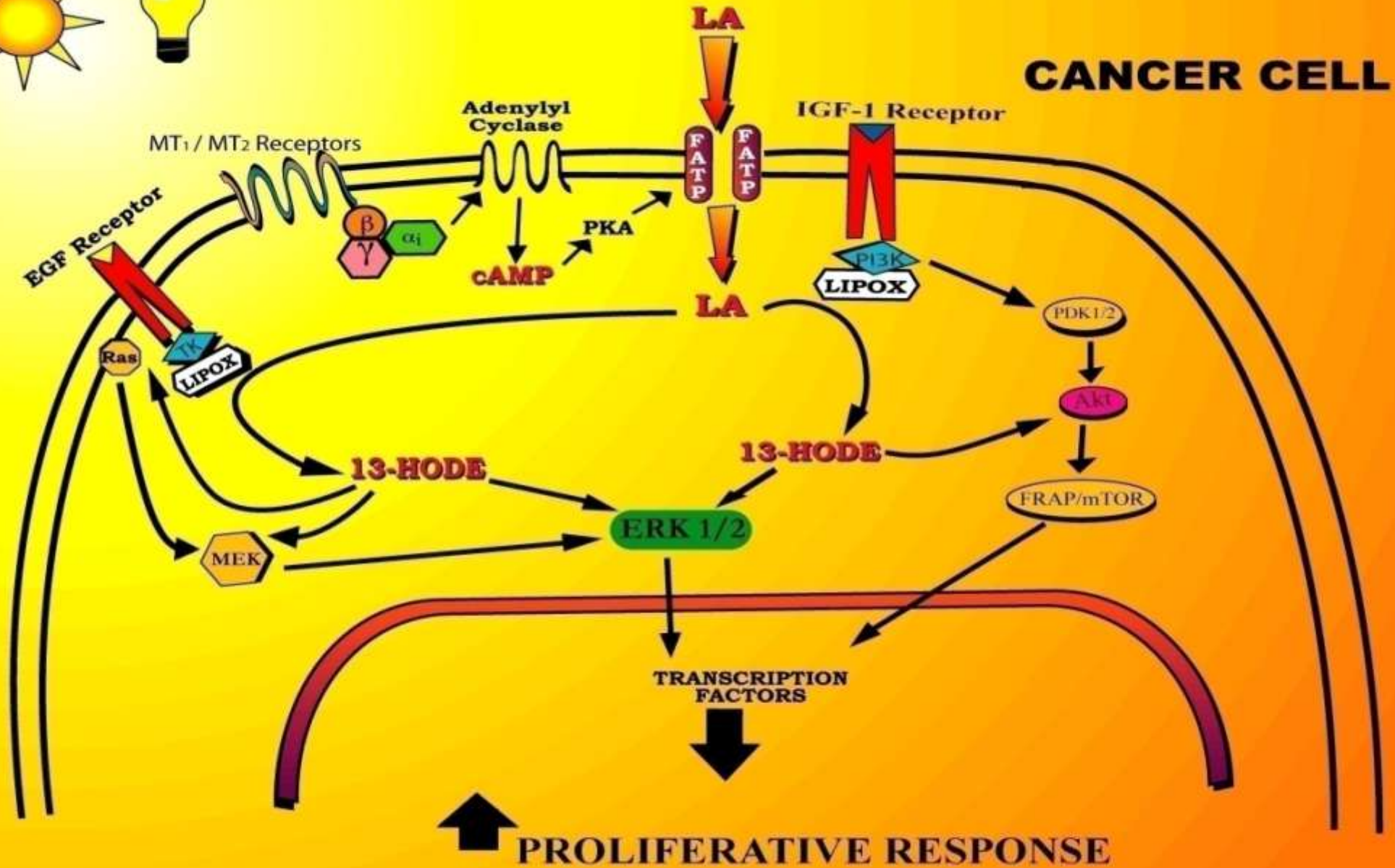
**NOCTURNAL
MELATONIN**



**FATTY ACIDS
(LA) &
GLUCOSE**



CANCER CELL

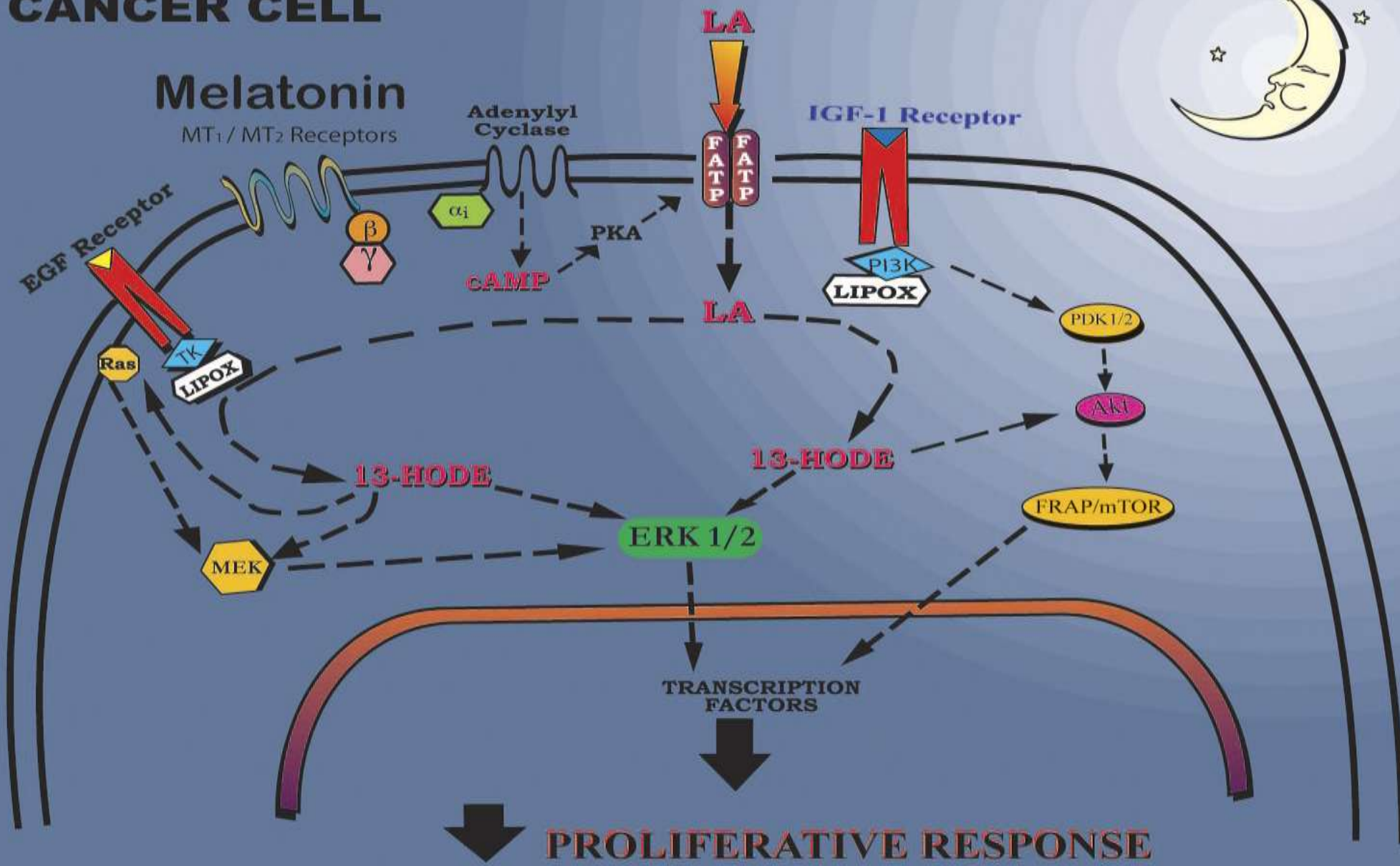


CANCER CELL

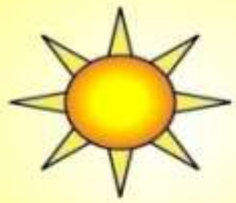


Melatonin

MT₁ / MT₂ Receptors



PROLIFERATIVE RESPONSE

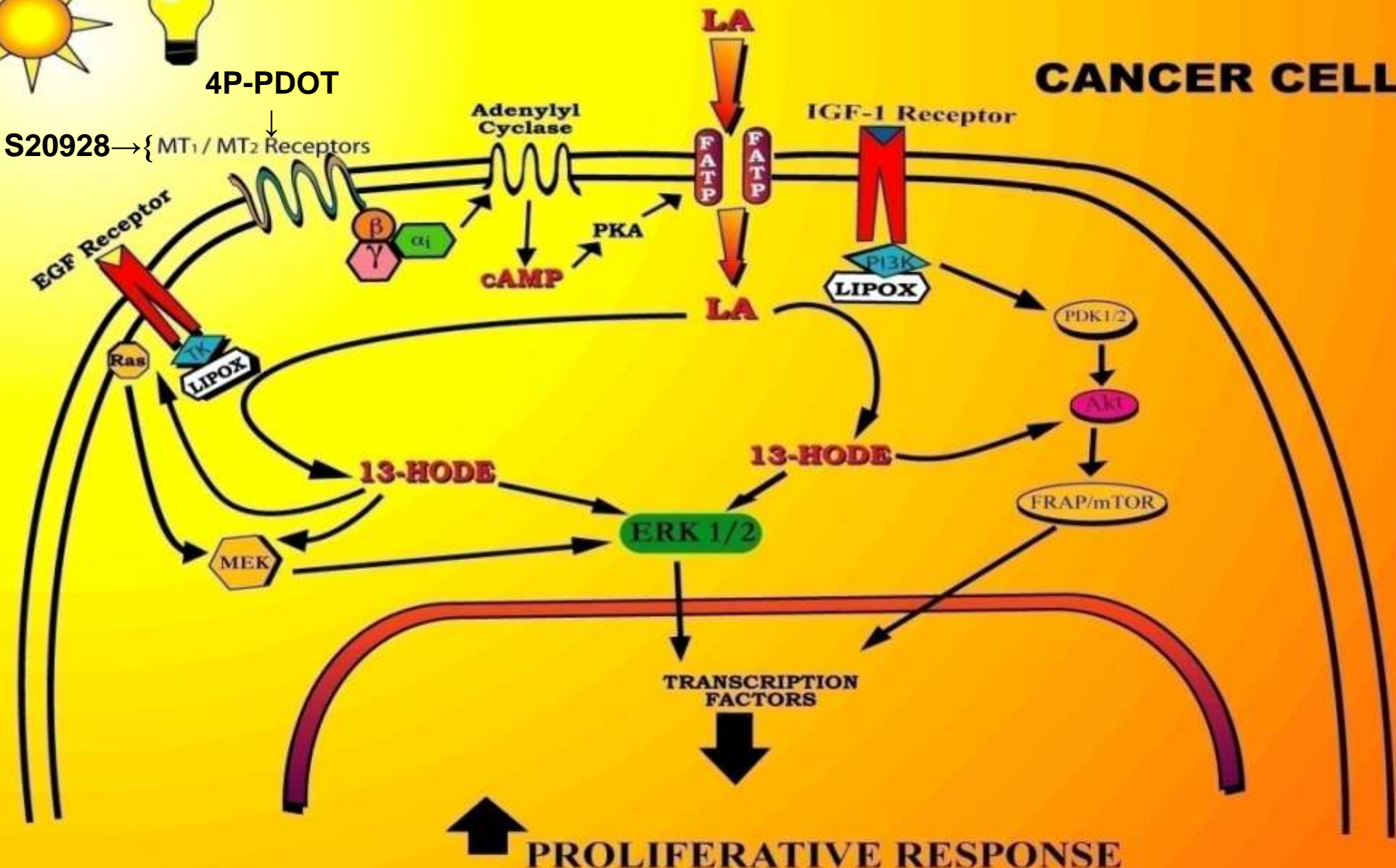


4P-PDOT

S20928

MT₁ / MT₂ Receptors

CANCER CELL



PROLIFERATIVE RESPONSE

PHOTOBIOLOGICAL EXPOSURE CHAMBER

Total Darkness

0.02 $\mu\text{W}/\text{cm}^2$

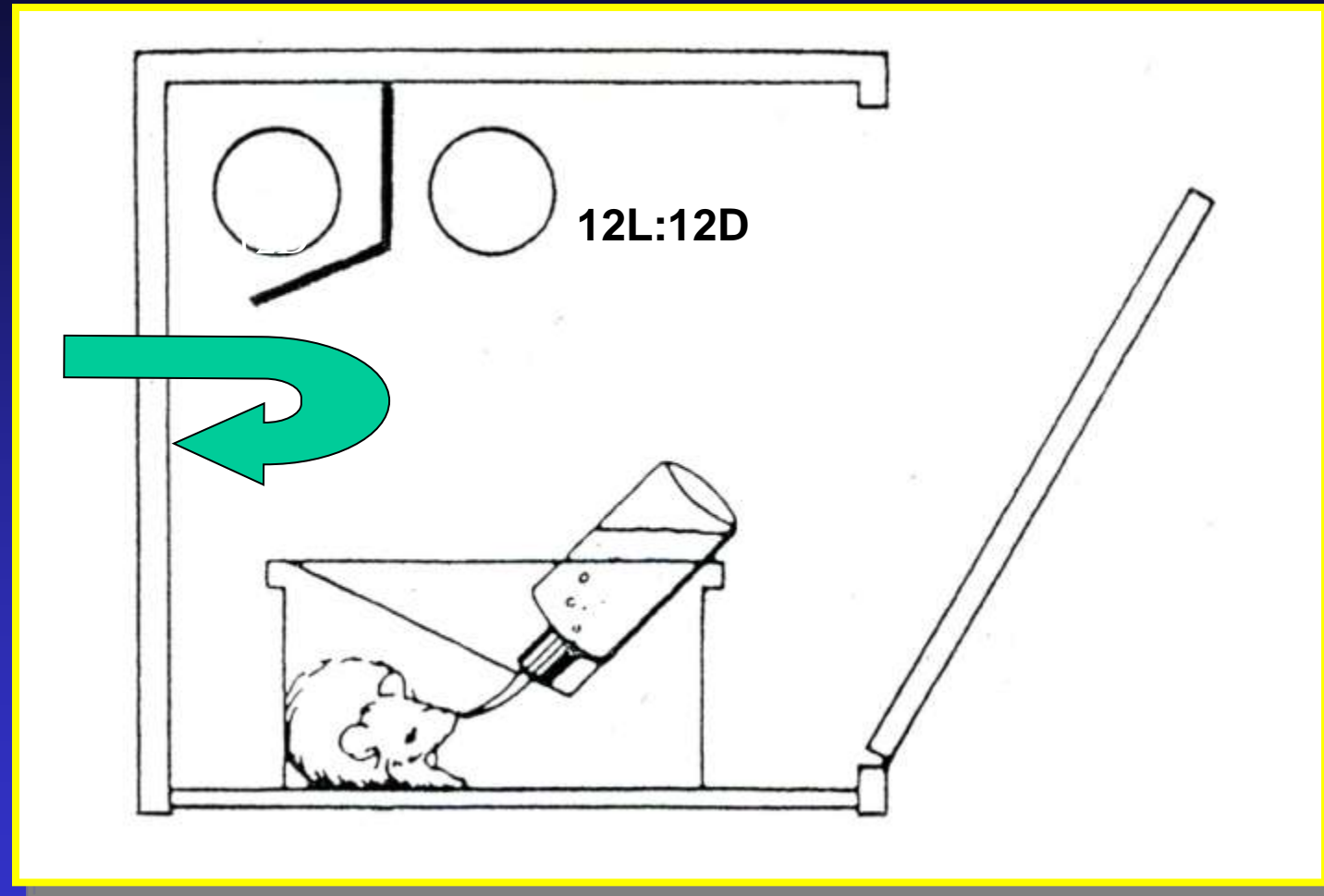
0.05 $\mu\text{W}/\text{cm}^2$

0.06 $\mu\text{W}/\text{cm}^2$

0.08 $\mu\text{W}/\text{cm}^2$

345 $\mu\text{W}/\text{cm}^2$

Reflected light at
rodent eye level for
2 wks prior to and
throughout tumor
growth period.

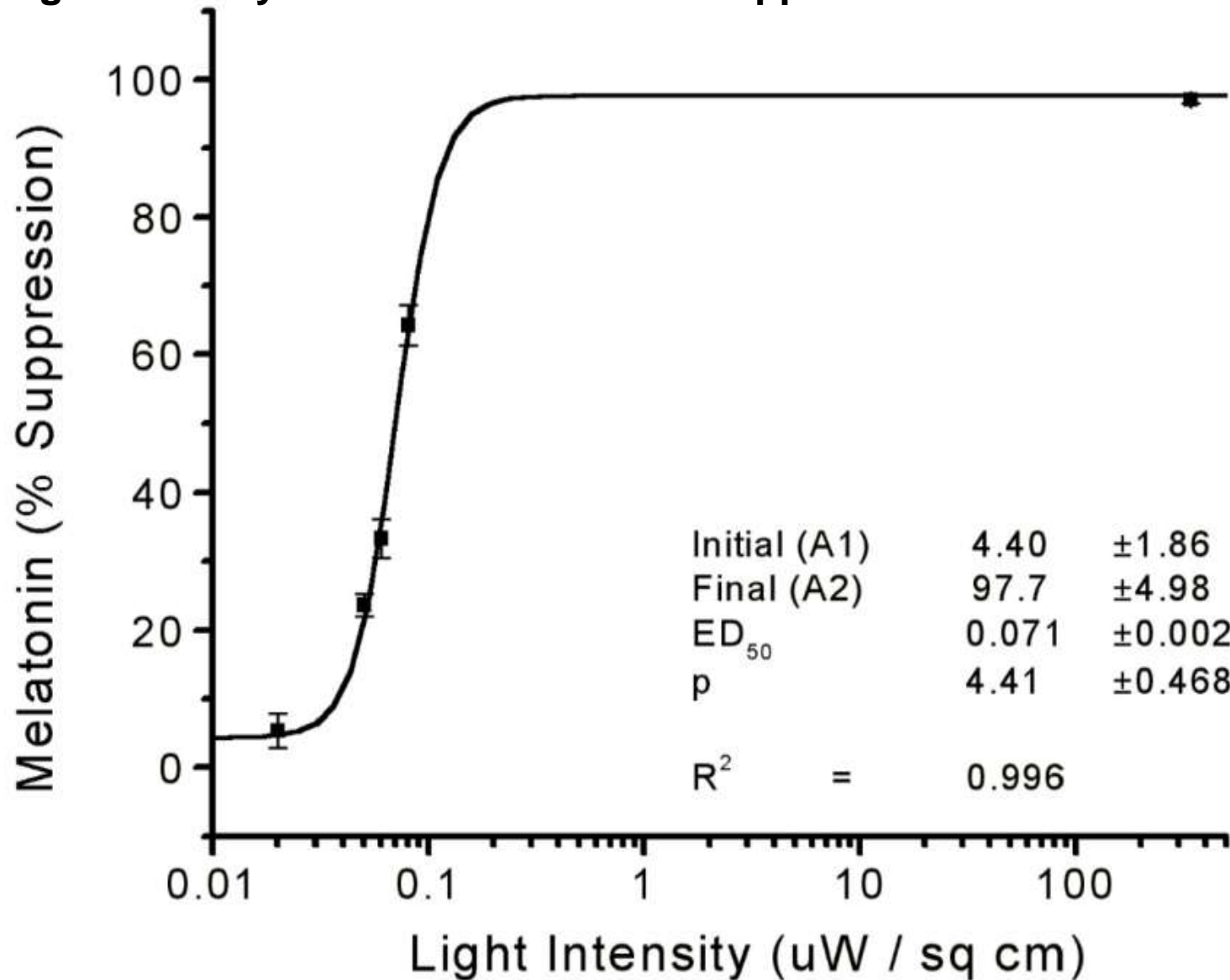


0.08 $\mu\text{W}/\text{cm}^2$ or 0.2 lux, or 0.02 ft.-candles

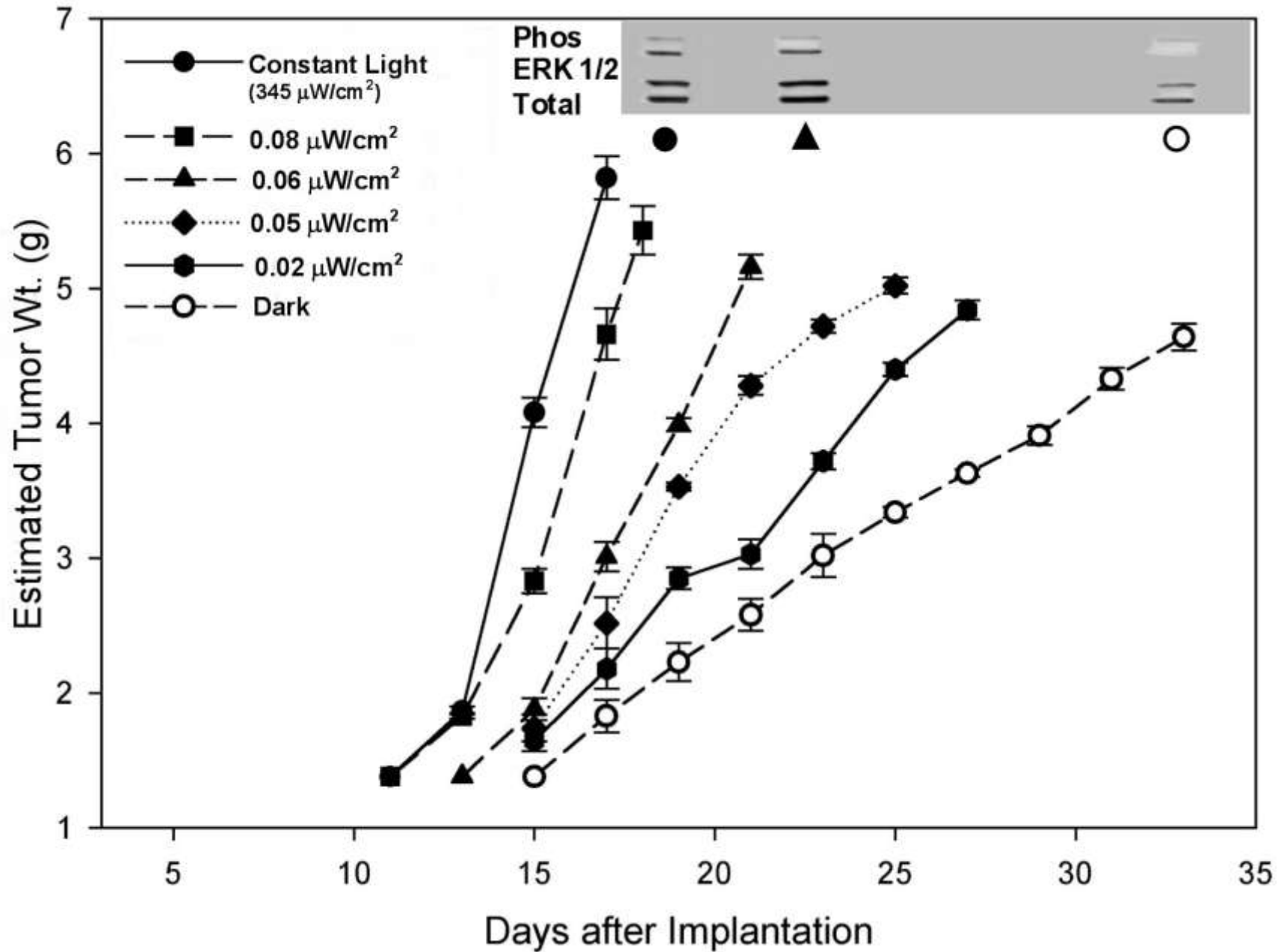


MELATONIN FLUENCE-RESPONSE CURVE - FEMALE NUDE RATS

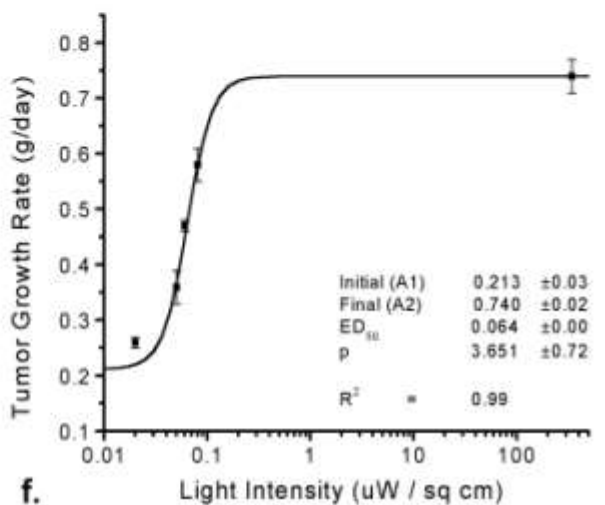
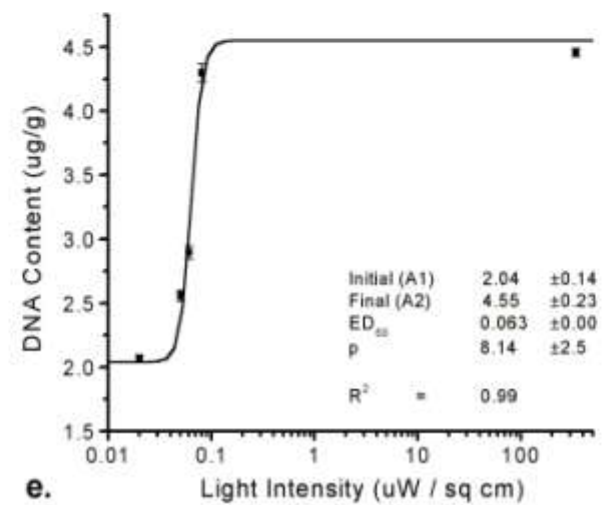
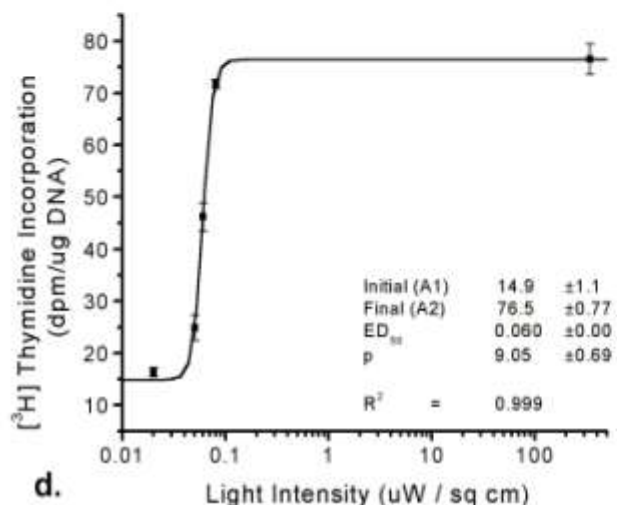
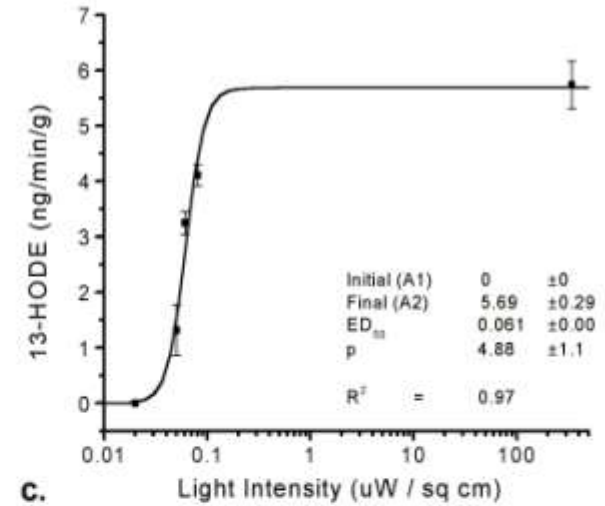
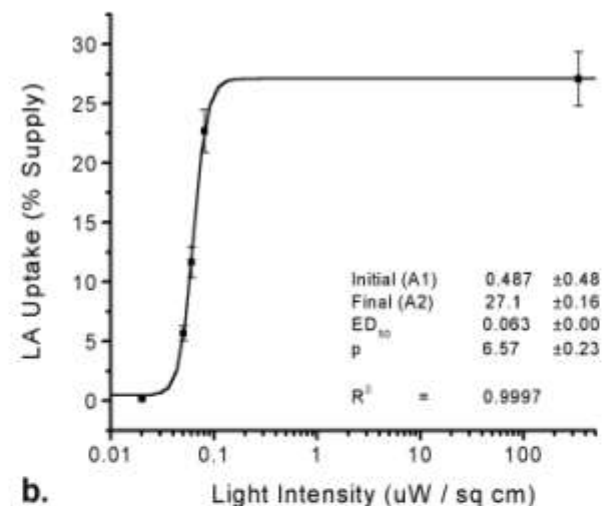
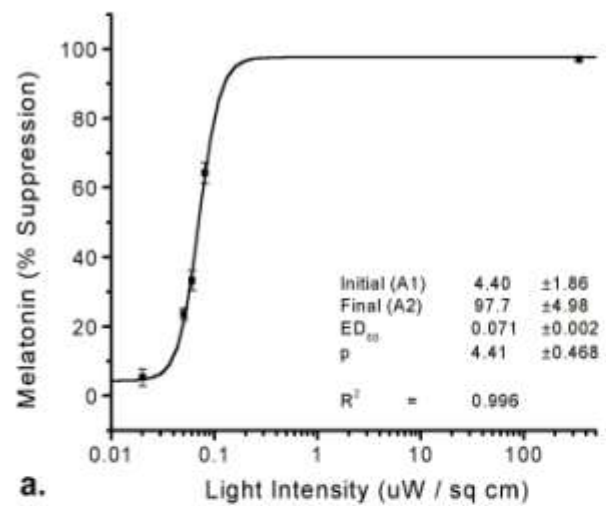
Light Intensity vs Percent Melatonin Suppression from Dark Condition



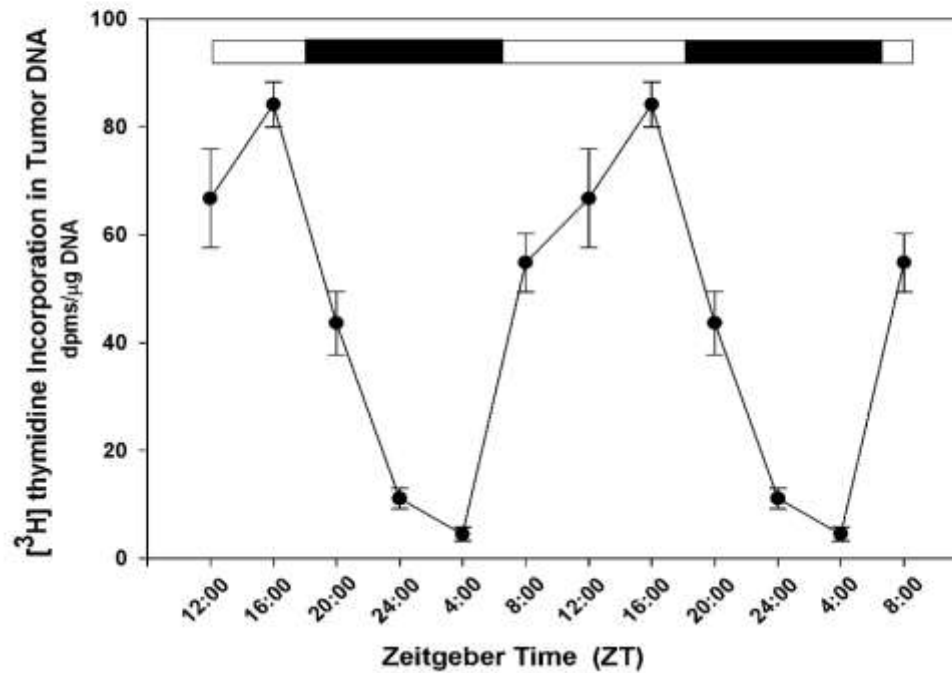
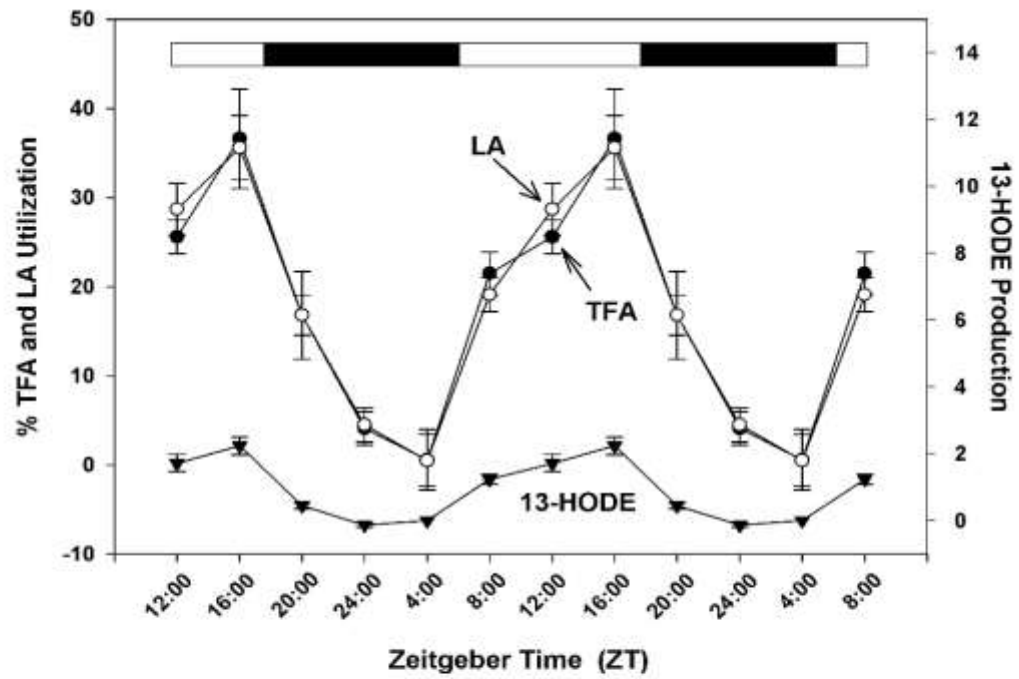
GROWTH RATES VS LIGHT INTENSITY FOR HUMAN BREAST CANCER XENOGRAFTS



FLUENCE-RESPONSE CURVES FOR HUMAN BREAST CANCER XENOGRAFTS (MCF-7 SR-)

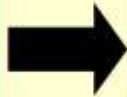


0.08 $\mu\text{W}/\text{cm}^2$ or 0.2 lux, or 0.02 ft.-candles





COLLECTION OF HUMAN BLOOD FOR PERFUSION OF TUMORS *IN SITU*



Light exposure of human subject at night (2800 lux)



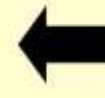
Venipuncture following light exposure



Overnight blood shipment



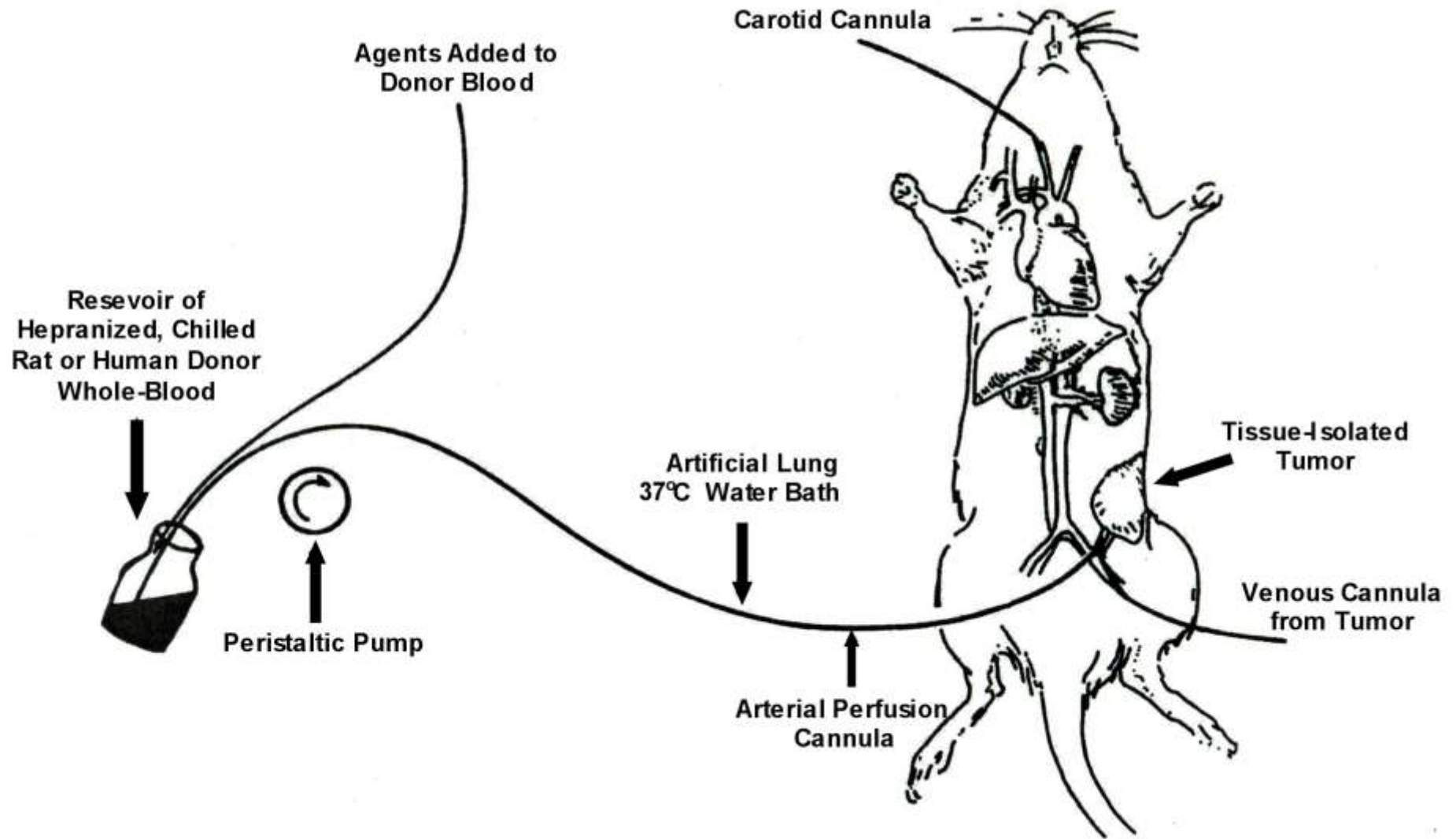
Bassett Research Institute



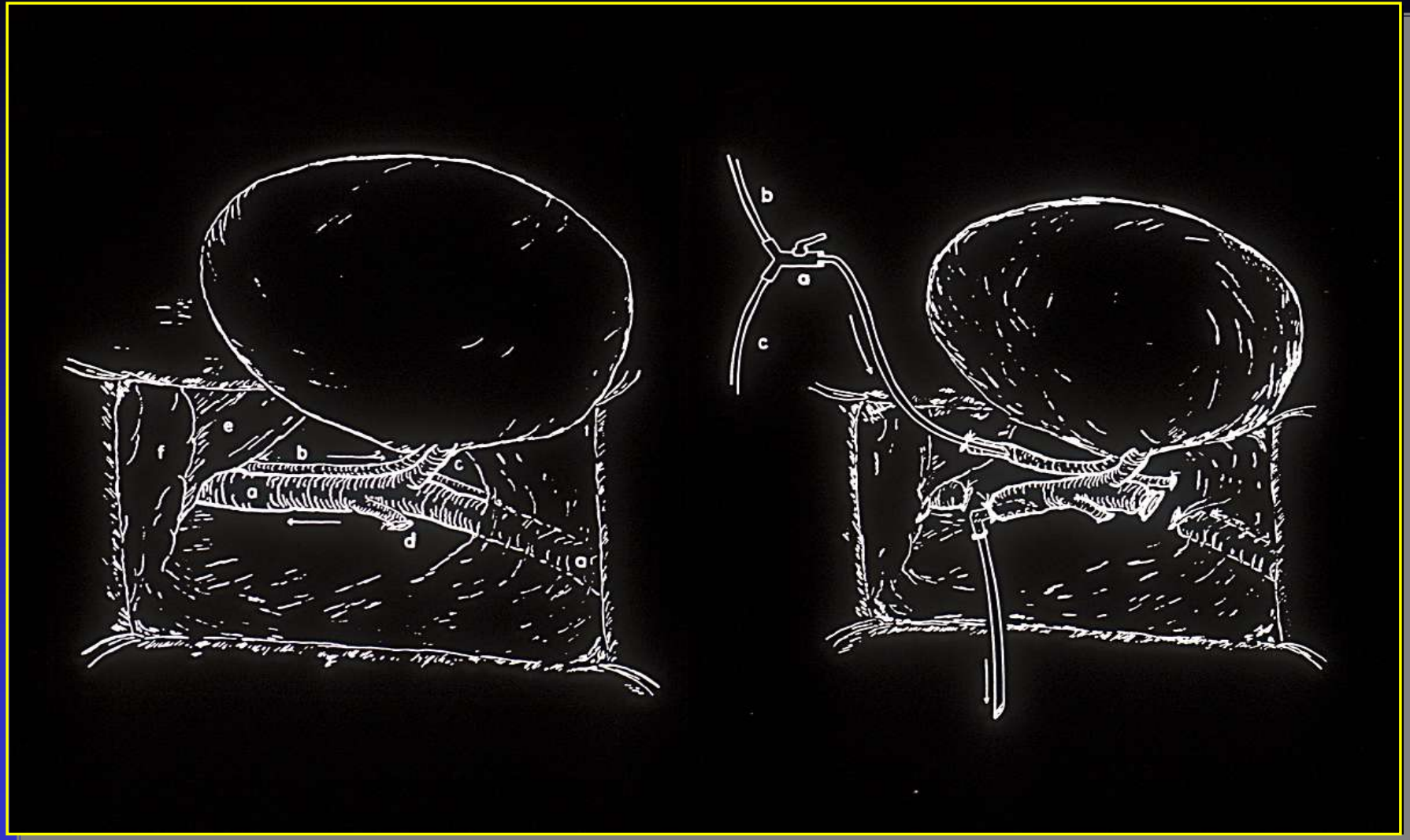
Tumor perfusion with human blood



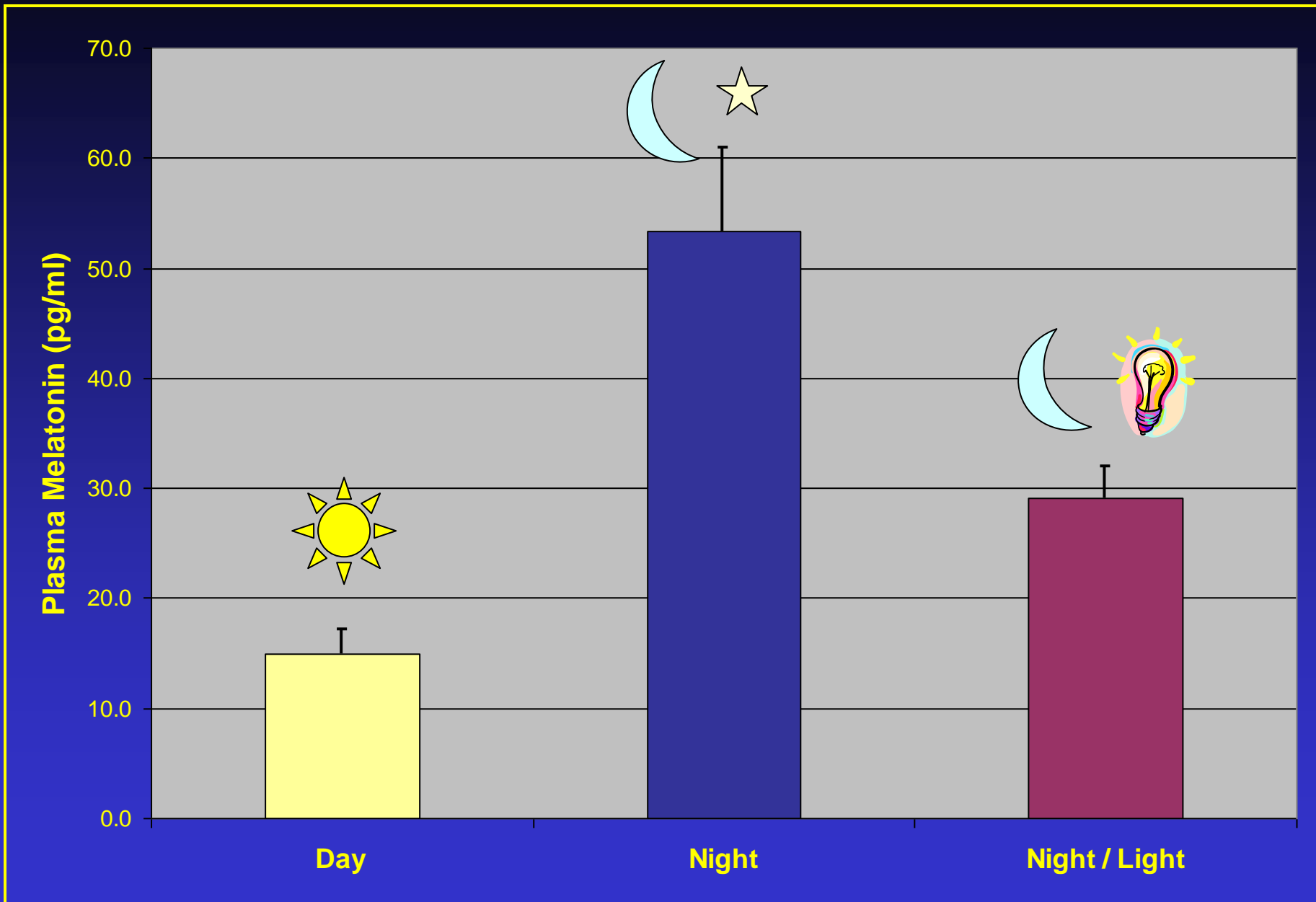
SYSTEM FOR PERFUSION OF TISSUE-ISOLATED TUMORS *IN SITU*



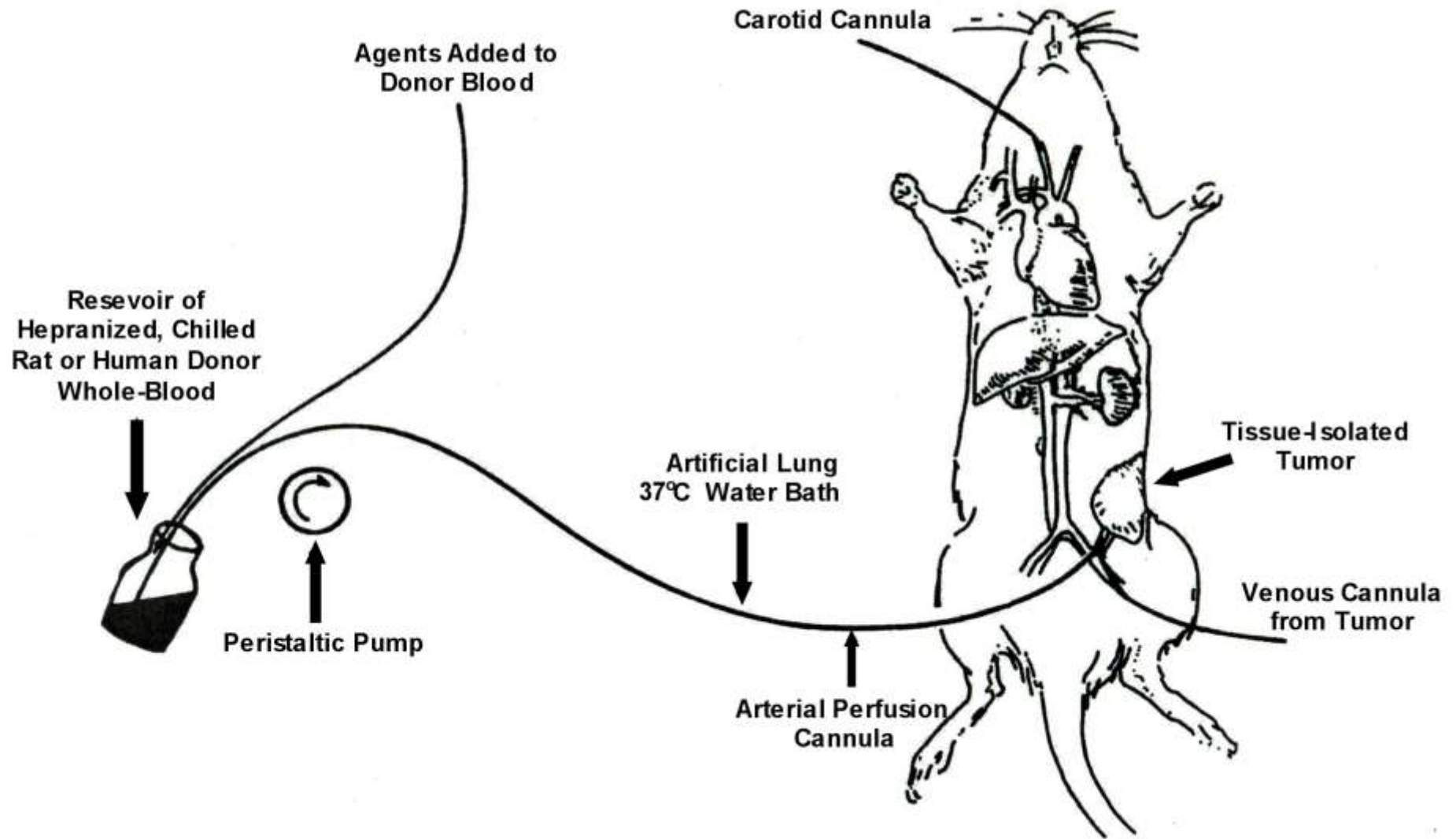
CANNULATION OF TISSUE-ISOLATED TUMOR



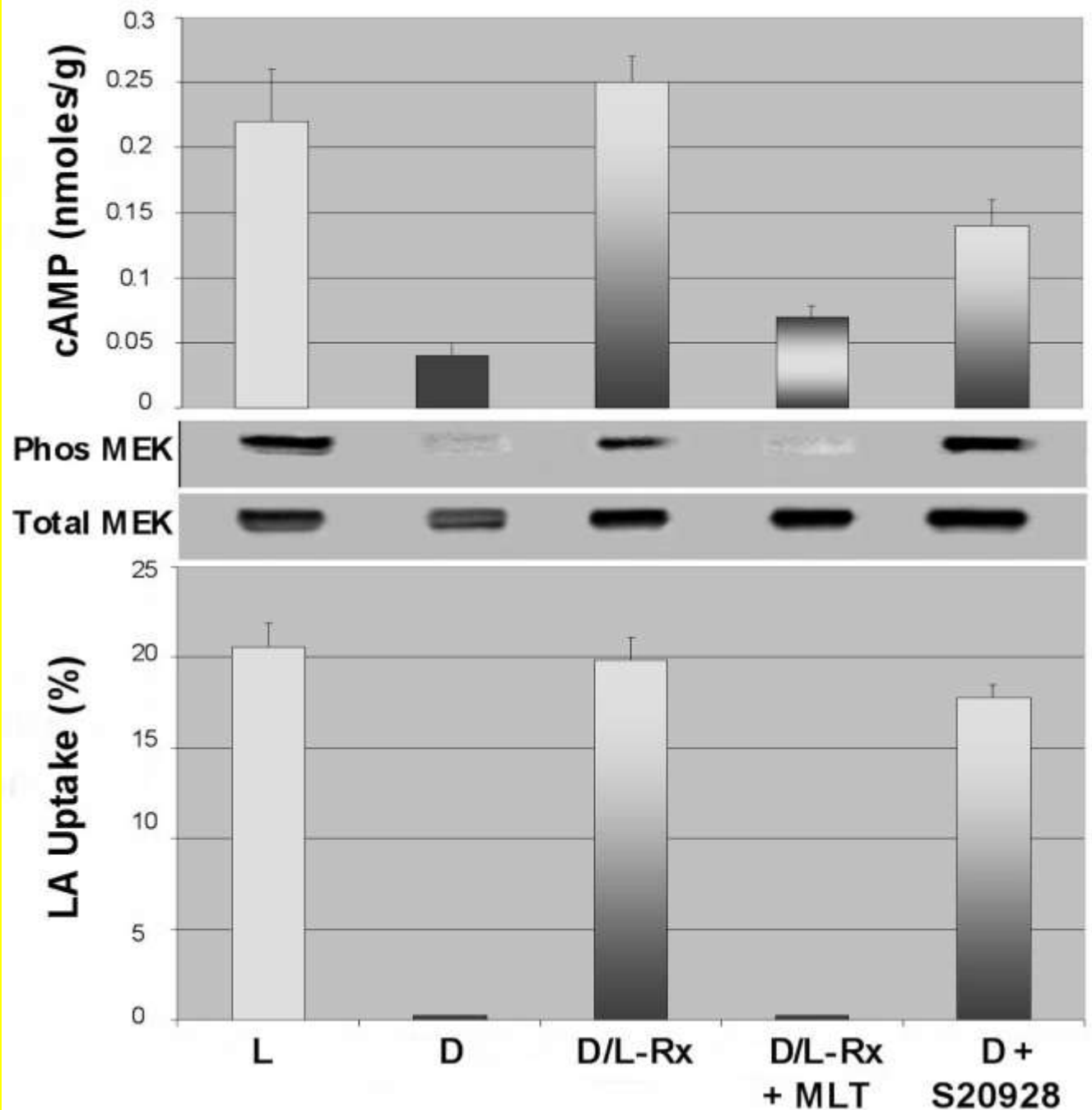
HUMAN BLOOD PLASMA MELATONIN LEVELS BEFORE AND AFTER LEAN



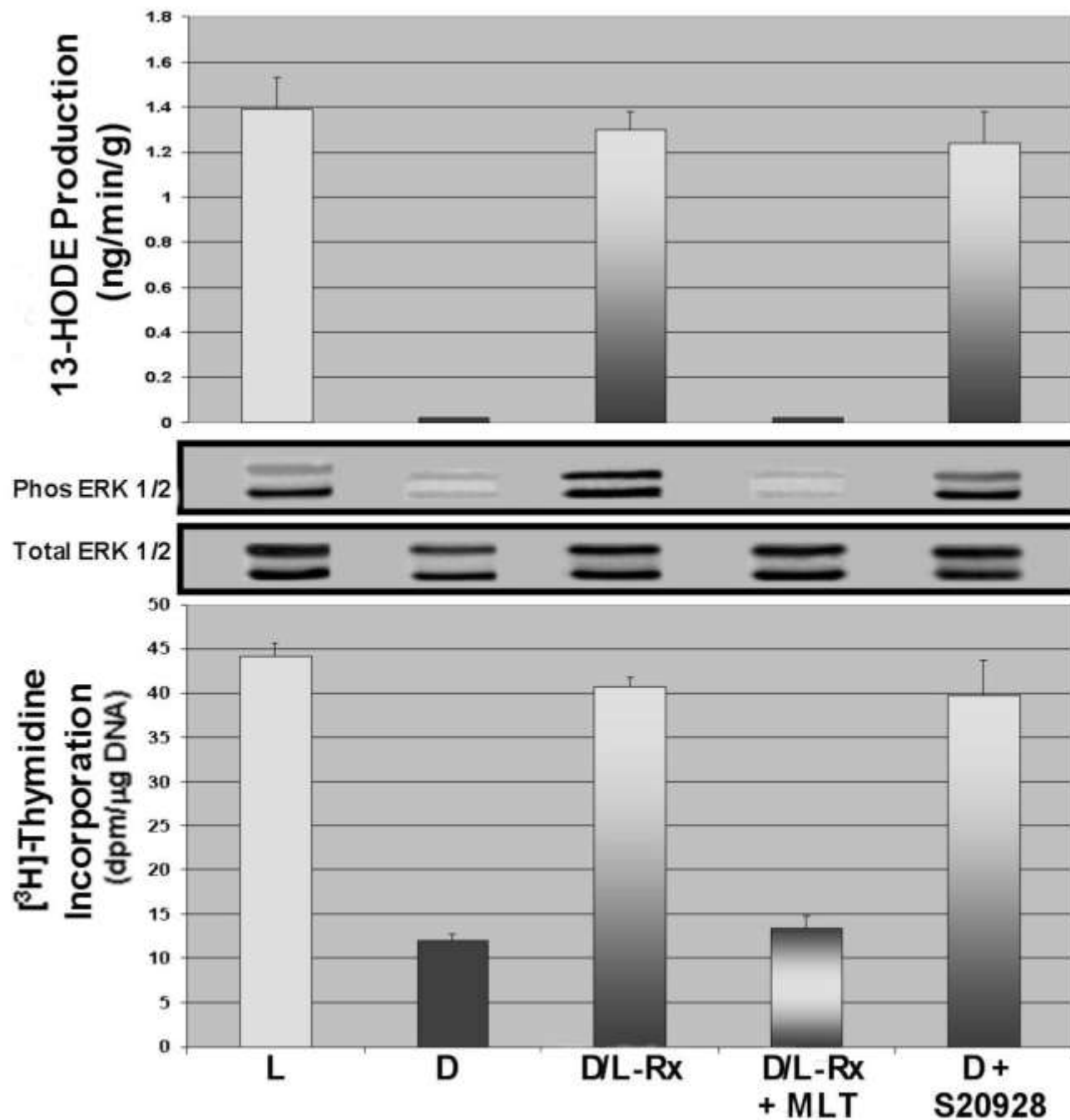
SYSTEM FOR PERFUSION OF TISSUE-ISOLATED TUMORS *IN SITU*



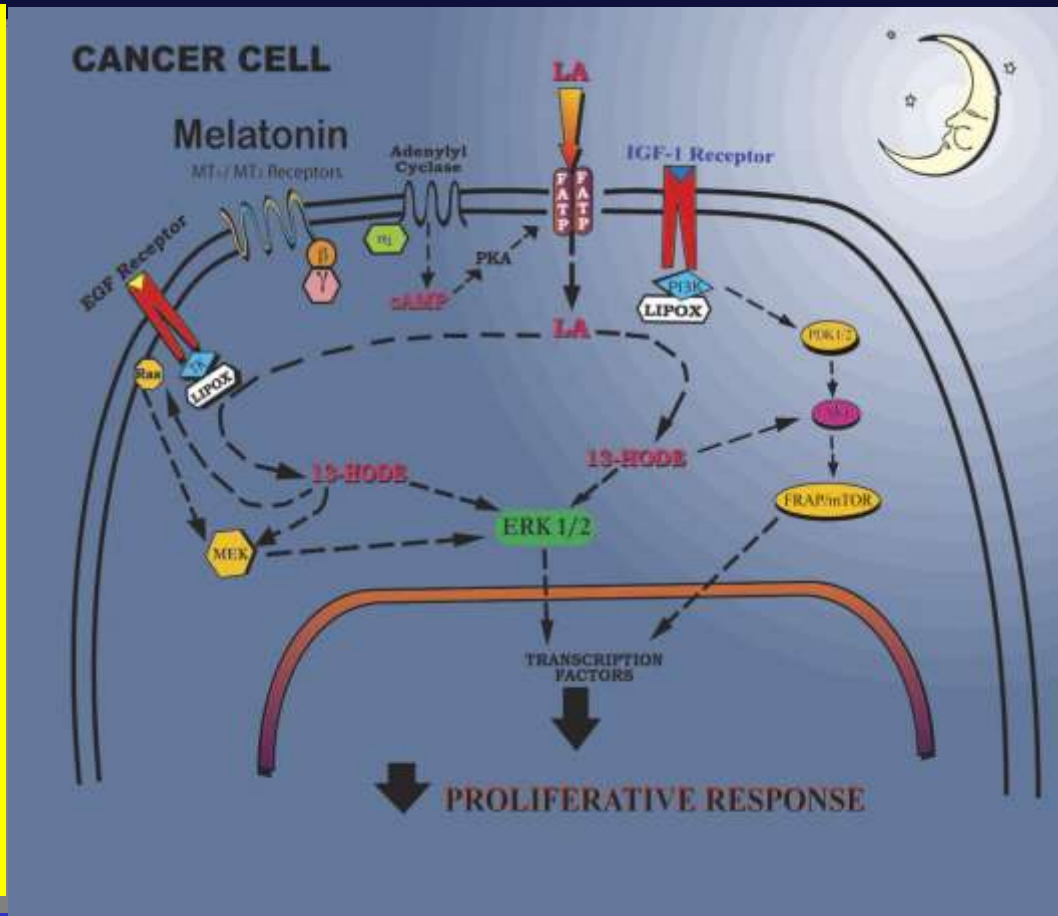
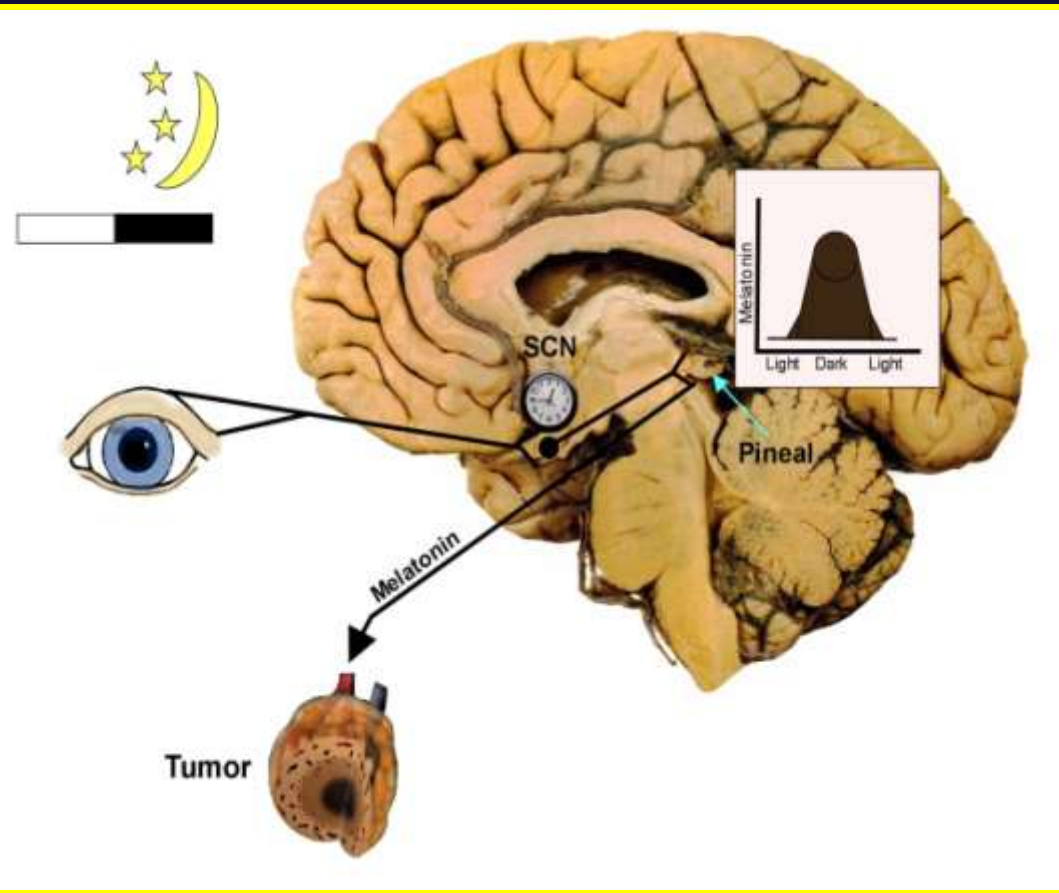
**SIGNAL TRANSDUCTION
AND PROLIFERATIVE
ACTIVITY IN MCF-7 (SR-)
HUMAN BREAST
CANCER XENOGRAFTS
PERFUSED *IN SITU* WITH
BLOOD COLLECTED
FROM HUMAN
VOLUNTEERS (n = 4)**



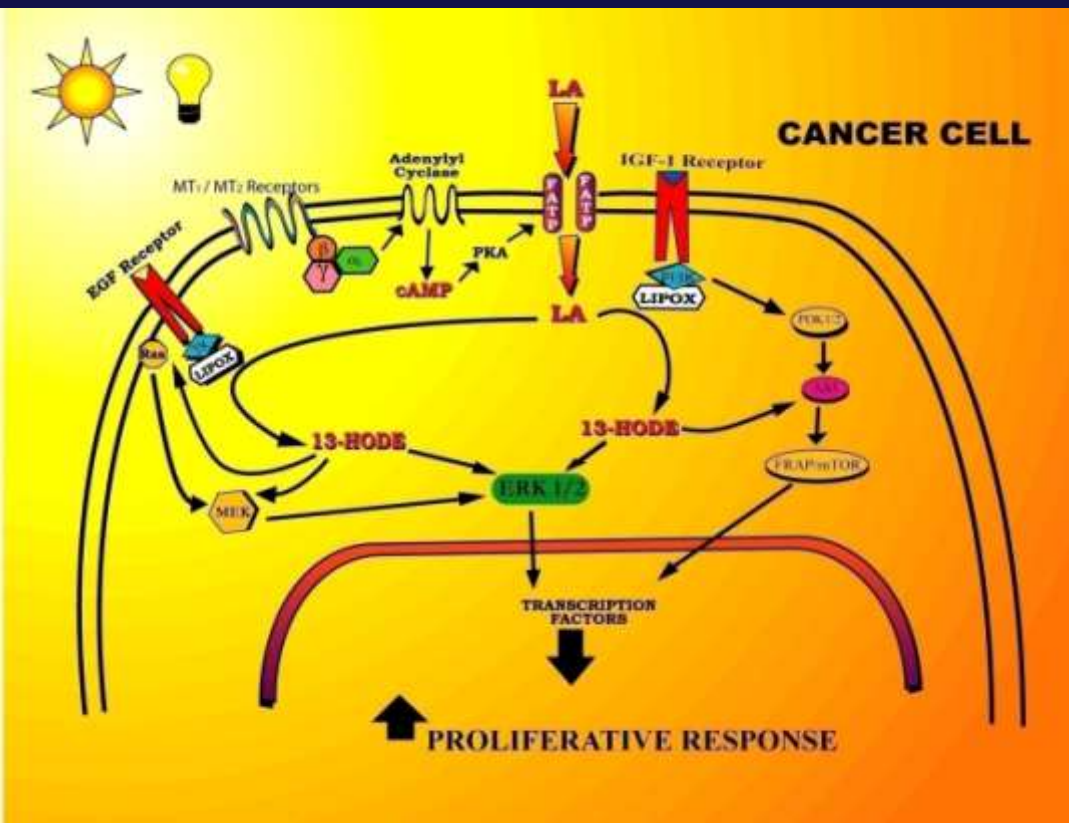
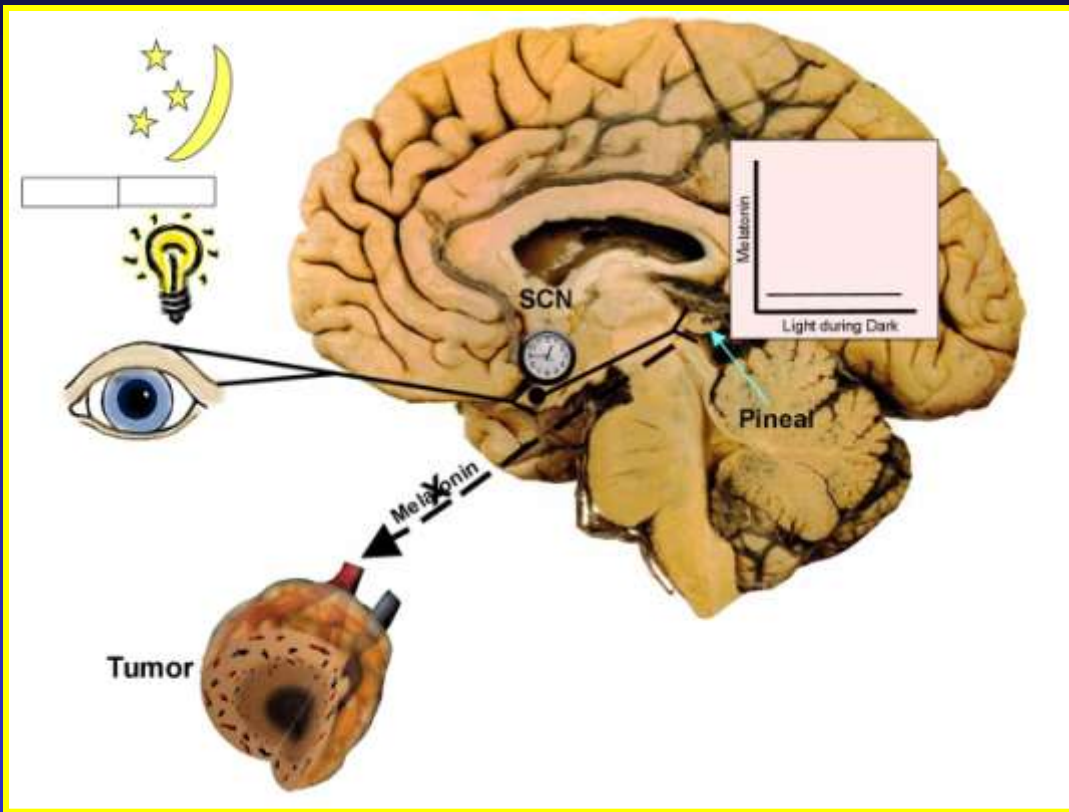
**SIGNAL TRANSDUCTION
AND PROLIFERATIVE
ACTIVITY IN MCF-7 (SR-)
HUMAN BREAST
CANCER XENOGRAFTS
PERFUSED *IN SITU* WITH
BLOOD COLLECTED
FROM HUMAN
VOLUNTEERS**



LIGHTS OFF AT NIGHT



LIGHTS ON AT NIGHT



Limitations and Recommendations

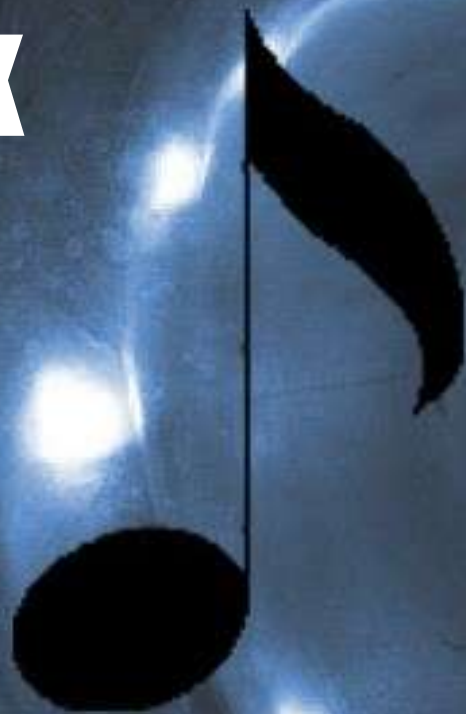
- Determine what aspects of light exposure at night (i.e., intensity spectrum, spatial pattern, duration and timing) result in the highest and lowest breast cancer risk in humans via circadian/melatonin disruption.**
- Test the effects of light-exposure regimens that mimic the light exposure patterns actually experienced by shift workers at night on breast cancer development and growth in the experimental setting in animals.**
- Examine the relative short- and long-term contributions of light at night-induced suppression of the nocturnal circadian melatonin signal versus circadian phase disruption to breast cancer initiation, growth, progression, and invasion and metastasis.**

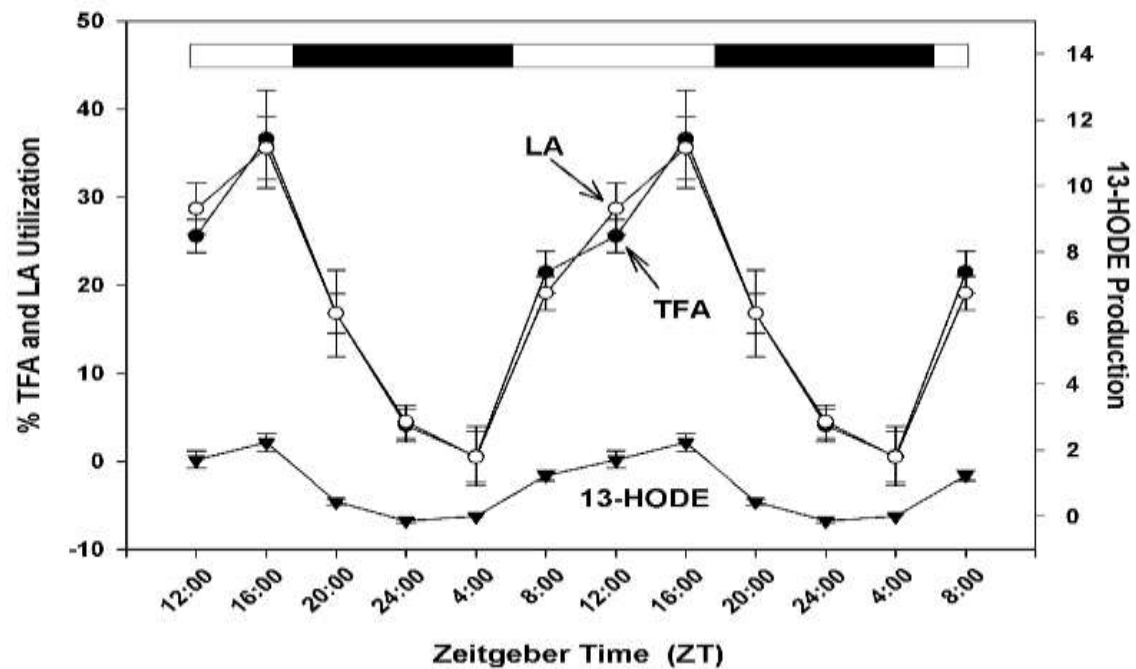
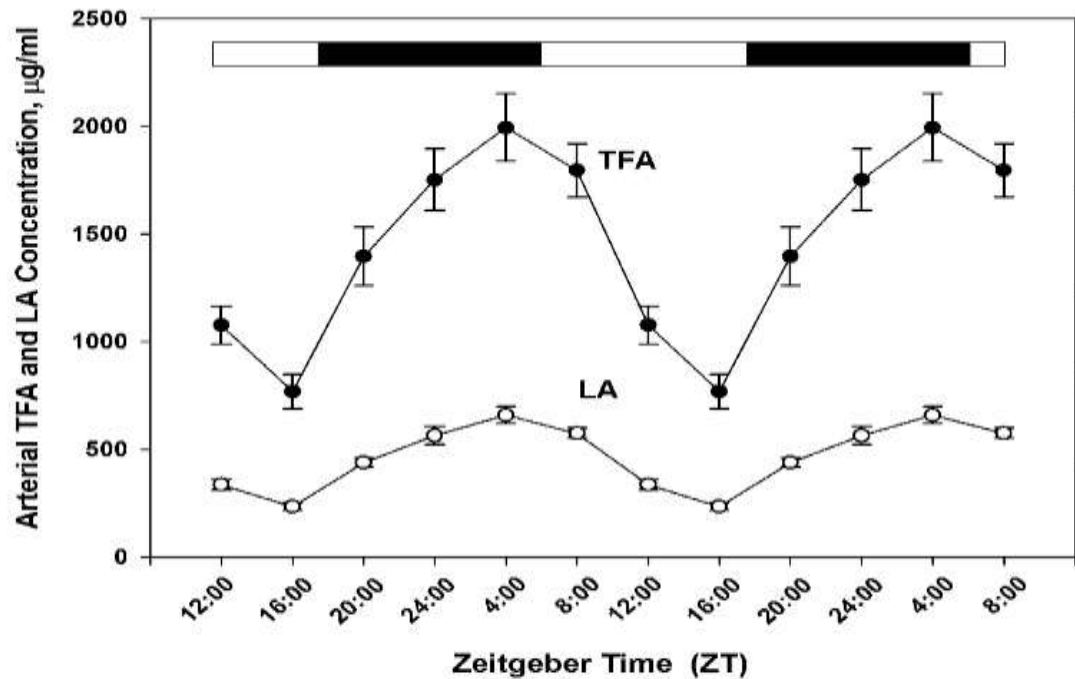
Limitations and Recommendations

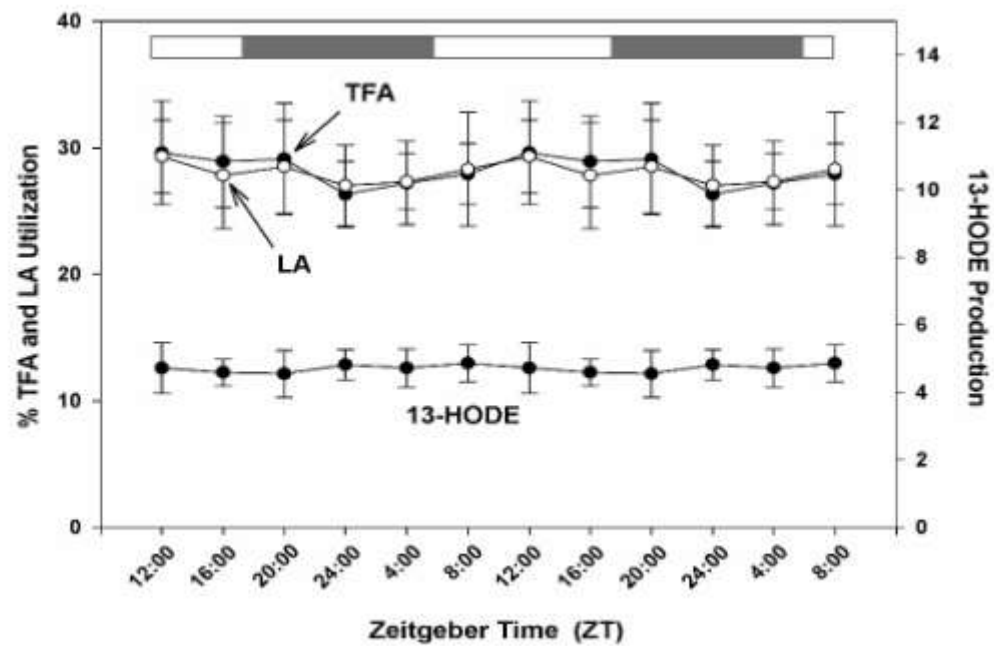
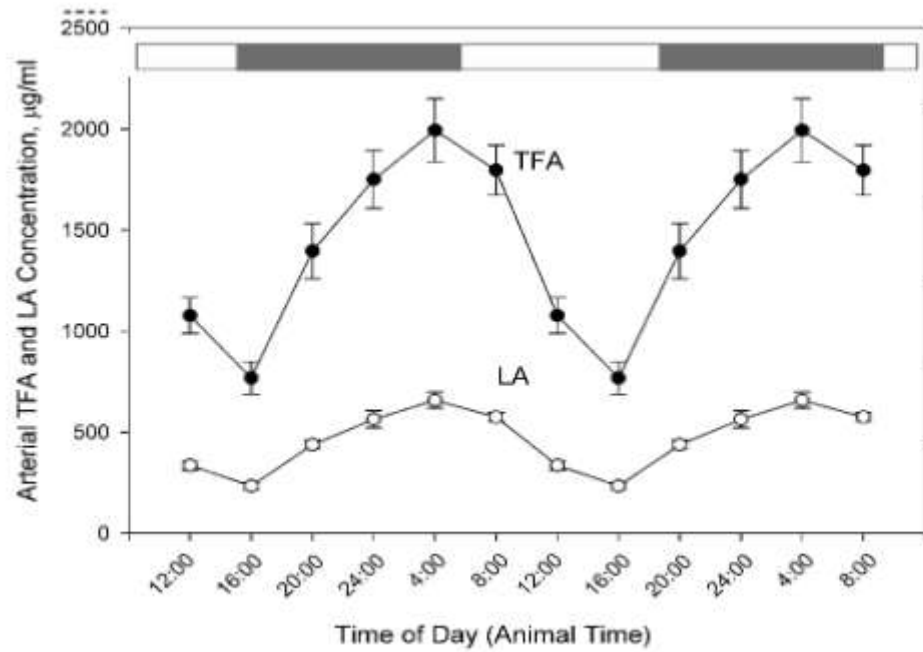
- Determine the balance between circadian melatonin signal strength, melatonin receptor expression sensitivity, and dietary cancer-stimulatory/inhibitory factors in cancer-susceptible target tissues that determines whether these tissues will be at more or less risk for developing breast cancer in response to circadian disruption of melatonin by light at night.
- Examine the interactions between nocturnal circadian melatonin signal disruption by light at night and melatonin receptors, clock genes and cell proliferation/survival pathways that may be involved in regulating oncogenesis and/or tumor suppressor genes .

Stevens et al. Environ Health Perspect 115:1357-1362, 2007

**THANK
YOU!**







LIGHT AT NIGHT WORLDWIDE 400 YEARS AGO

beautiful
Edison
lamp

New Type Edison Lamp,
Patented June 14, 1881, Dec.
27, 1881, Sept. 19, 1882.
OTHER PATENTS.

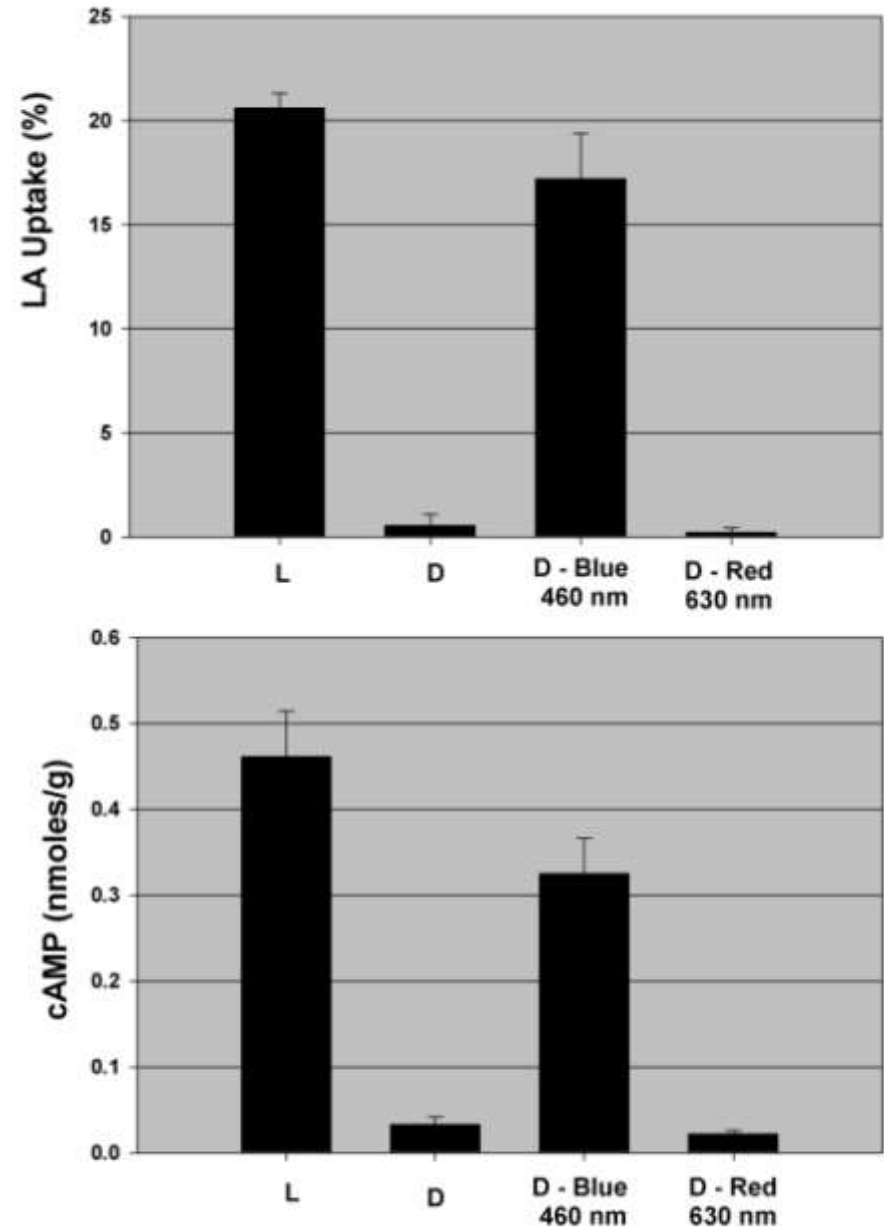
perfect
carbon
filament



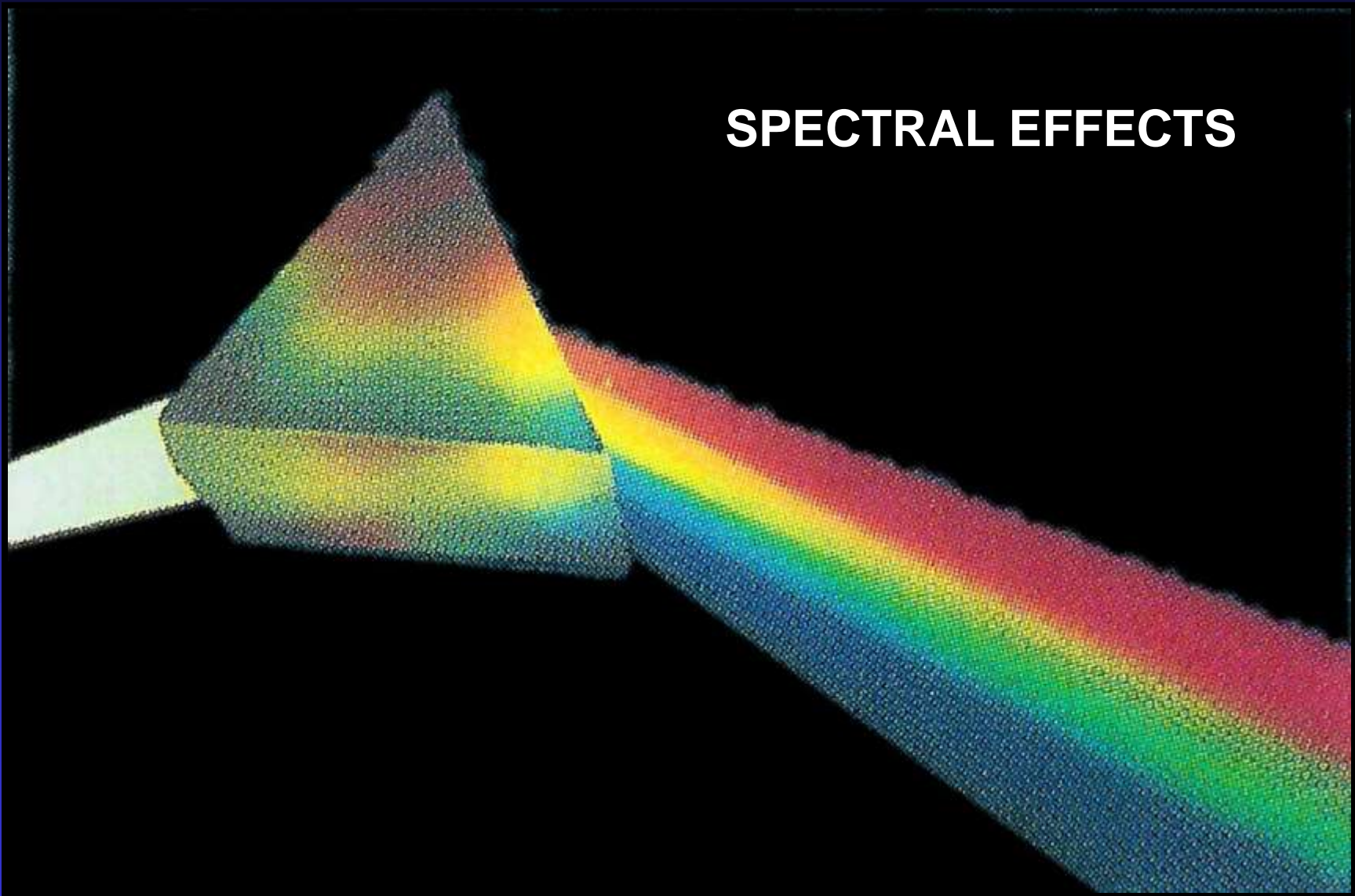
Thomson Houston Base



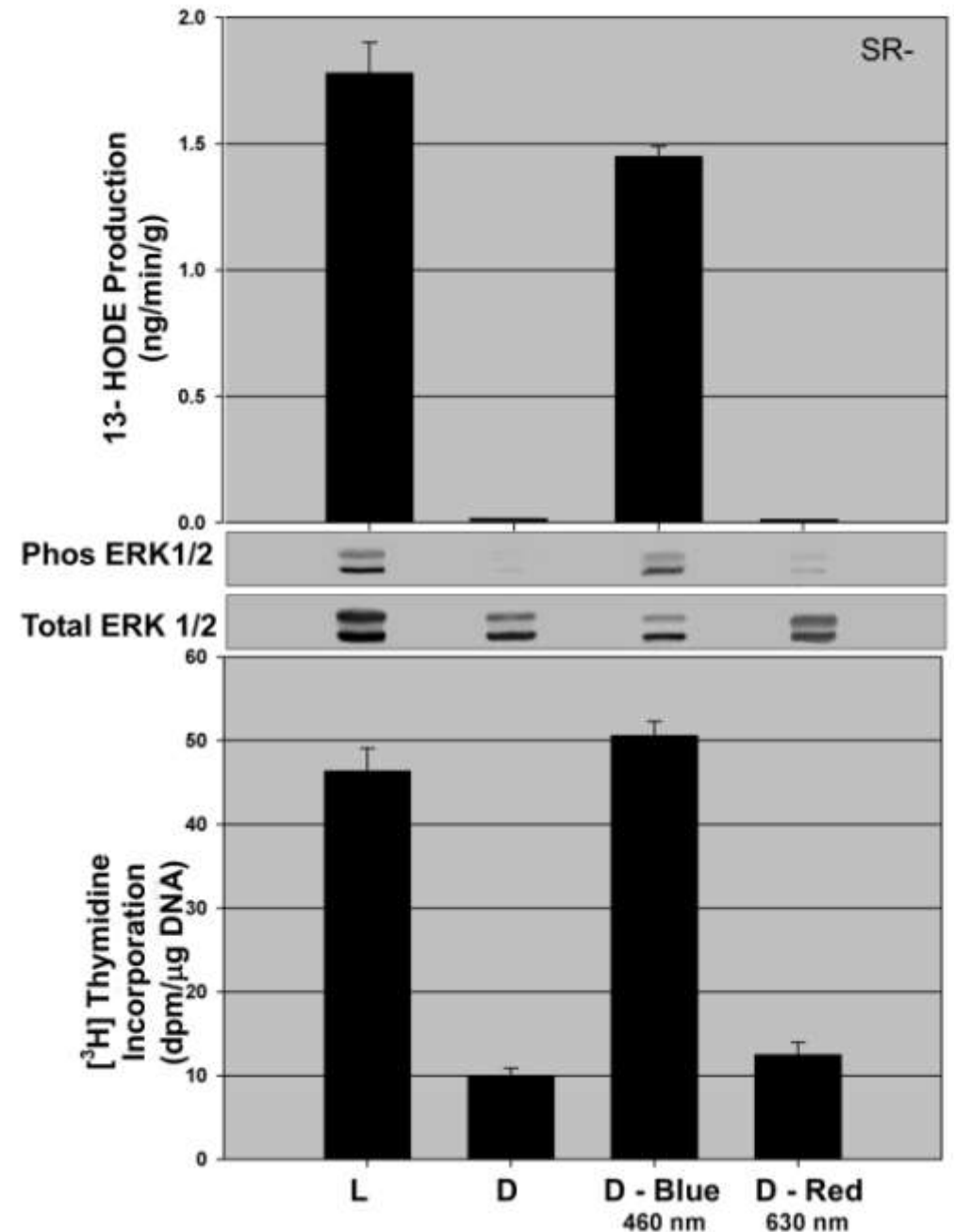
SIGNAL TRANSDUCTION AND PROLIFERATIVE ACTIVITY IN MCF-7 (SR-) HUMAN BREAST CANCER XENOGRAPTS PERFUSED *IN SITU* WITH BLOOD COLLECTED FROM HUMAN VOLUNTEERS (n = 2) EXPOSED TO EITHER BLUE MONOCHROMATIC (460 nm peak, 12 nm half-peak band width, 54.0 $\mu\text{W}/\text{cm}^2$) OR RED MONOCHROMATIC (630 nm peak, 12 nm half-peak bandwidth, 39.4 $\mu\text{W}/\text{cm}^2$) ; equal photon densities (1.54×10^{14} photons / cm^2/sec)

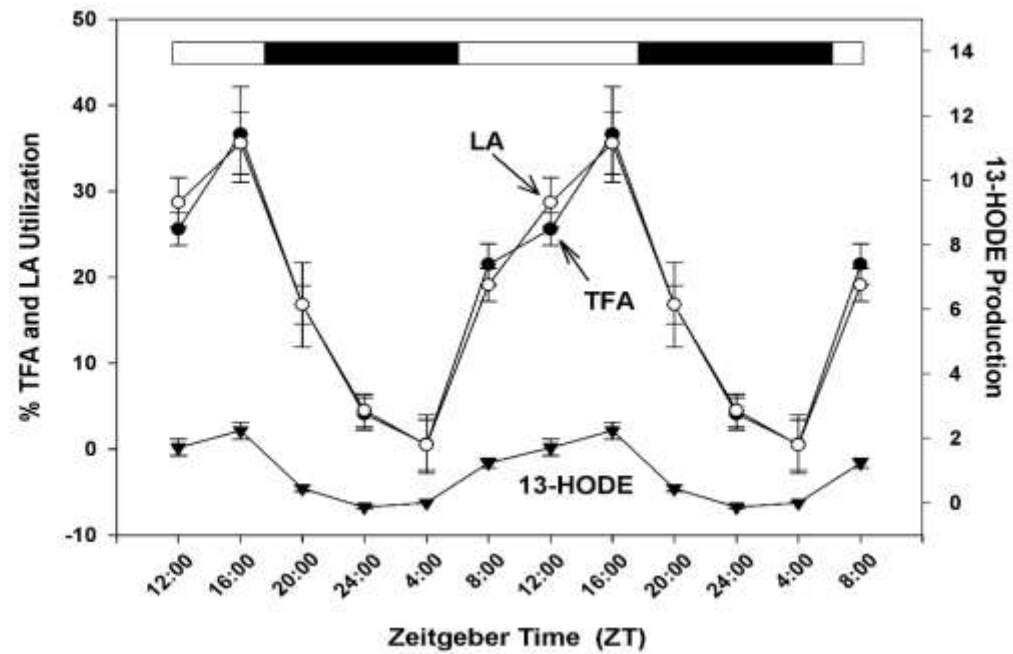
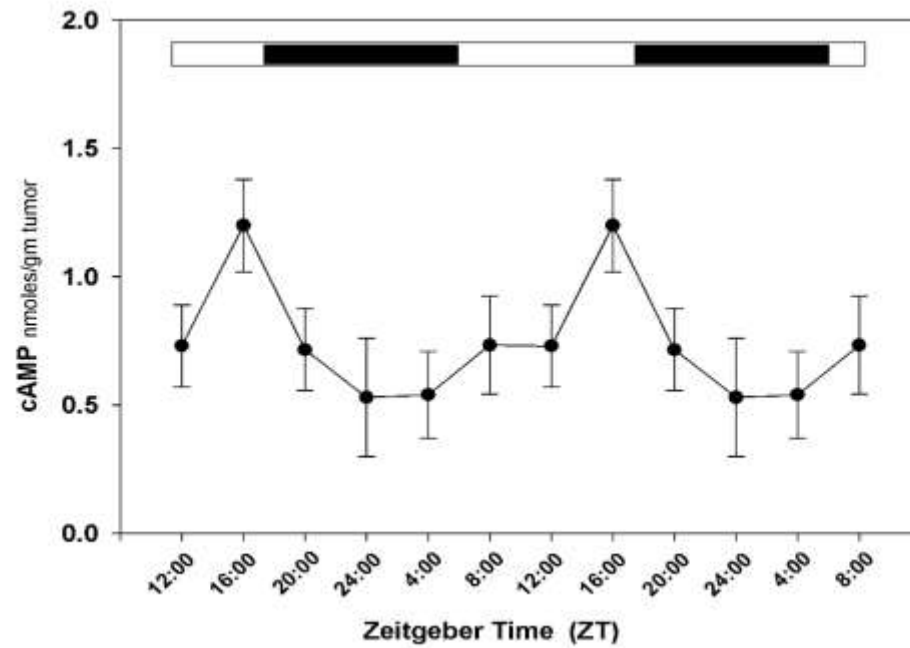


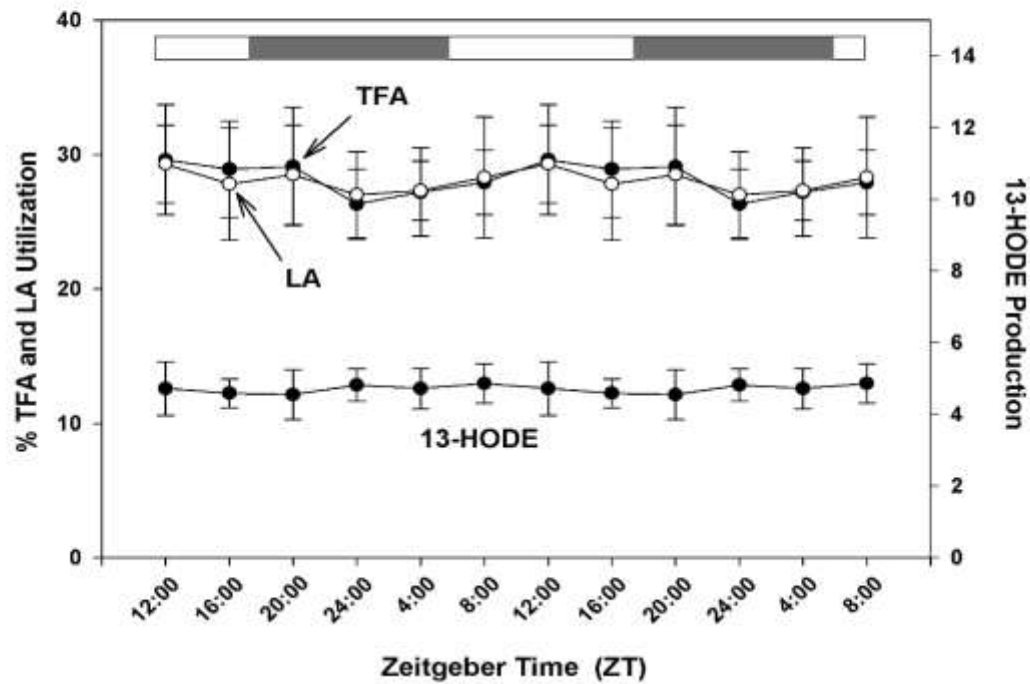
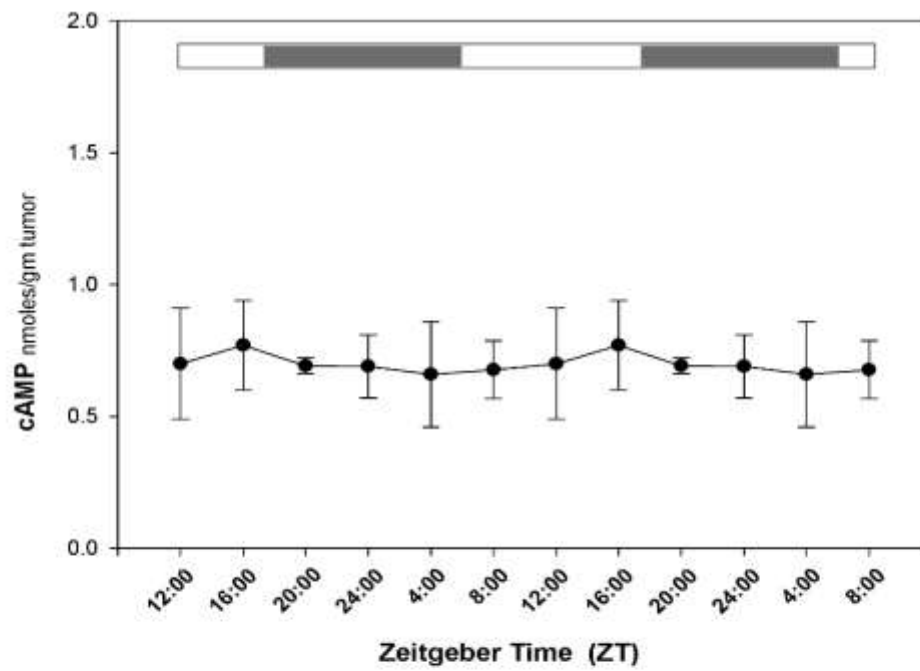
SPECTRAL EFFECTS



SIGNAL TRANSDUCTION AND PROLIFERATIVE ACTIVITY IN MCF-7 (SR-) HUMAN BREAST CANCER XENOGRAFTS PERFUSED *IN SITU* WITH BLOOD COLLECTED FROM HUMAN VOLUNTEERS (n = 2) EXPOSED TO EITHER BLUE MONOCHROMATIC (460 nm peak, 12 nm half-peak band width, 54.0 $\mu\text{W}/\text{cm}^2$) OR RED MONOCHROMATIC (630 nm peak, 12 nm half-peak bandwidth, 39.4 $\mu\text{W}/\text{cm}^2$)

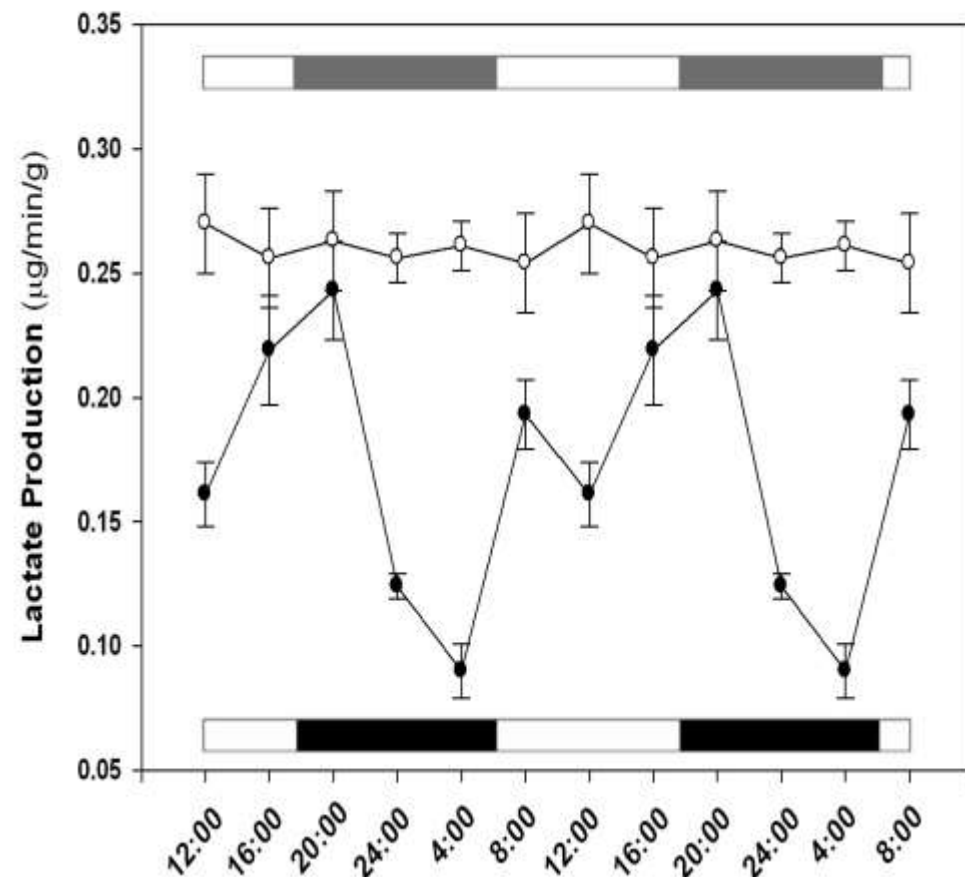
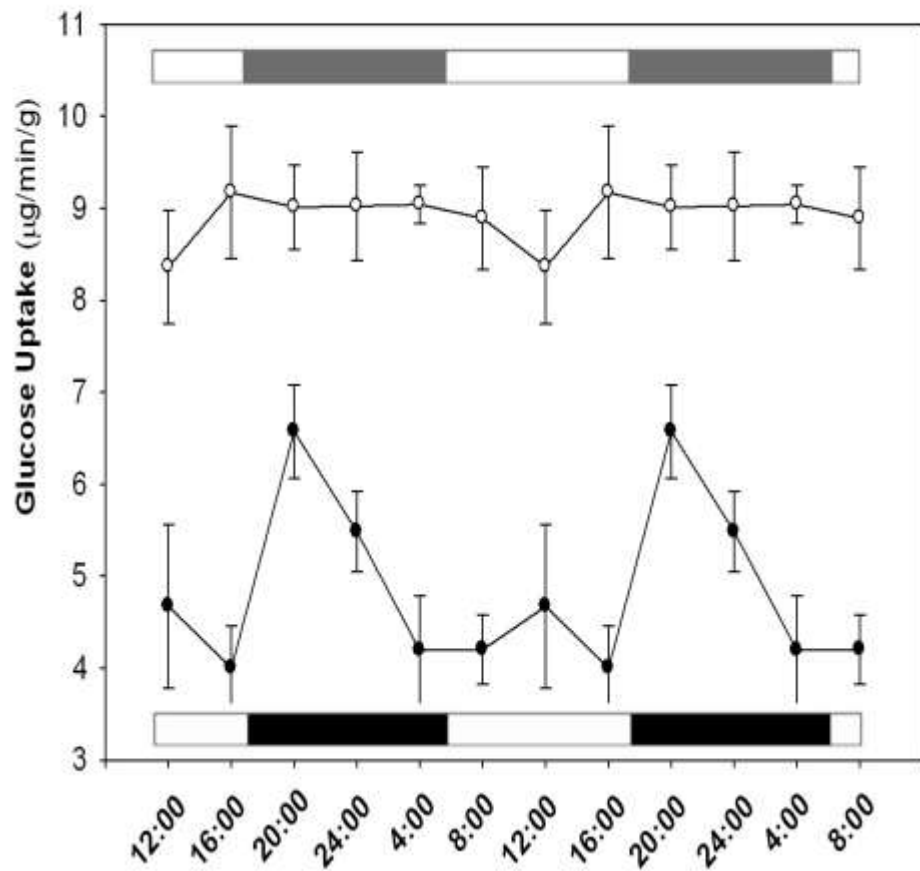






Light At Night Worldwide



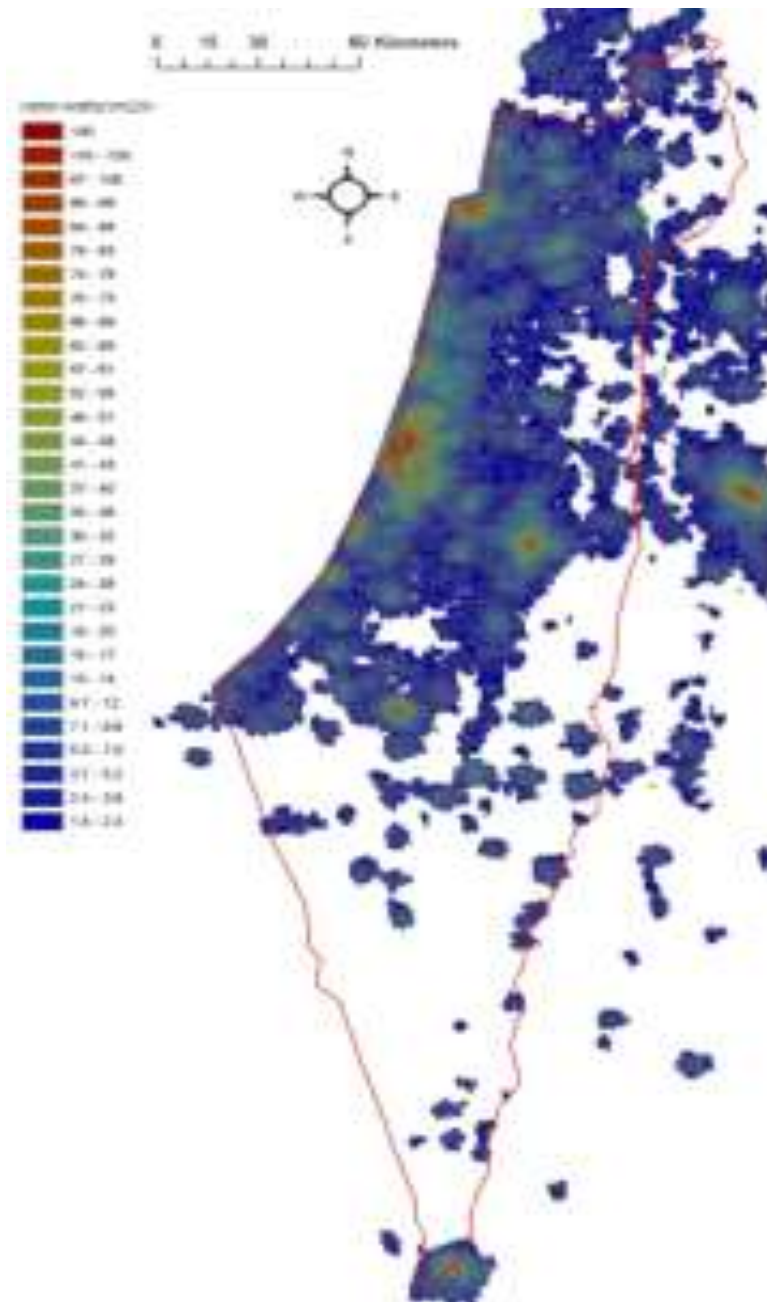


Light at night in U.S. at today

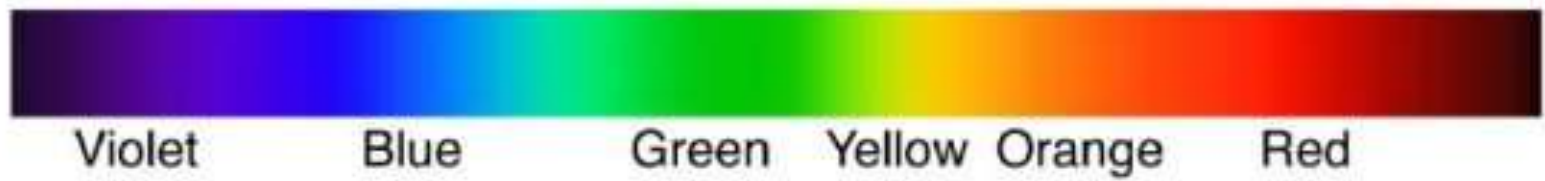
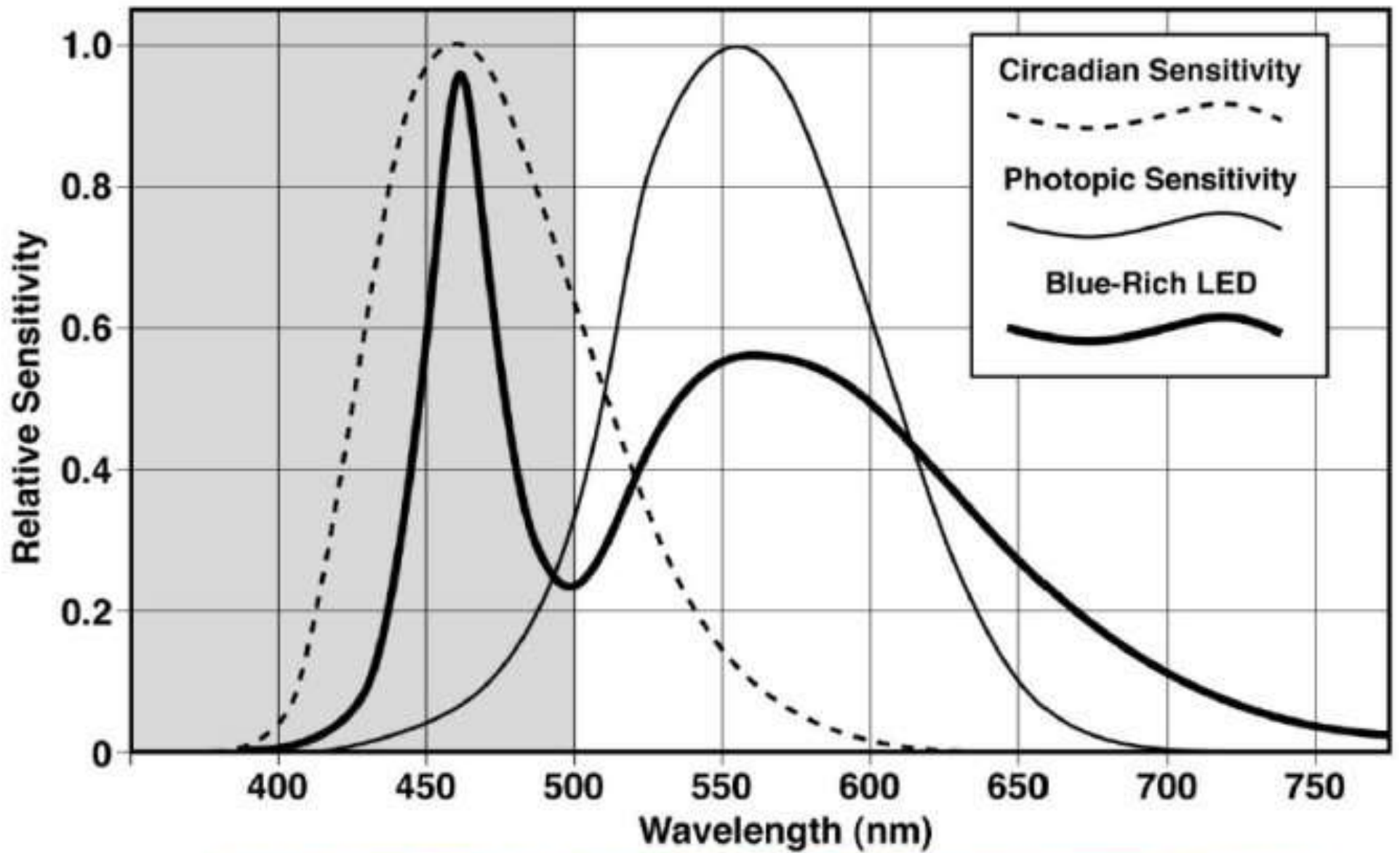


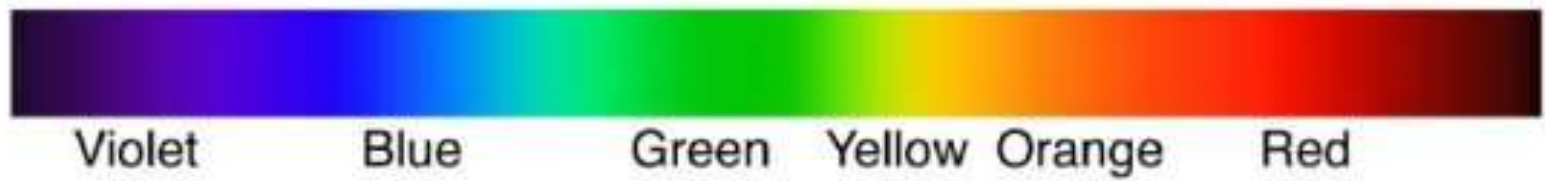
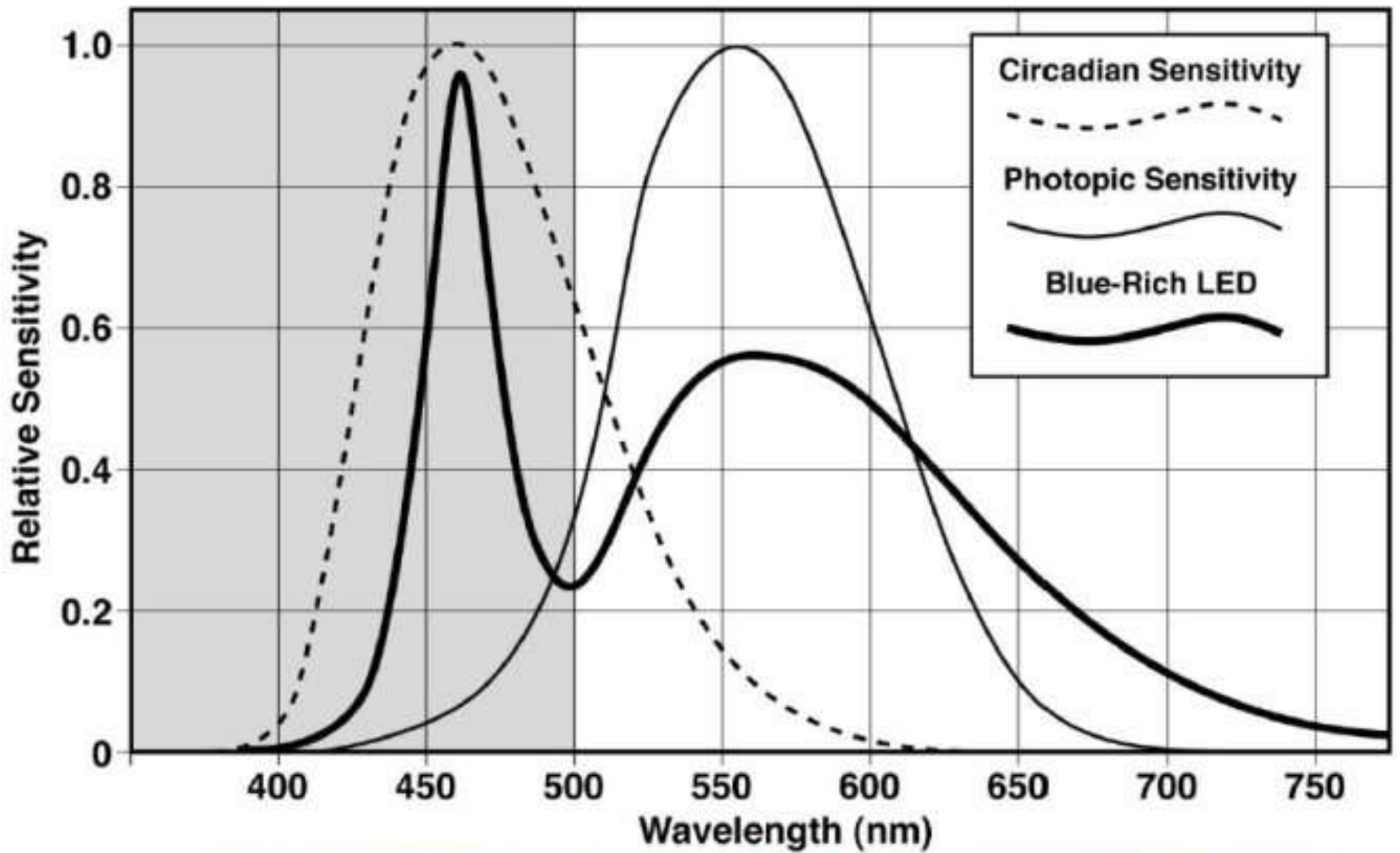
EPIDEMIOLOGICAL EVIDENCE

- **Women working nightshifts are at a 50% to 80% increased risk of developing breast cancer - risk increases with the number of years worked**
- **Blind women are at a 50% decreased risk of developing breast cancer**
- **Women with higher levels of melatonin have a decreased risk of developing breast cancer**
- **Women who sleep ≥ 9 hours per night are at a decreased risk of developing breast cancer**



LAN intensity levels
(nanowatts/cm²/sr)
according to nighttime
satellite image data

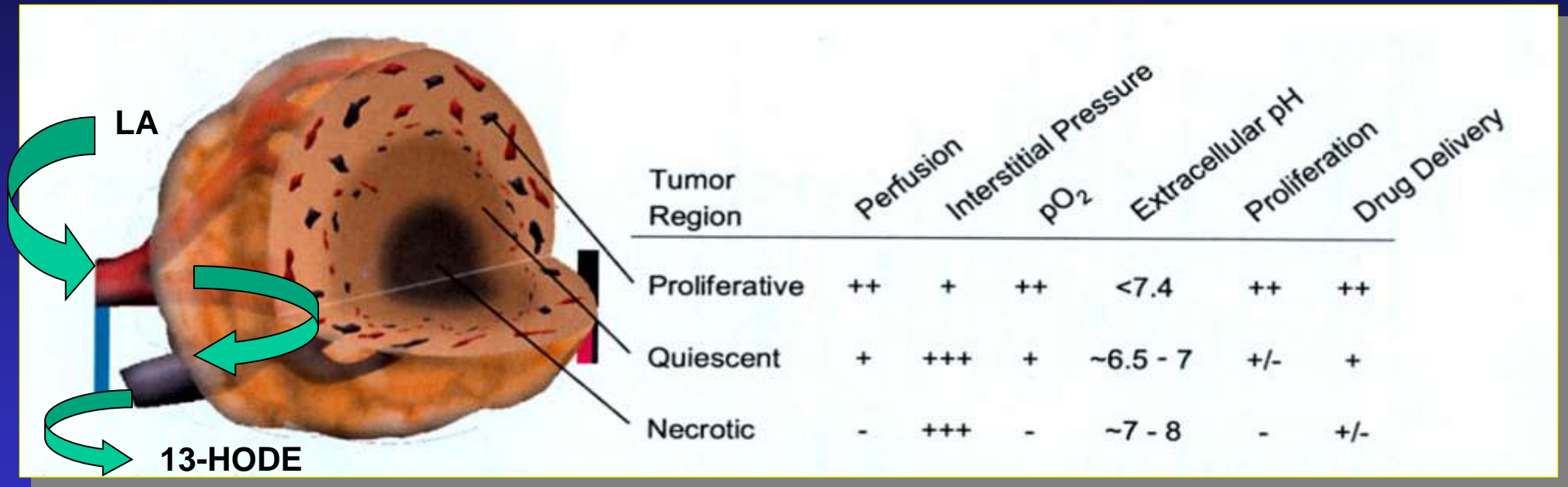


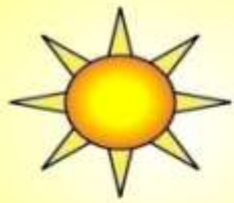


ASSUMPTION

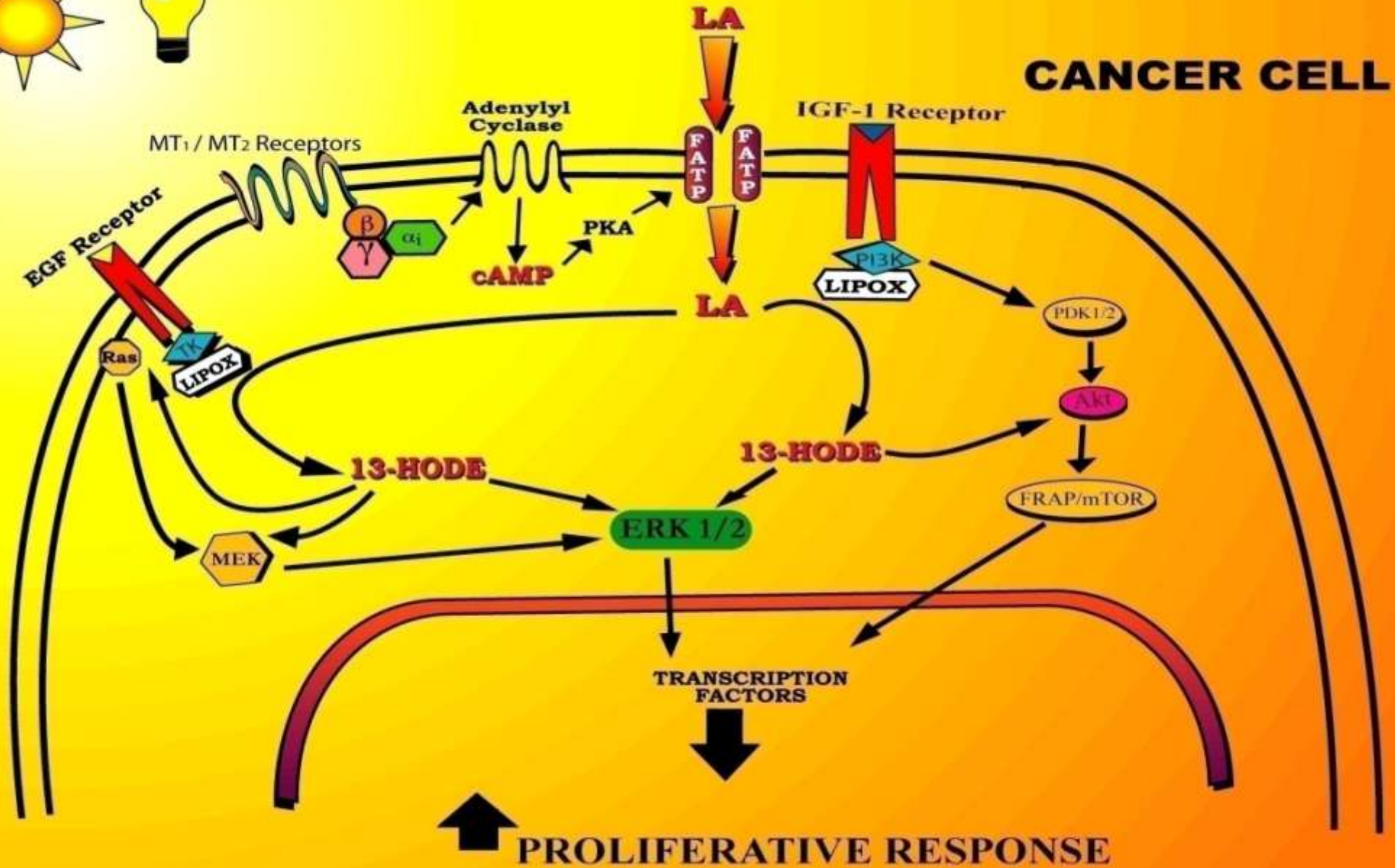
**Night shift work is a surrogate for
light at night-induced melatonin
suppression**

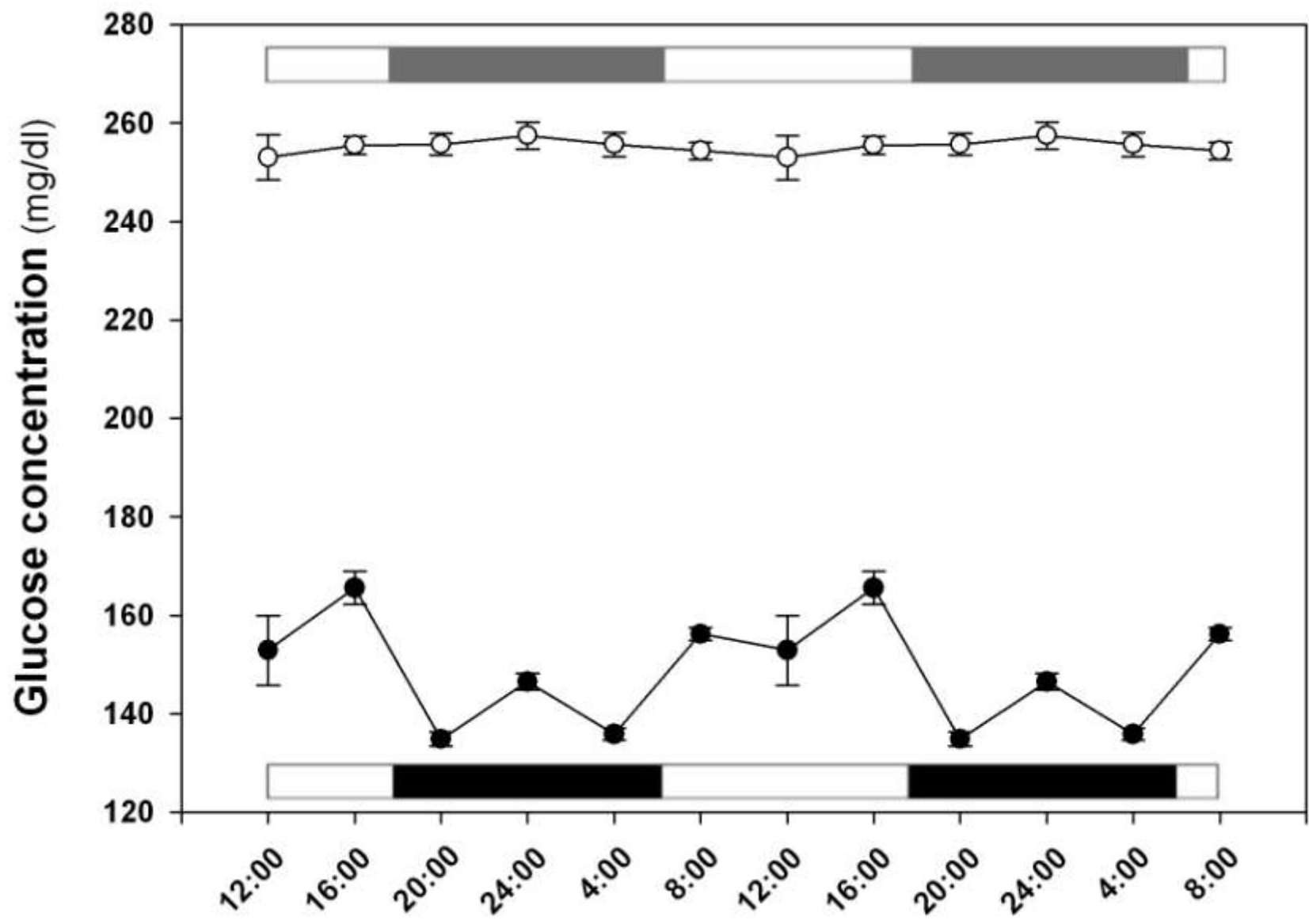
LA UPTAKE/UTILIZATION AND 13-HODE PRODUCTION/RELEASE BY TISSUE-ISOLATED TUMORS





CANCER CELL

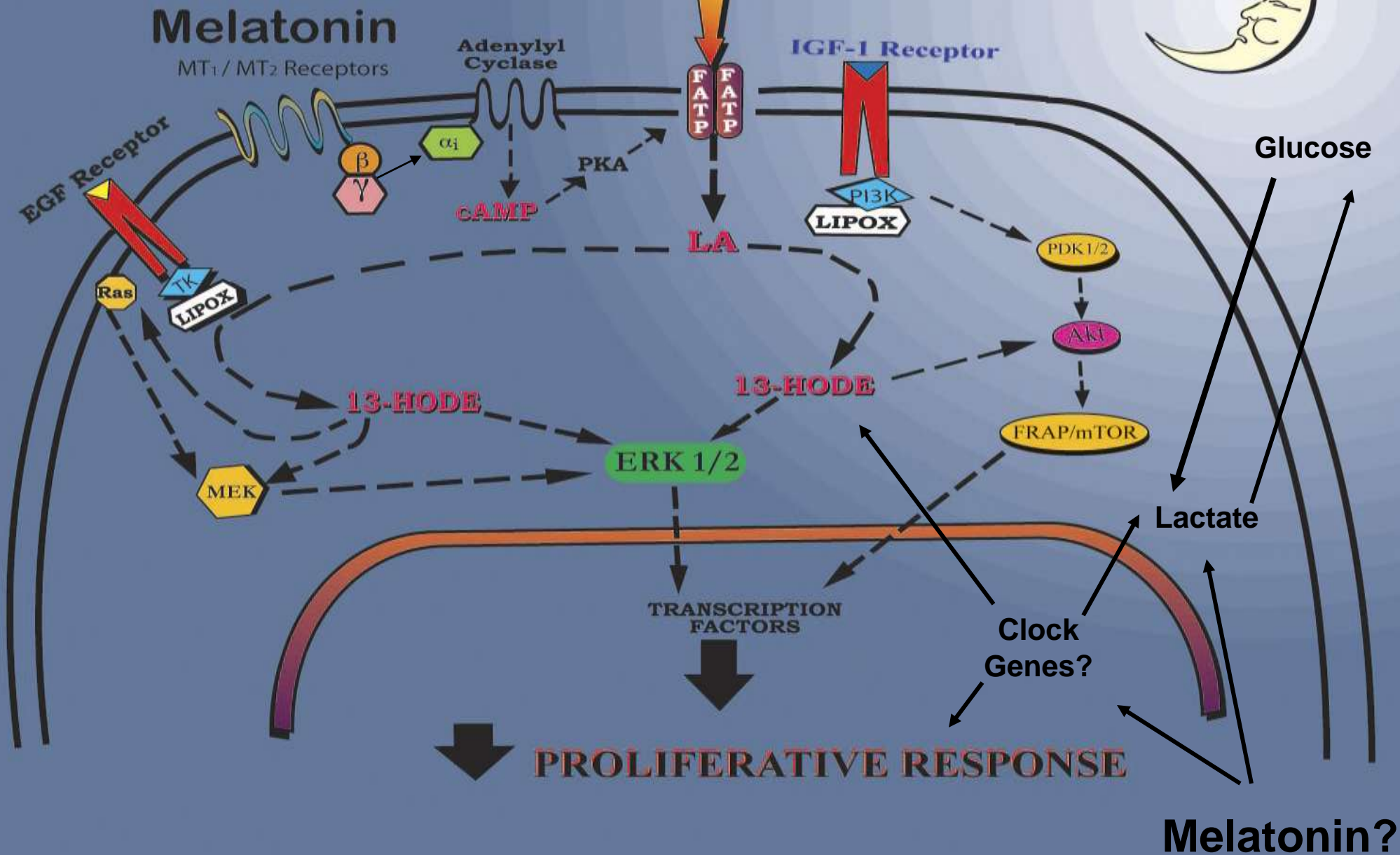


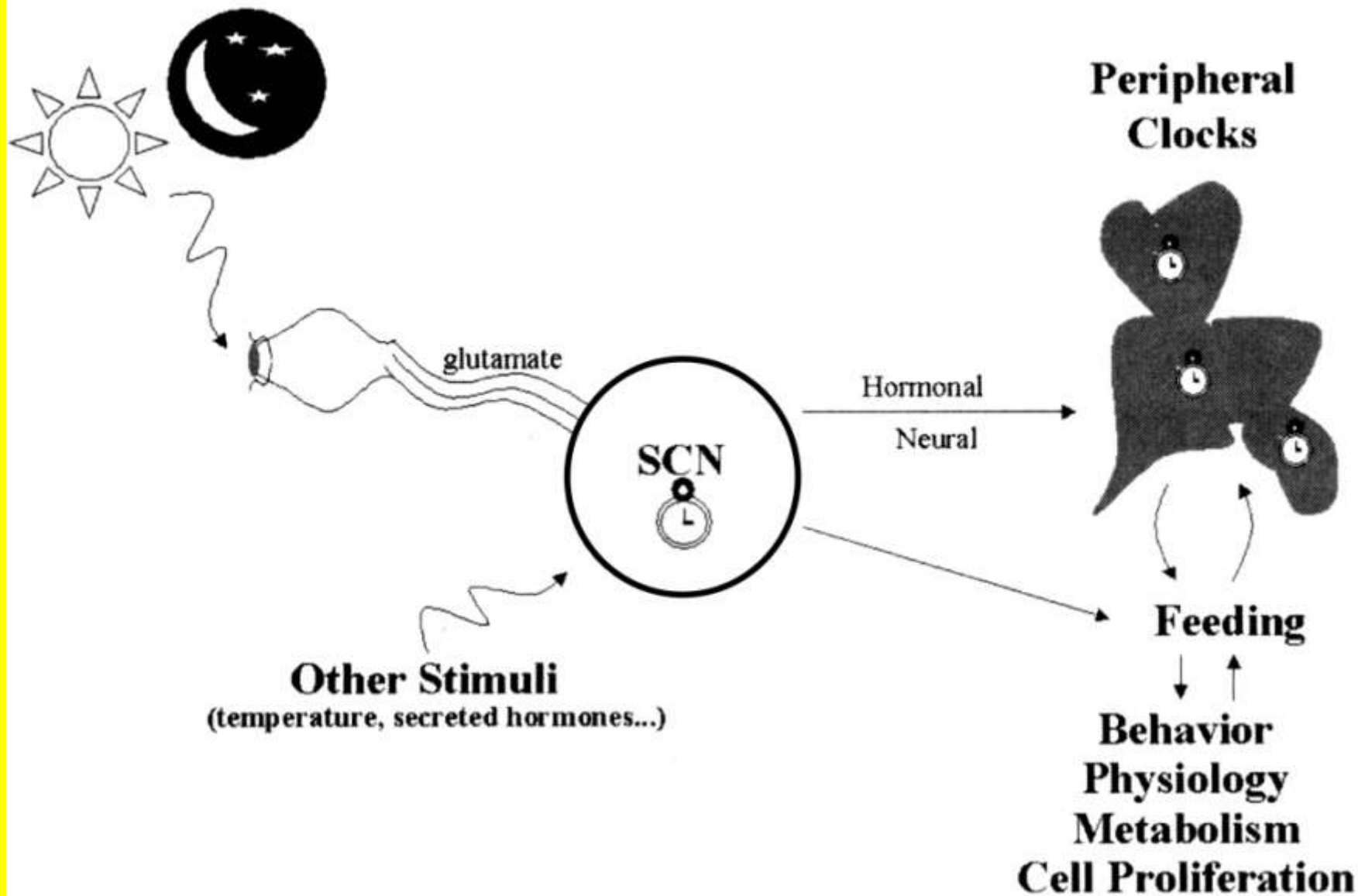


PHOTOBIOLOGICAL EXPOSURE CHAMBERS



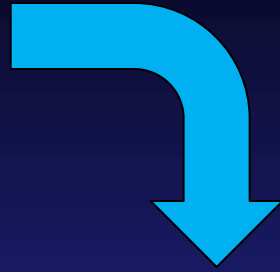
CANCER CELL



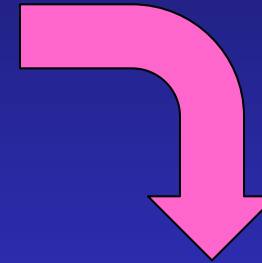




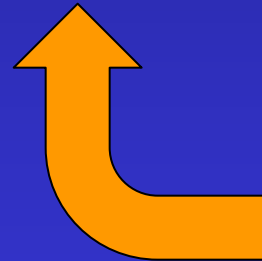
LIGHT AT NIGHT



BROKEN TIMING



CANCER



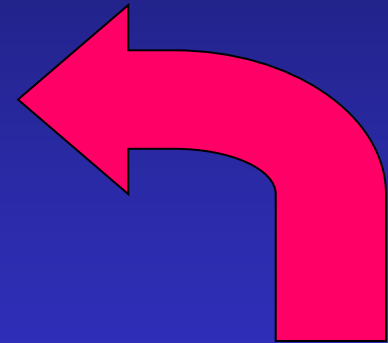
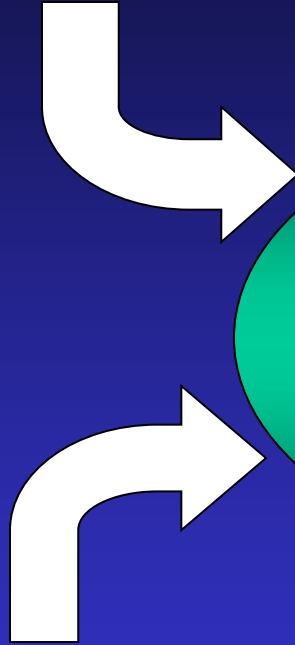
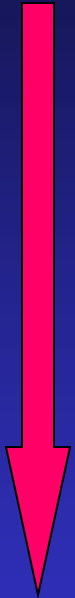
**CIRCADIAN
SYSTEM
(BIOLOGICAL
CLOCK)**

**NOCTURNAL
LIGHT
EXPOSURE**

**BREAST &
PROSTATE
CANCER**

**NOCTURNAL
MELATONIN**

**FATTY ACIDS
(LA) &
GLUCOSE**



COOH



Linoleic acid

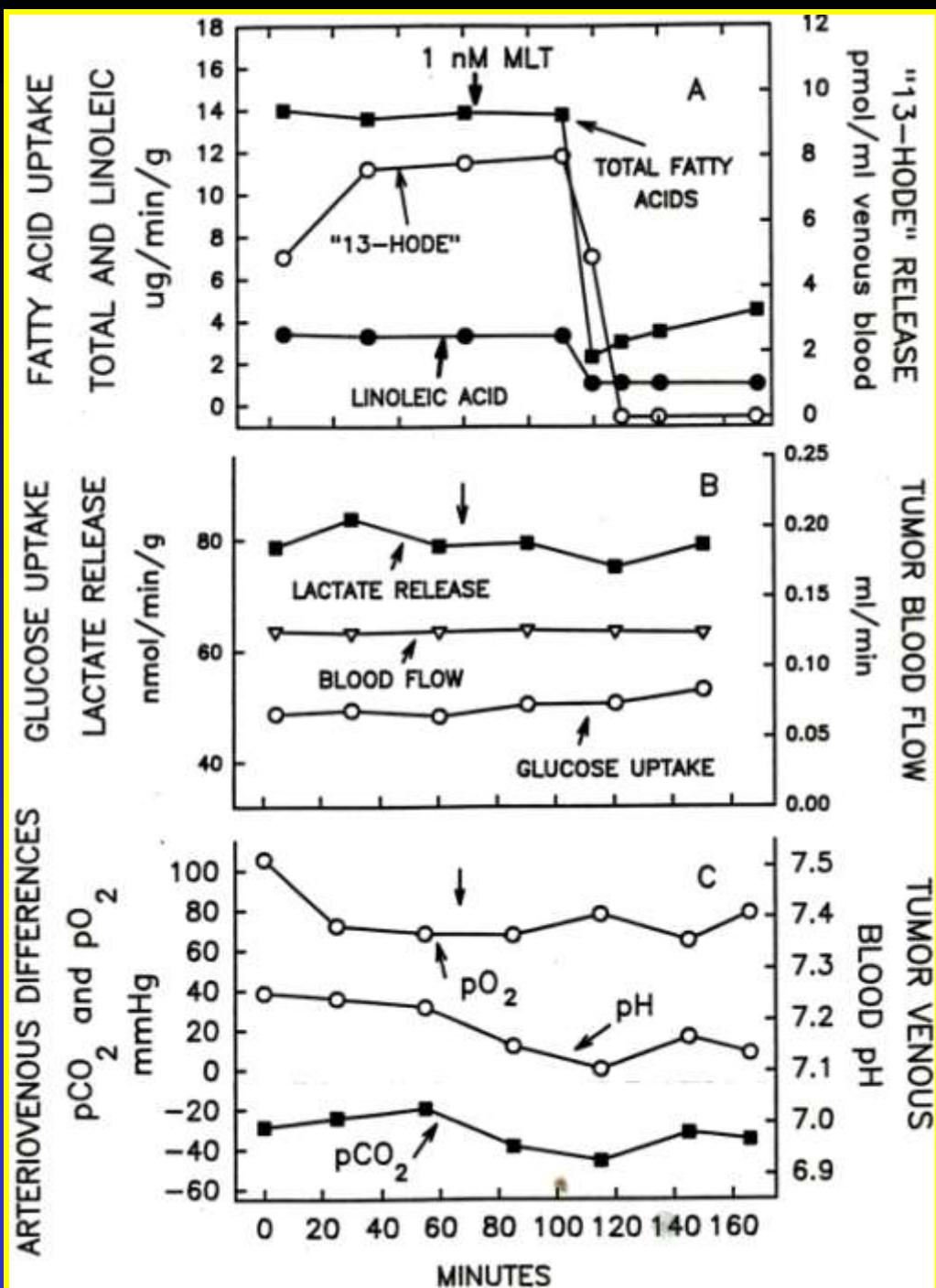
COOH



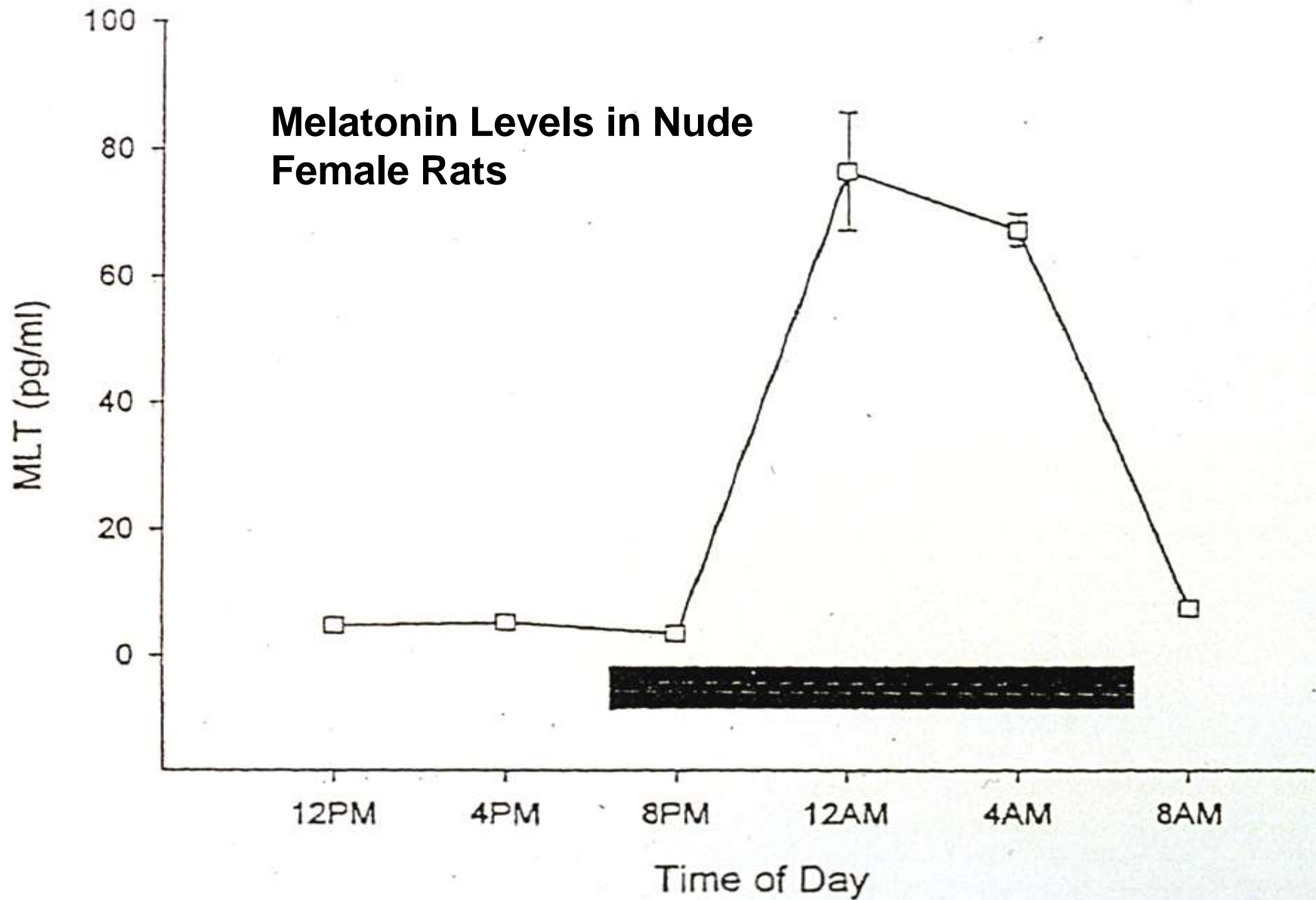
Linolenic acid



**EFFECT OF PHYSIOLOGICAL
MELATONIN ON KINETICS OF
FATTY ACID UPTAKE AND
METABOLISM IN TISSUE-
ISOLATED RAT HEPATOMA
7288CTC PERFUSED *IN SITU***



Melatonin Levels in Nude Female Rats



**DOSE-RESPONSE
EFFECTS OF
MELATONIN ON TUMOR
FATTY ACID
UPTAKE/METABOLISM
AND PROLIFERATIVE
ACTIVITY**

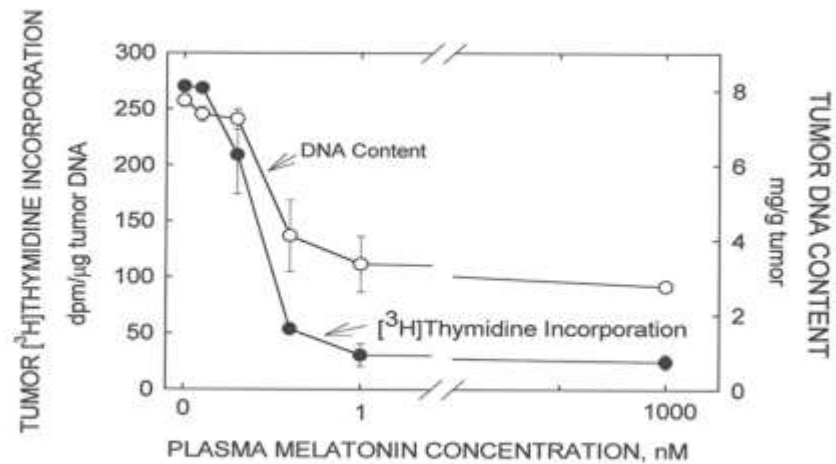
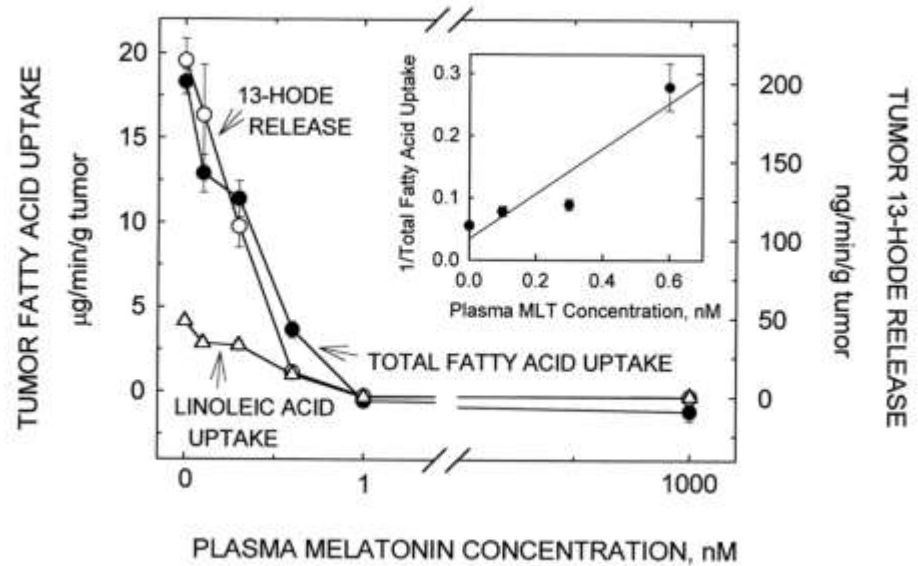
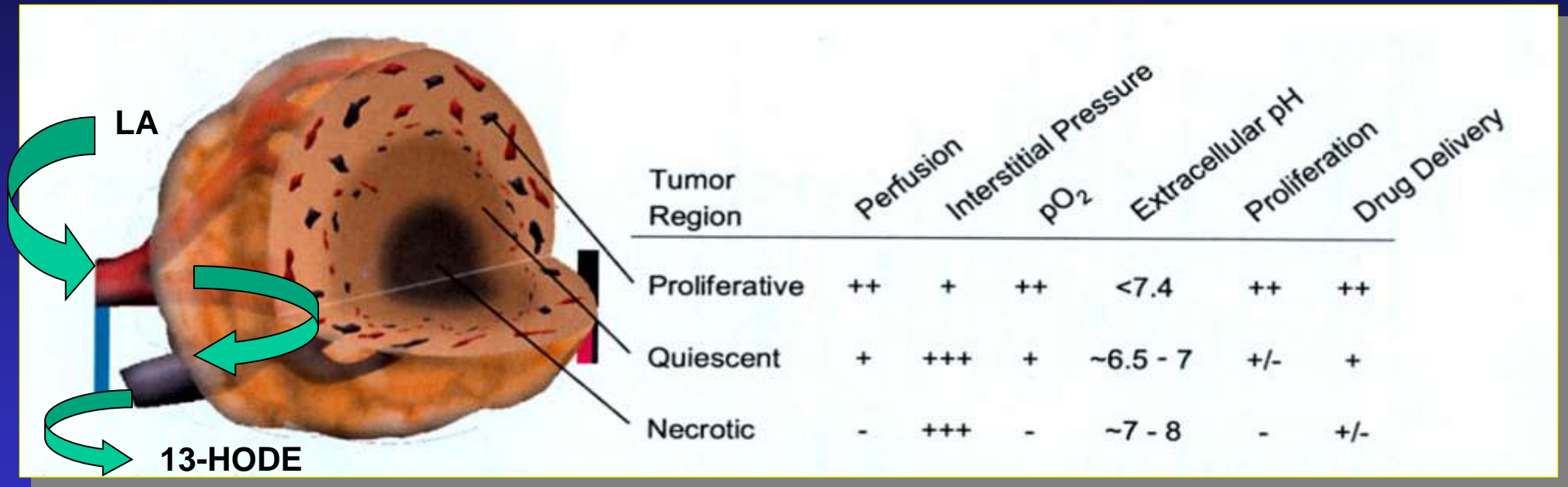
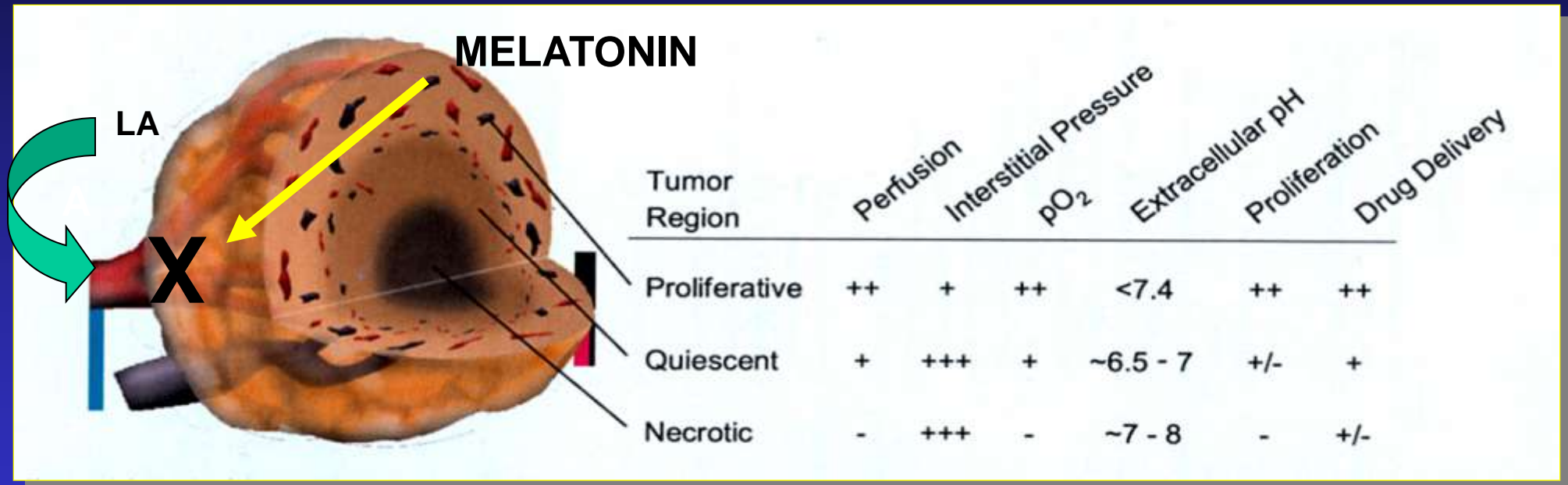


Figure 4 Upper and Lower

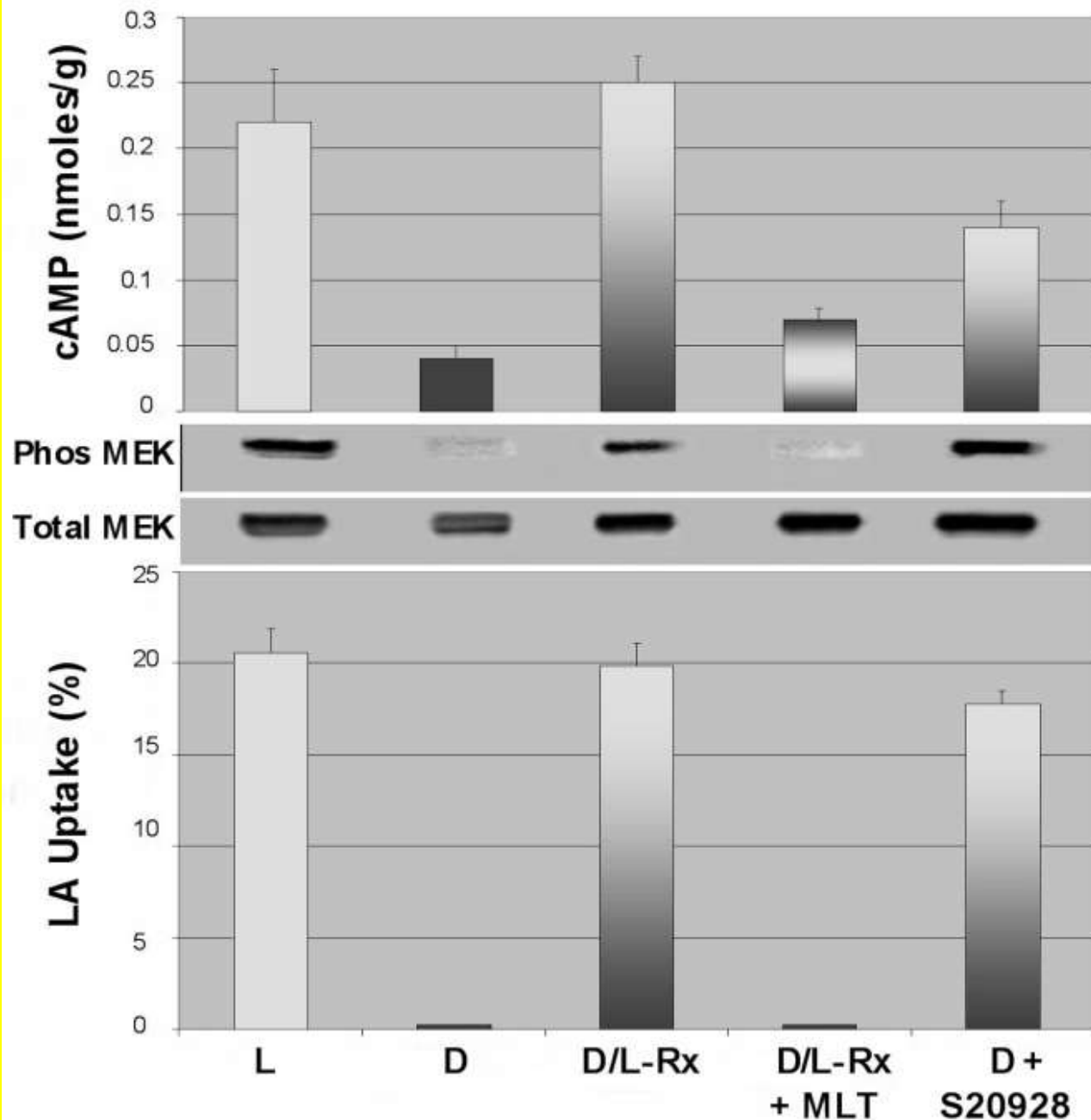
LA UPTAKE/UTILIZATION AND 13-HODE PRODUCTION/RELEASE BY TISSUE-ISOLATED TUMORS



LA UPTAKE/UTILIZATION AND 13-HODE PRODUCTION/RELEASE BY TISSUE-ISOLATED TUMORS



**SIGNAL TRANSDUCTION
AND PROLIFERATIVE
ACTIVITY IN MCF-7 (SR-)
HUMAN BREAST
CANCER XENOGRAPHS
PERFUSED *IN SITU* WITH
BLOOD COLLECTED
FROM HUMAN
VOLUNTEERS (n = 4)**



Total tumors perfused/lighting condition

12

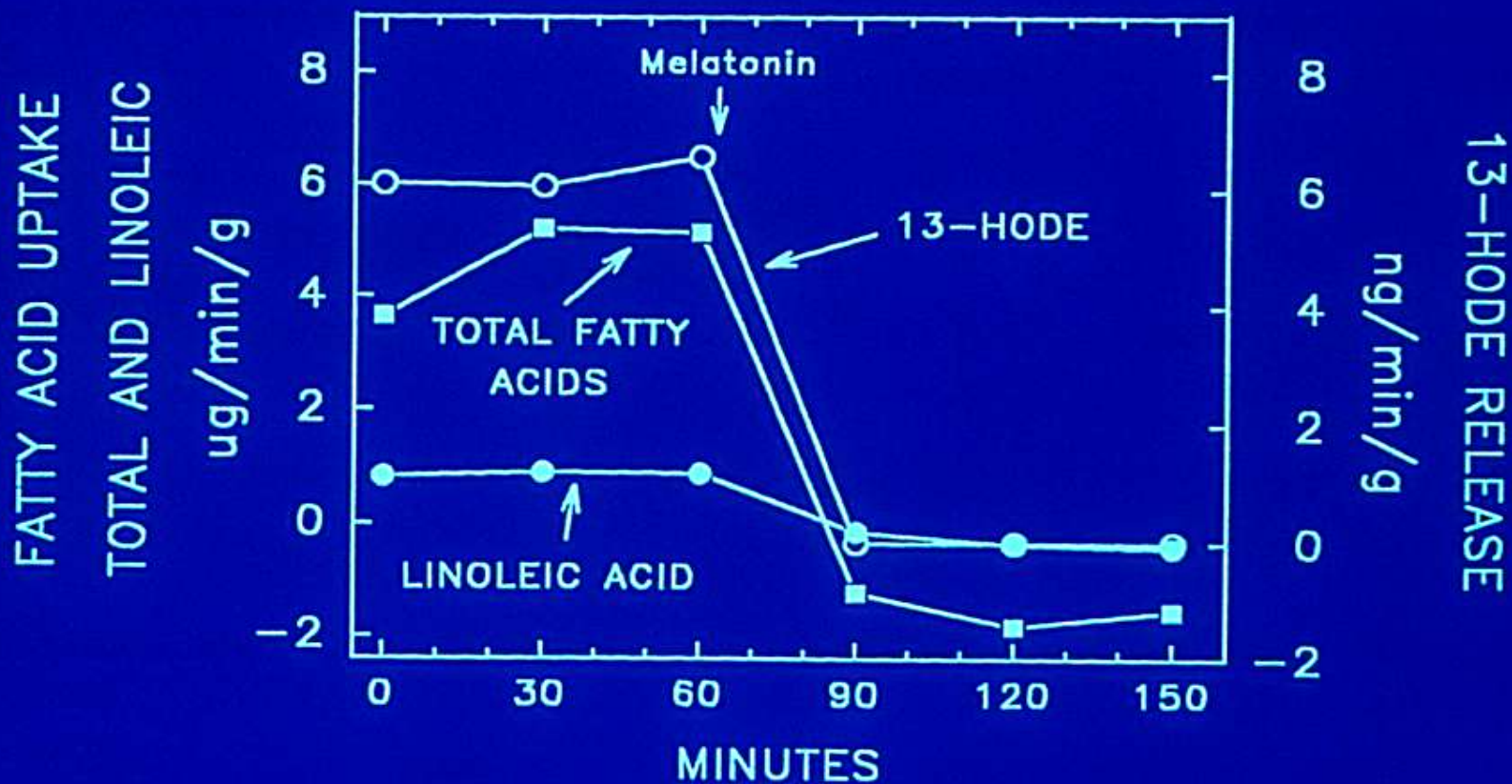
12

12

3

3

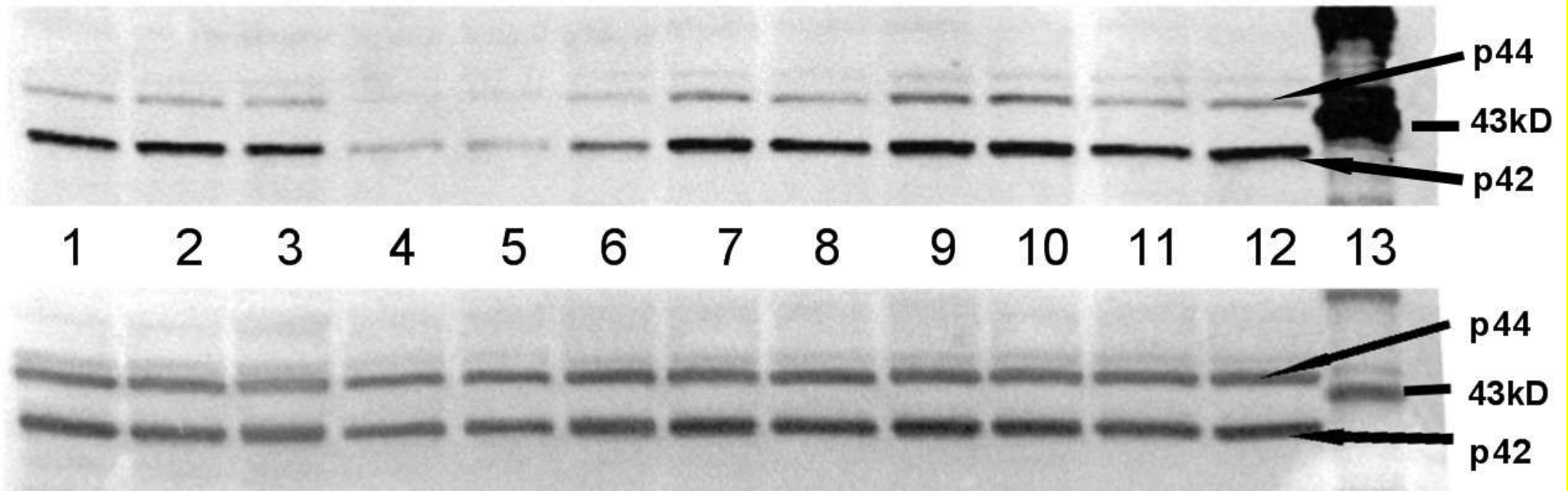
EFFECT OF MELATONIN ON FATTY ACID UPTAKE AND 13-HODE RELEASE
IN A MCF-7 HUMAN MAMMARY CANCER XENOGRAFT PERFUSED IN SITU



Perfusion of Tissue-Isolated MCF-7 Human Breast Cancer (SR-) Xenografts with Melatonin

Treatment	DNA Content (mg/g)	[³ H] Thymidine Incorp. (dpms/ug DNA)	Total FA Uptake (% Supply)	LA Uptake (% Supply)	13-HODE Production (ng/min/g)	cAMP (nmoles/g)
Controls	2.79 ± 0.63	47.4 ± 3.9	15.5 ± 2.2	16.7 ± 1.7	0.97 ± 0.17	0.55 ± 0.11
Melatonin (1 nM)	1.85 ± 0.10*	13.6 ± 1.6*	0	0	0	0.33 ± 0.12*
Melatonin + 13-HODE	4.32 ± 0.33	74.8 ± 6.3	0	0	333.17 ± 19.02	0.68 ± 0.06
13-HODE	3.96 ± 0.21	78.0 ± 6.5	17.2 ± 3.2	16.2 ± 2.5	363.18 ± 10.62	0.78 ± 0.17

ERK1/2 (MAPK p44/p42) in MCF-7 (SR-) Human Breast Cancer Xenografts



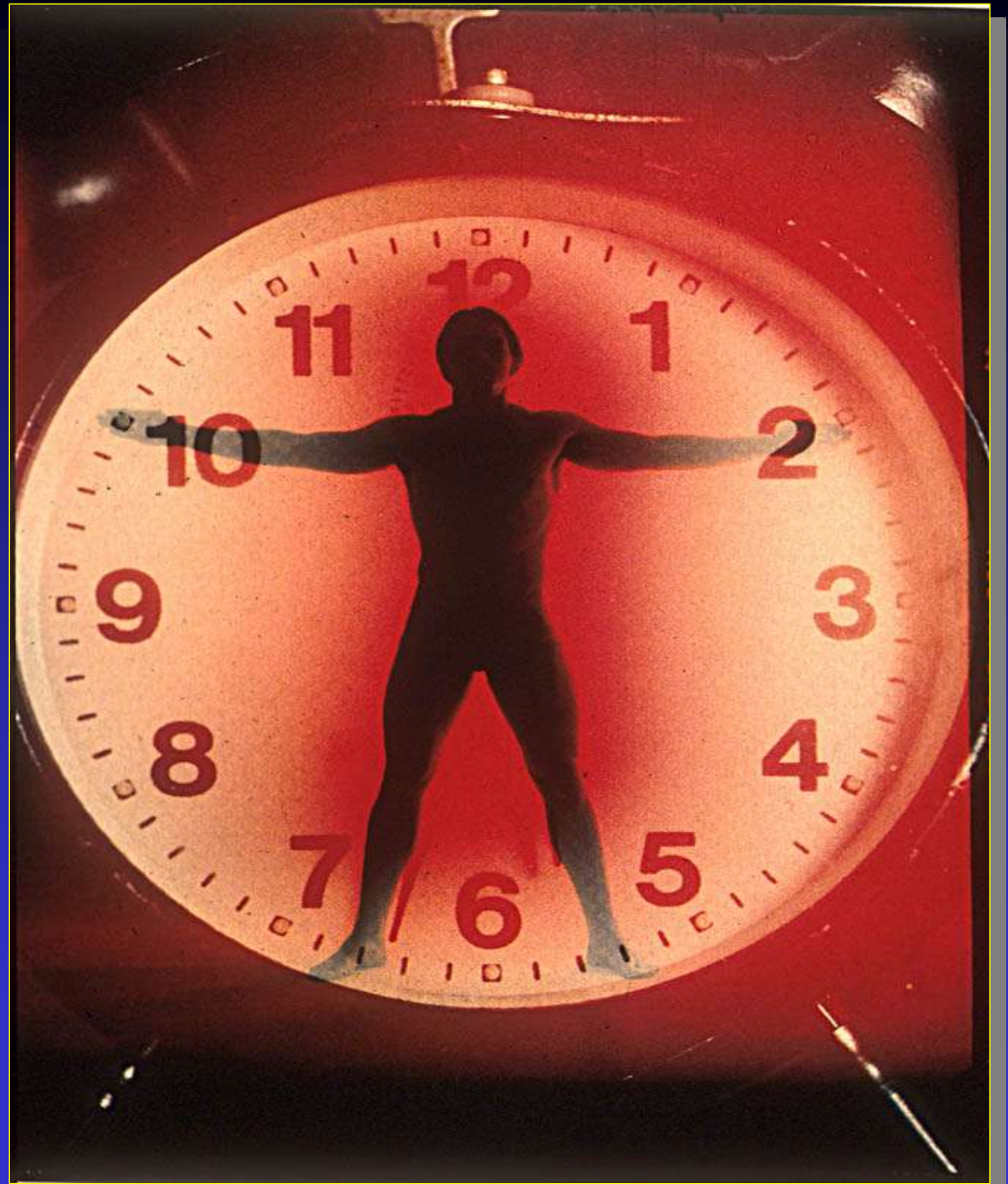
CONTROL

MLT

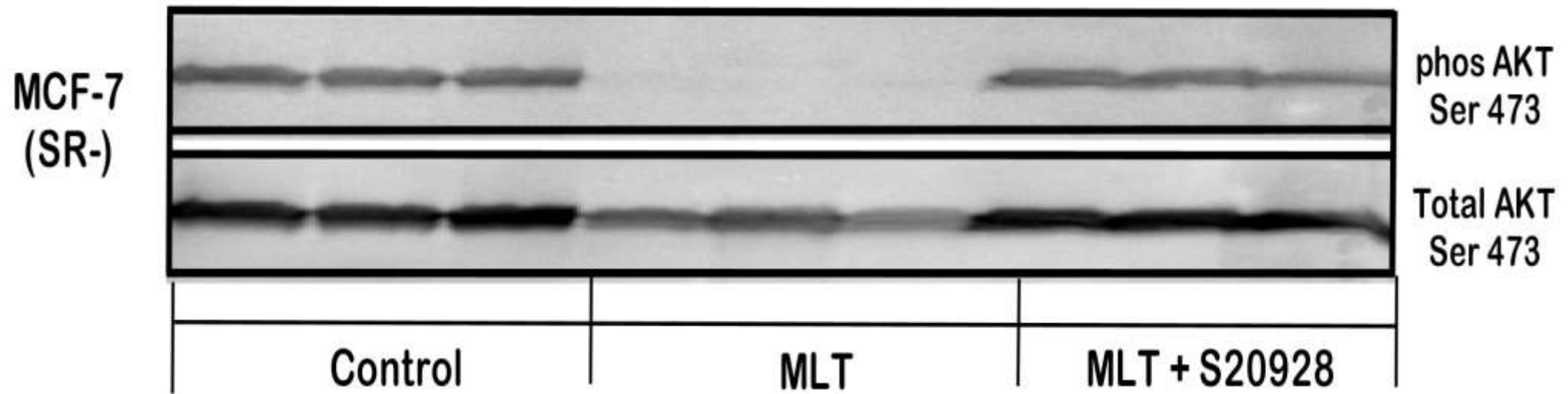
MLT/13-HODE

13-HODE

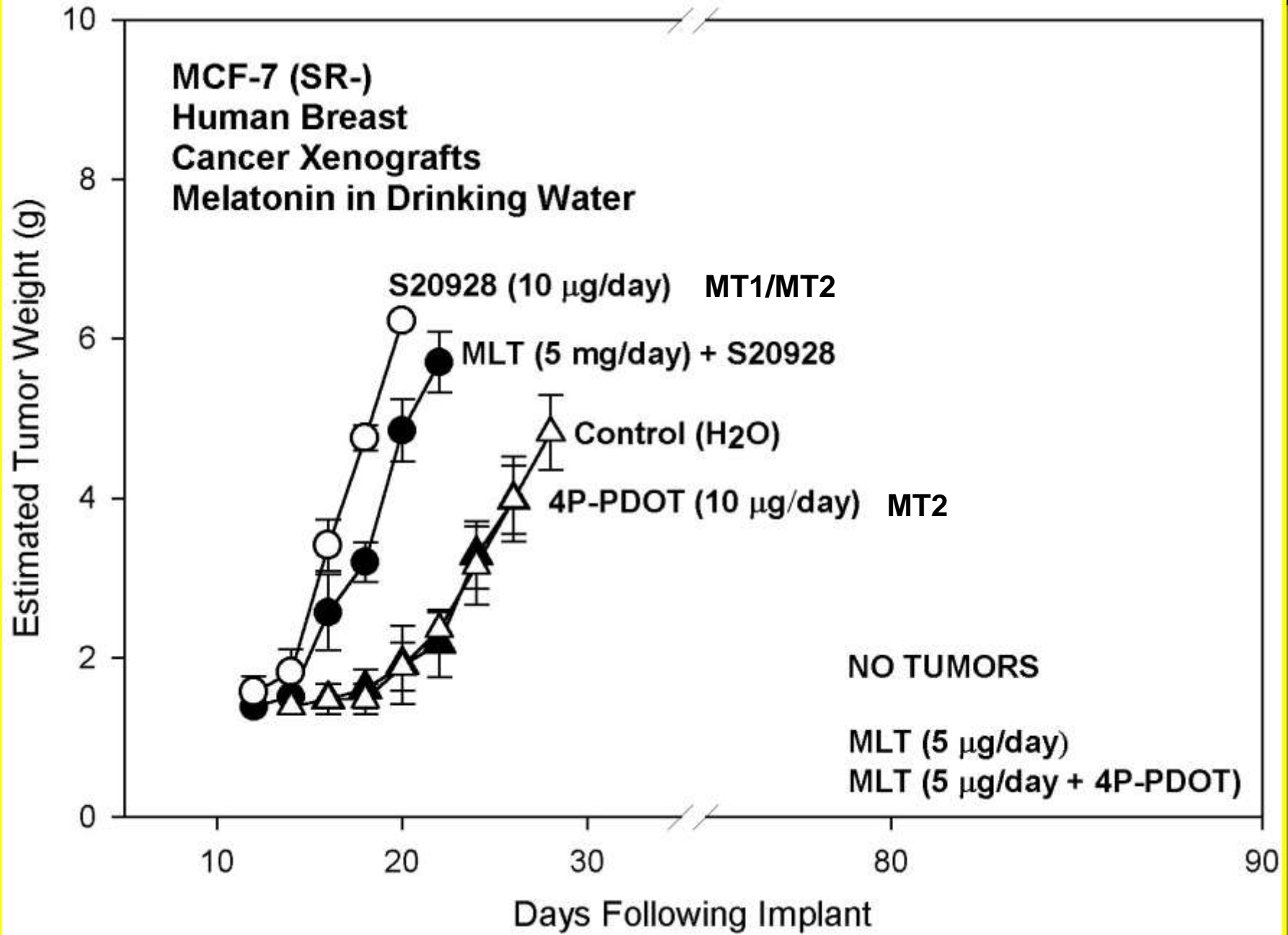
**Timing is
Everything**



Effects of Melatonin (1 nM) on Akt Activation in Tissue-Isolated (SR-) MCF-7 Human Breast Cancer Xenografts Perfused *In Situ*



**MCF-7 (SR-)
Human Breast
Cancer Xenografts
Melatonin in Drinking Water**



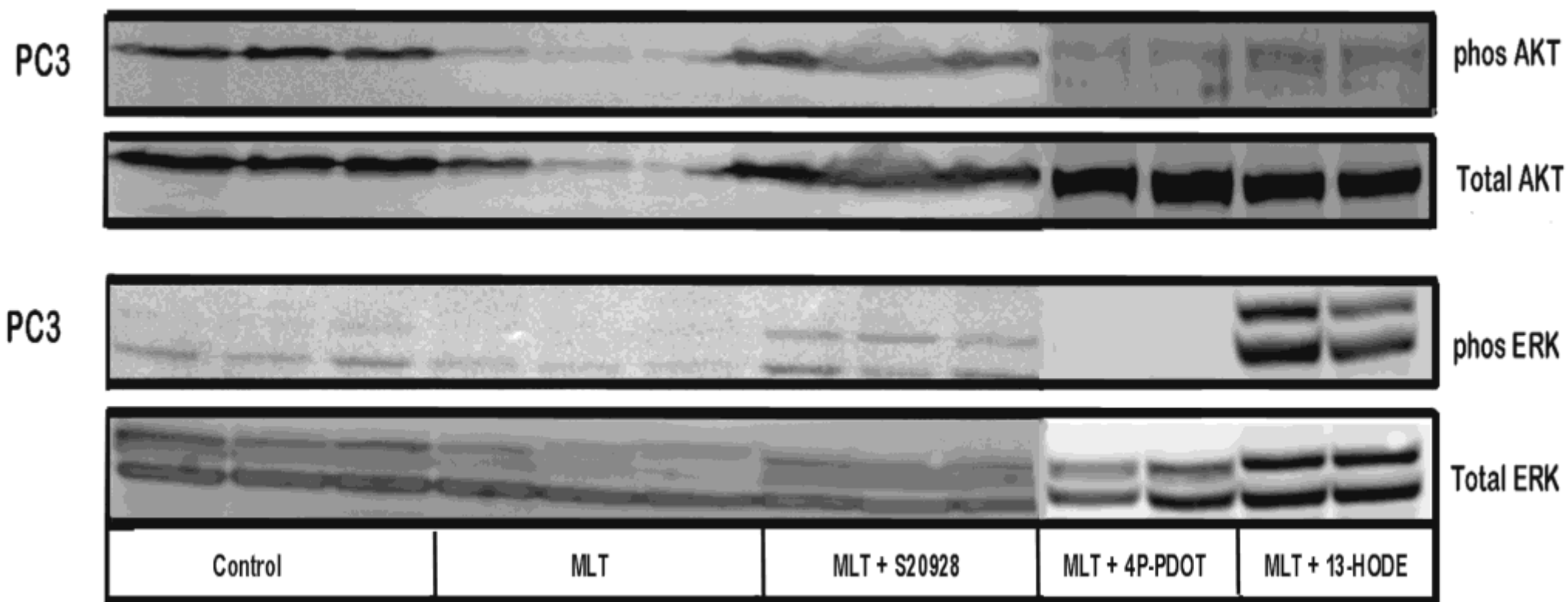
Effects of melatonin and melatonin receptor antagonist supplementation in the drinking water on signal transduction activity, LAuptake/metabolism and proliferative activity in (SR-) human breast cancer xenografts

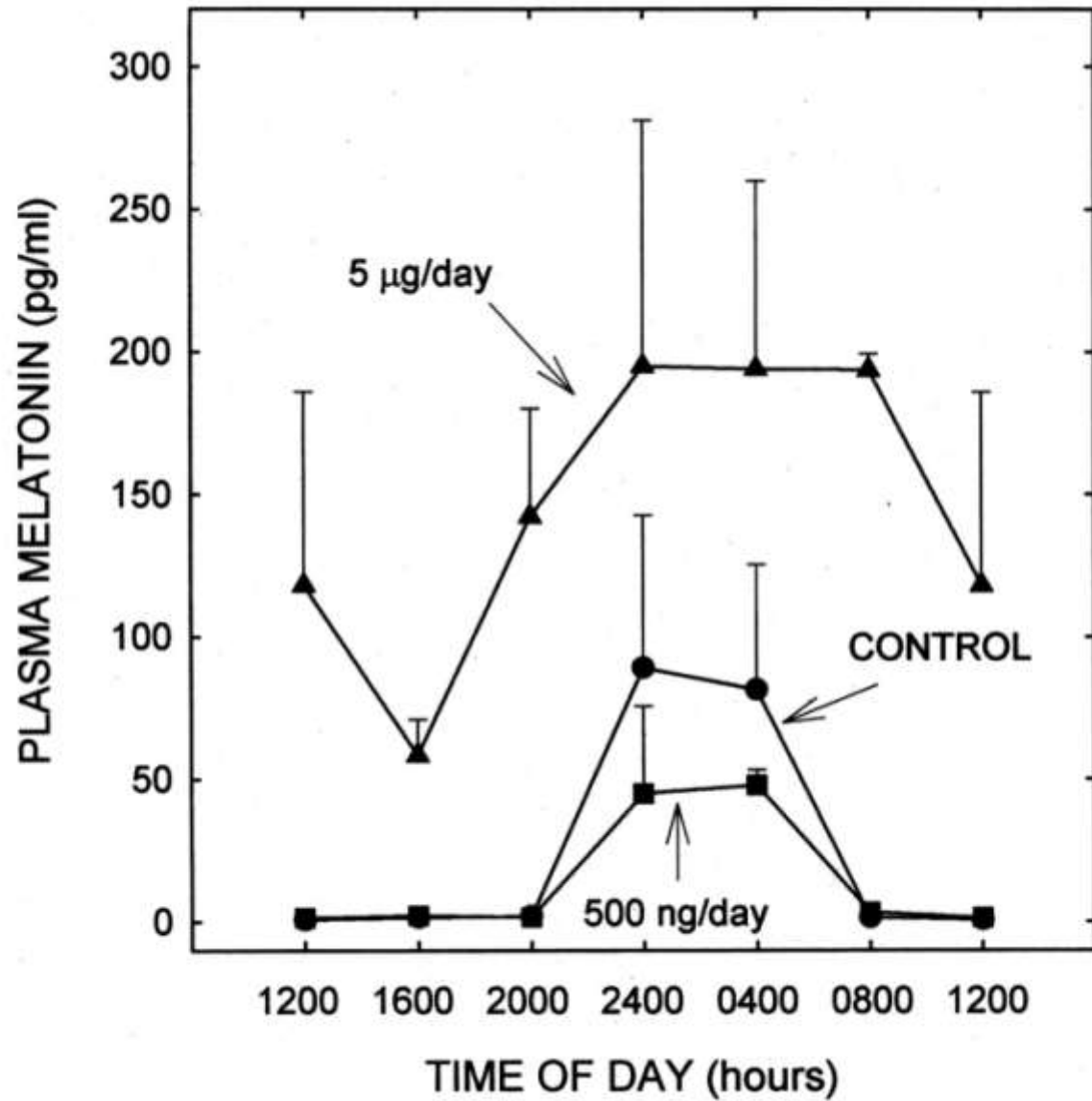
Treatment (n= 6)	DNA Content (mg/g)	[³H] Thymidine Incorporation (dpms/μg DNA)	LA Uptake (% of supply)	13-HODE Production (ng/min/g)	CAMP (nmoles/g)
Controls	3.16 \pm 0.25	37.8 \pm 3.5	21.4 \pm 4.4	1.54 \pm 0.47	0.173 \pm 0.05
S20928	4.34 \pm 0.15*	72.6 \pm 5.4*	30.3 \pm 6.9*	4.45 \pm 1.05*	0.521 \pm 0.15*
Melatonin	----	----	----	----	----
Melatonin + S20928	4.29 \pm 0.20*	73.7 \pm 1.8*	28.2 \pm 7.6*	3.38 \pm 0.48*	0.758 \pm 0.29*
Melatonin + 4P-PDOT	----	----	----	----	----
4P-PDOT	3.10 \pm 0.22	37.8 \pm 4.9	19.4 \pm 5.2	1.48 \pm 0.17	0.223 \pm 0.06

Effects of Melatonin on Proliferative Activity and LA Metabolism in Tissue-Isolated PC3 Human Prostate Cancer Xenografts Perfused In Situ

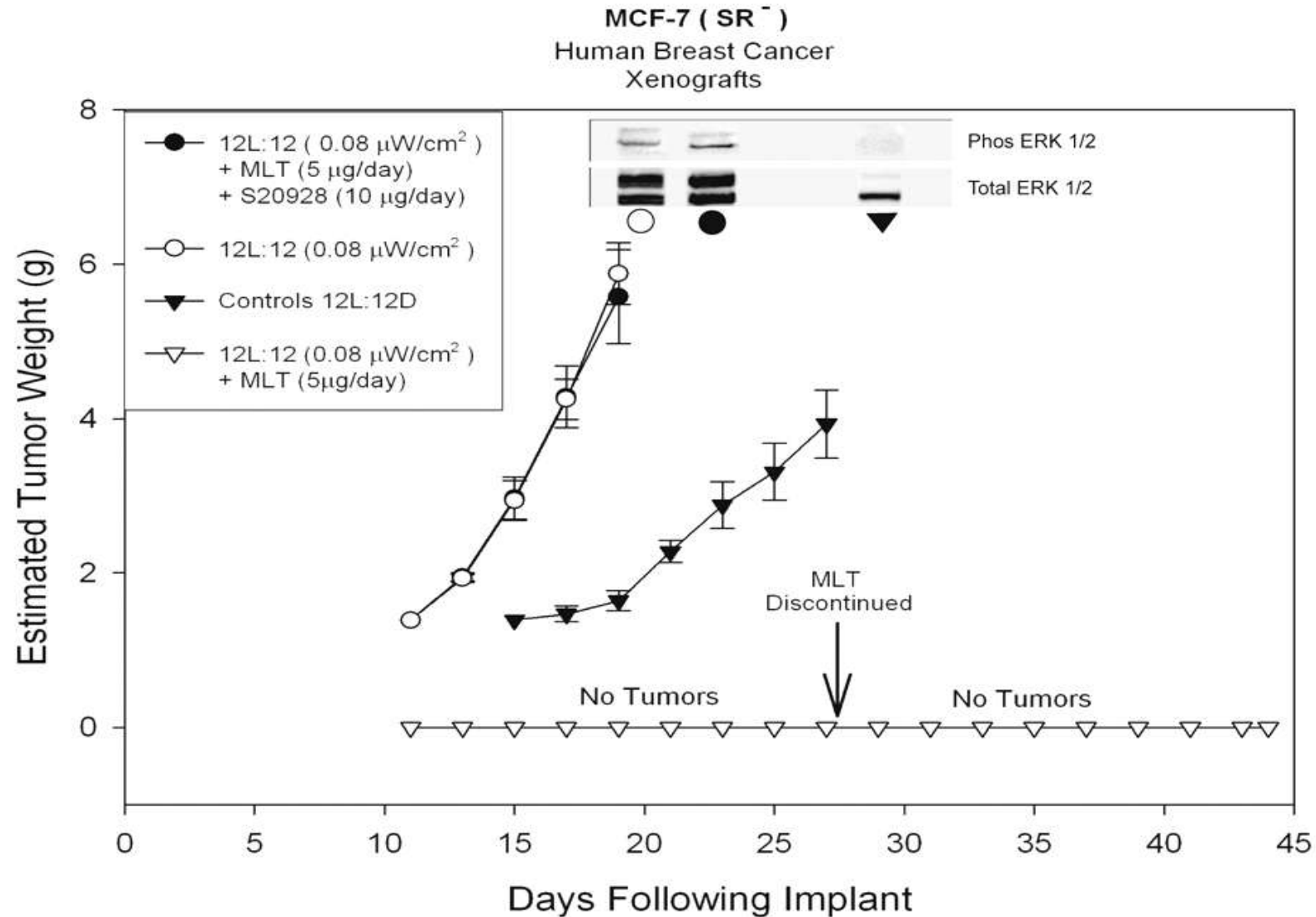
Treatment (n=3) tumor)	[³ H]-Thymidine Incorporation (dpms/μg DNA)	LA Uptake Production (% of Supply)	13-HODE (ng/min/g)	cAMP (nmol/g)
Controls	37.6 ± 0.7	26.9 ± 4.6	23.4 ± 2.6	1.66 ± 0.44
Melatonin (1 nM)	5.1 ± 0.2*	0	0	0.434 ± 0.05*
Melatonin + S20928	37.5 ± 5.1***	23.0 ± 7.6***	25.72 ± 6.5***	2.43 ± 0.47***
Melatonin + 4P-PDOT	7.8 ± 0.5*	0	0	0.583 ± 0.14*
Melatonin + 13-HODE	59.1 ± 5.7**	0	289.8 ± 26.5	0.684 ± 0.07*

Effects of Melatonin (1 nM) on Akt and ERK1/2 Activation in Tissue-Isolated PC3 Human Prostate Cancer Xenografts Perfused *In Situ*



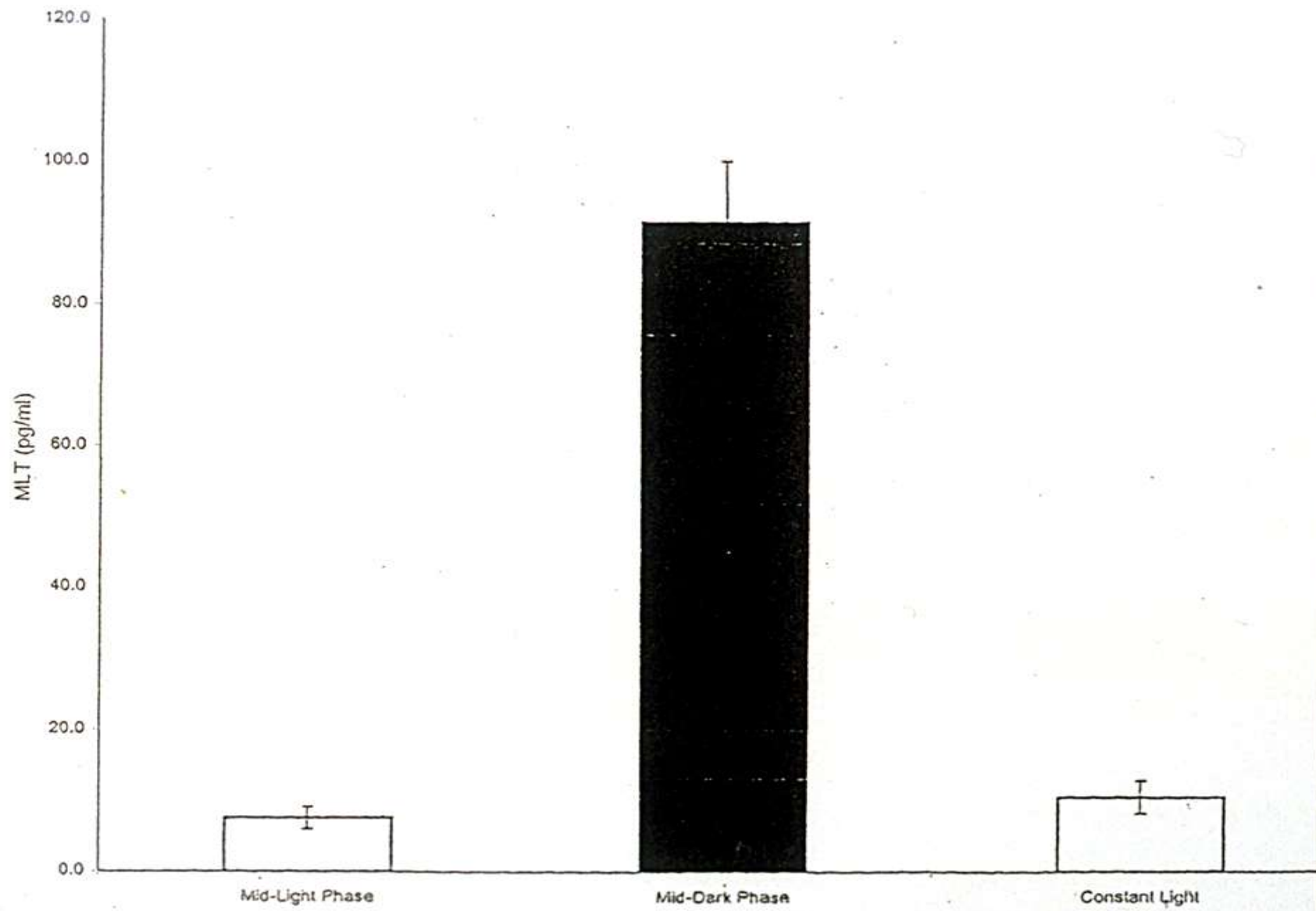


EFFECTS OF CIRCADIAN-BASED MELATONIN SUPPLEMENTATION ON GROWTH AND ERK1/2 ACTIVATION IN TISSUE-ISOLATED MCF-7 (SR-) HUMAN BREAST CANCER XENOGRAPHS DURING DIM LIGHT DURING DARKNESS

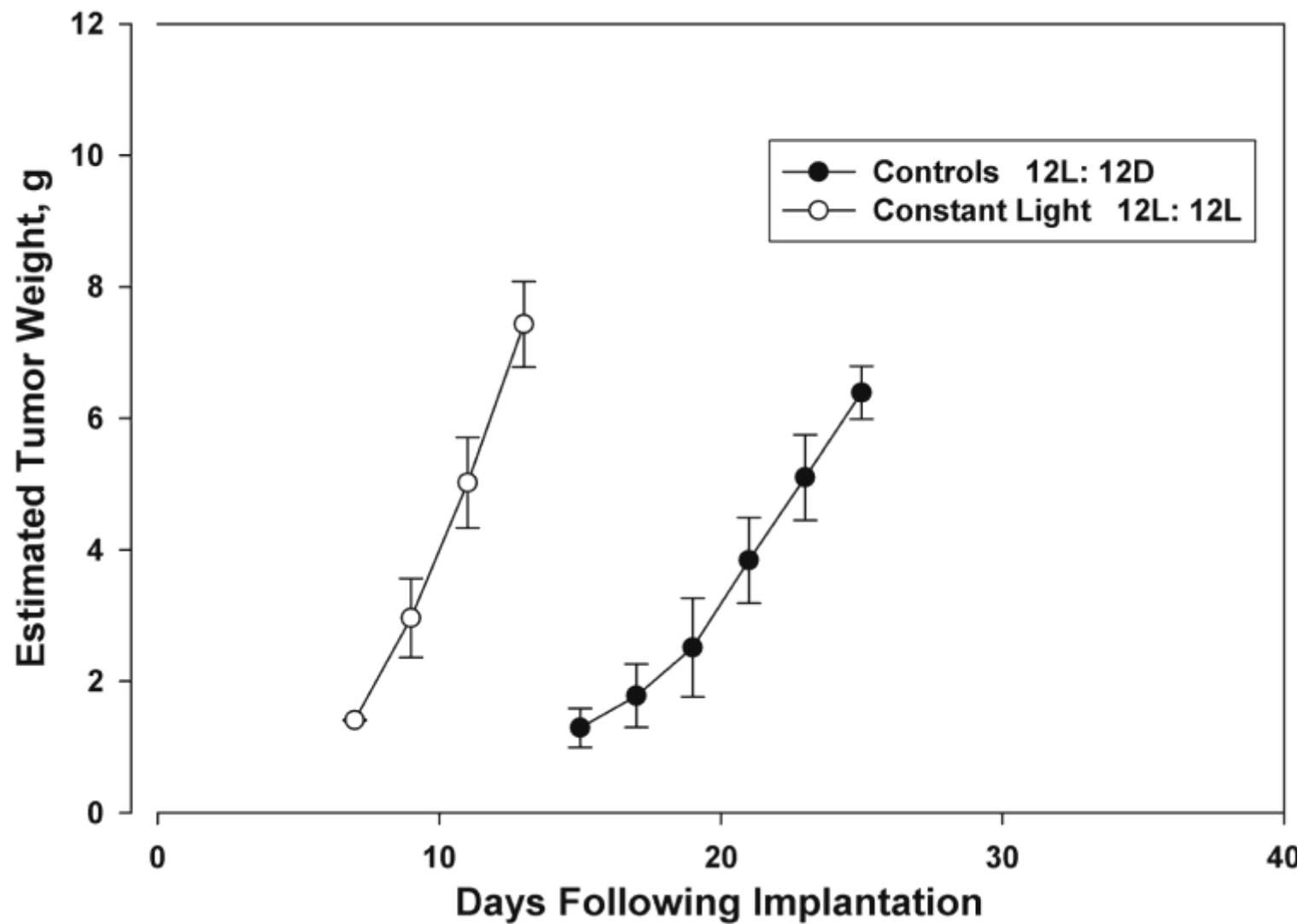


Effect of Circadian-Based Melatonin Supplementation on Signal Transduction, LA Metabolic and Proliferative Activities During Dim Light During Darkness (DLDD) in Tissue-Isolated SR- Human Breast Cancer Xenografts

Treatment (n=8)	DNA Content (mg/g)	[³ H]-Thymidine Incorporation (dpm/μg DNA)	LA Uptake (% of supply)	13-HODE Production (ng/min/g)	cAMP (nmoles/g)
Controls (12L:12D)	2.16 ± 0.11	13.7 ± 1.0	0	0	0
DLDD (0.08 μW/cm²)	4.36 ± 0.01	73.6 ± 1.1	23.5 ± 2.4	4.73 ± 0.35	0.42 ± 0.04
DLDD + Melatonin	---	---	---	---	---
DLDD + Melatonin + S20928	4.33 ± 0.14	74.6 ± 2.2	20.5 ± 0.32	4.7 ± 0.32	0.39 ± 0.02



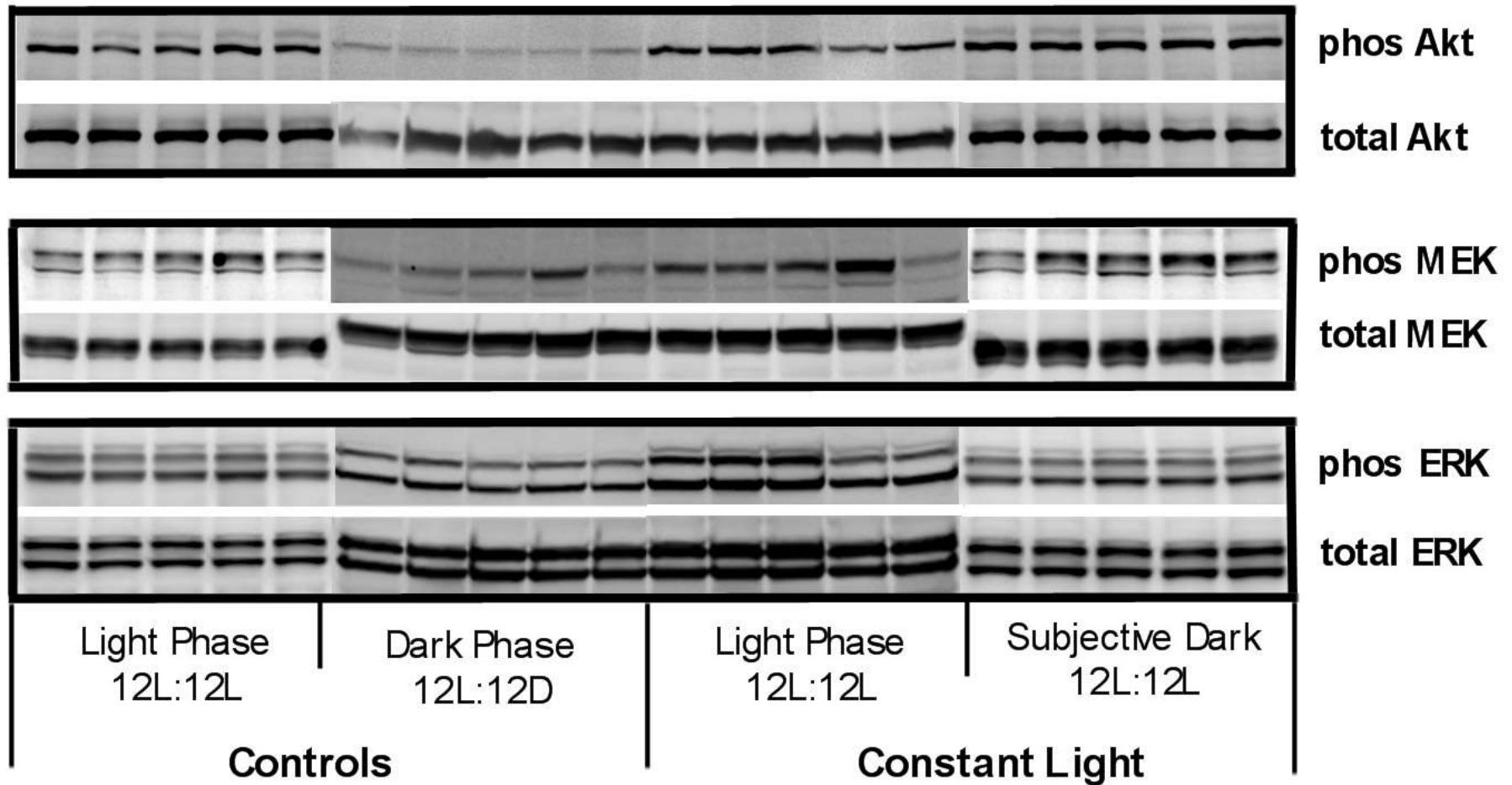
PC3 Human Prostate Xenograft

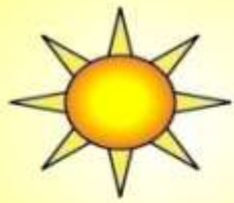


Effects of Bright Fluorescent Light at Night on Proliferative Activity and LA Metabolism in Tissue-Isolated PC3 Human Prostate Cancer Xenografts in Male Nude Rats

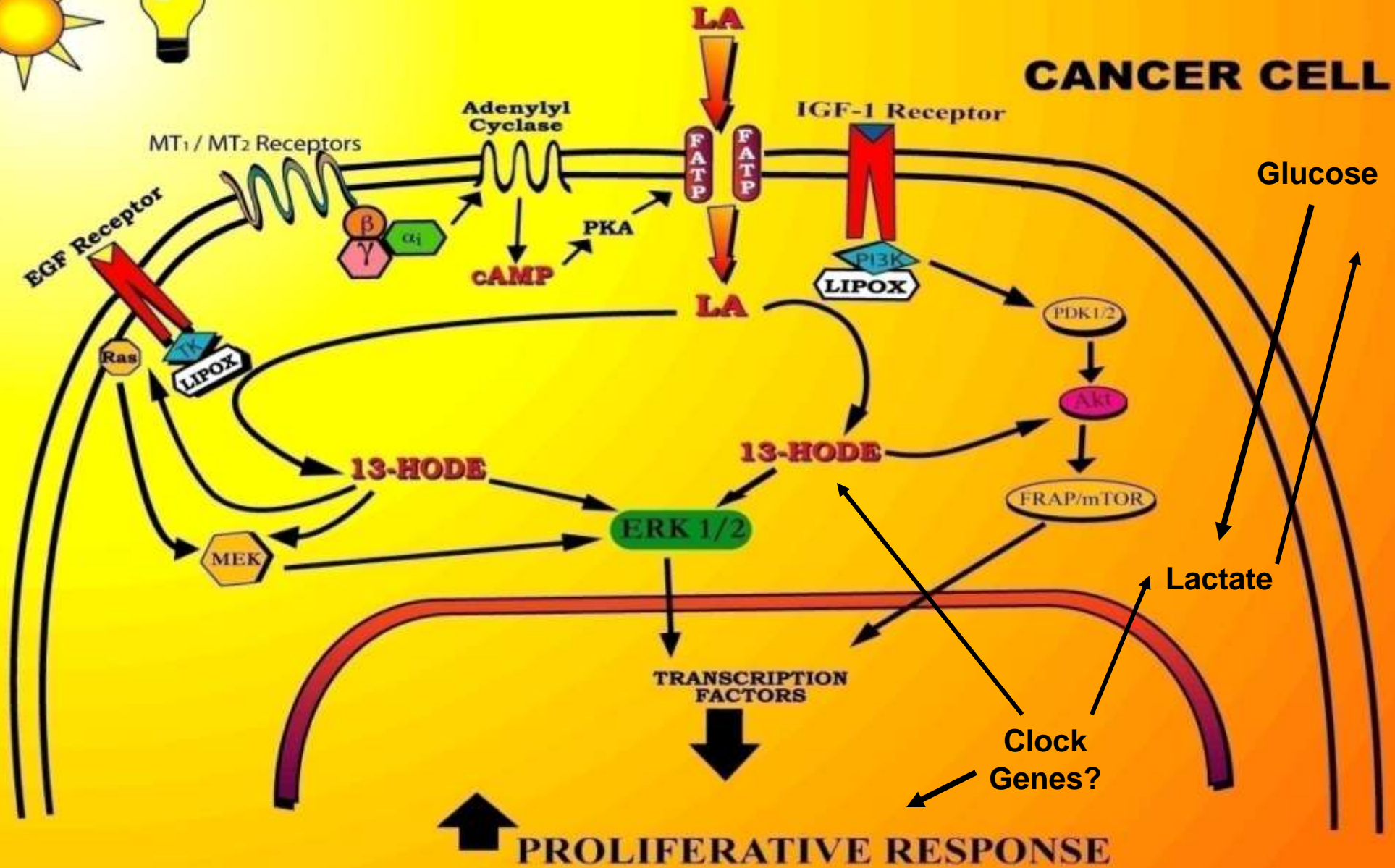
Treatment ^a	DNA Content (mg/g)	[³ H]Thymidine Incorporation (dpms/ μ gDNA)	LA Uptake (% Supply)	13-HODE Production (ng/min/g)	cAMP (nmoles/g)
Controls (Light Phase) 12L:12L	6.06 \pm 0.76	34.2 \pm 1.5	29.5 \pm 1.7	19.9 \pm 1.7	0.714 \pm 0.076
Controls (Dark Phase) 12L:12D)	2.53 \pm 0.12*	8.6 \pm 4.4*	0	0	0.286 \pm 0.024*
Constant Light (Light Phase) 12L:12L)	6.29 \pm 0.19	245.3 \pm 15.9	32.6 \pm 6.2	205.3 \pm 12.9	0.864 \pm 0.193
Constant Light (Subjective Dark) 12L:12L	6.57 \pm 0.13	245.6 \pm 4.7	31.0 \pm 4.7	210.9 \pm 6.3	1.074 \pm 0.274

Effects of Constant Bright Light on Akt, MEK and ERK1/2 Activation in Tissue-Isolated PC3 Human Prostate Cancer Xenografts

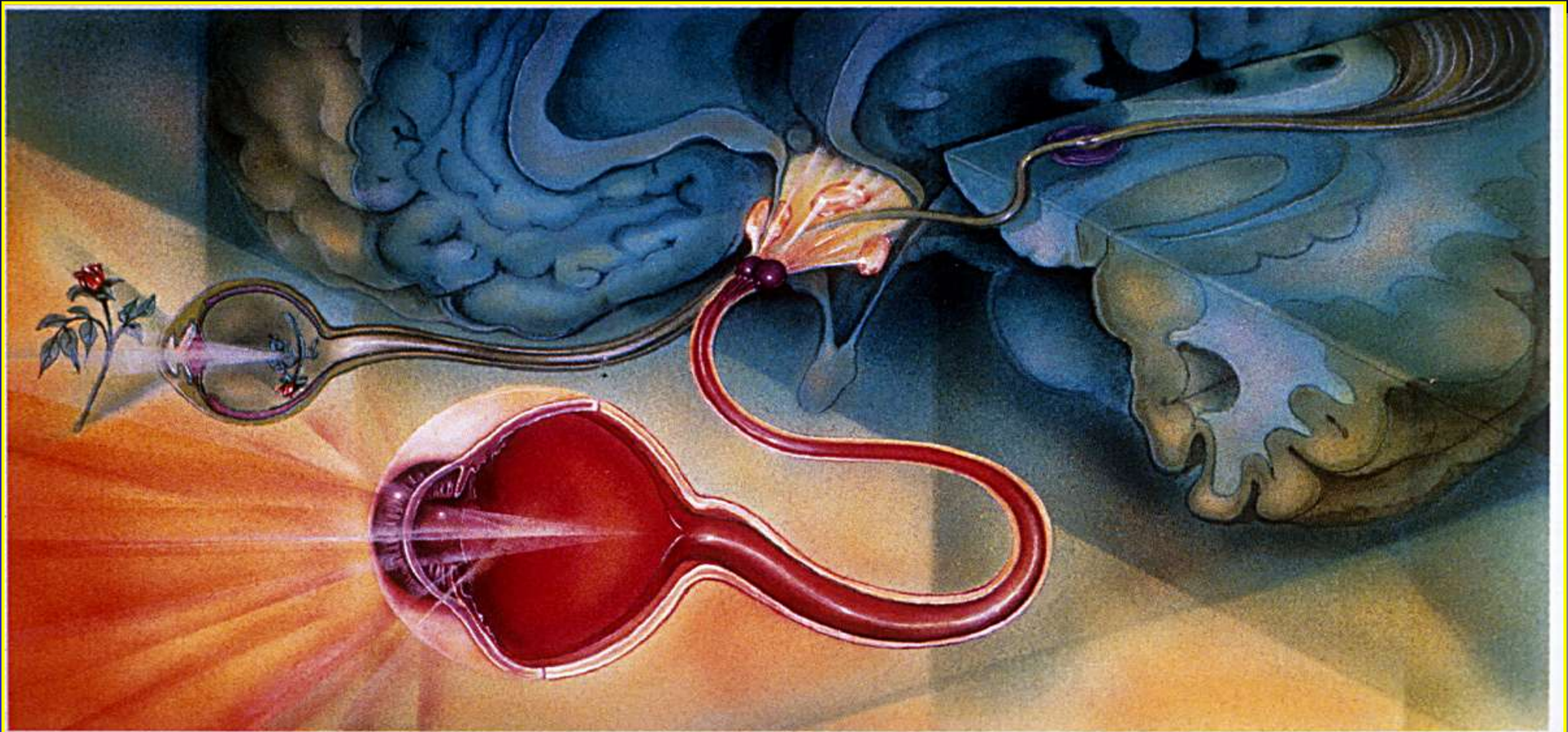




CANCER CELL

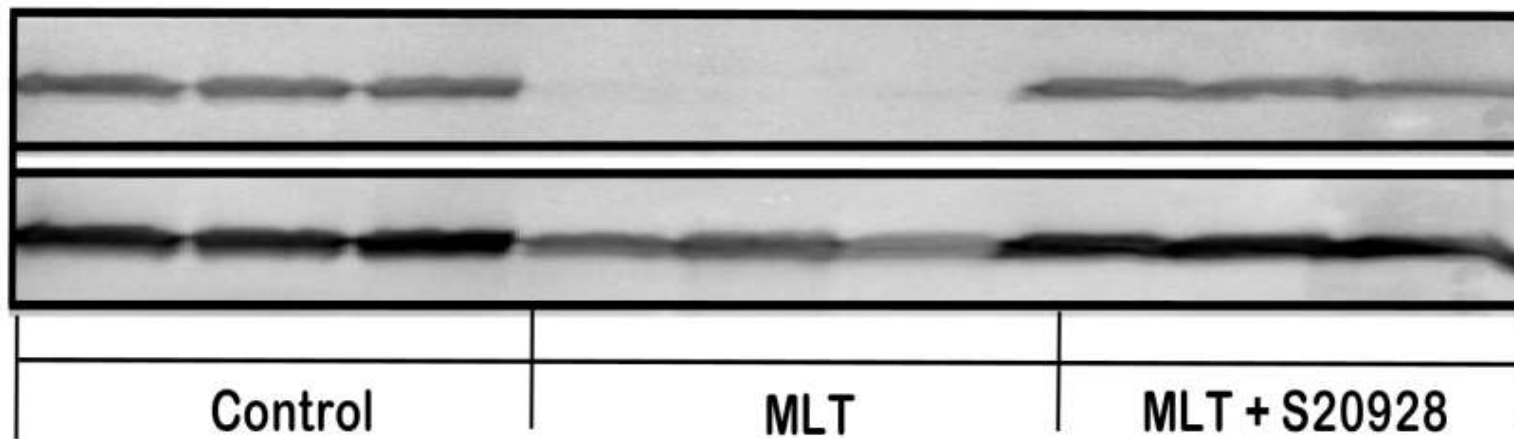






ROSEMARIE D'ALBA

MCF-7
(SR-)



phos AKT
Ser 473

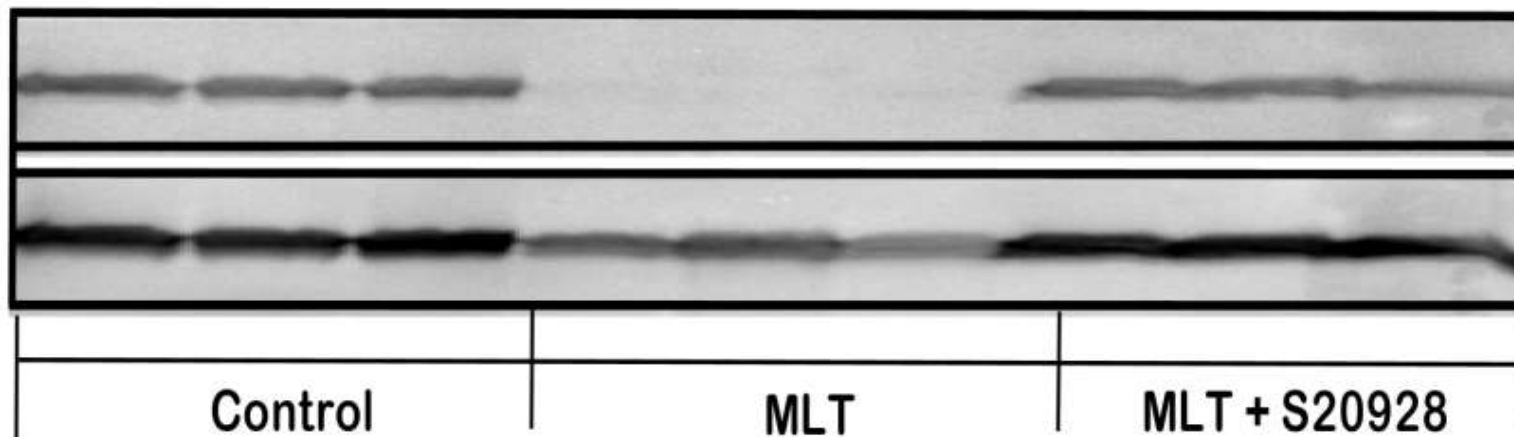
Total AKT
Ser 473

Control

MLT

MLT + S20928

MCF-7
(ER-)



phos AKT
Ser 473

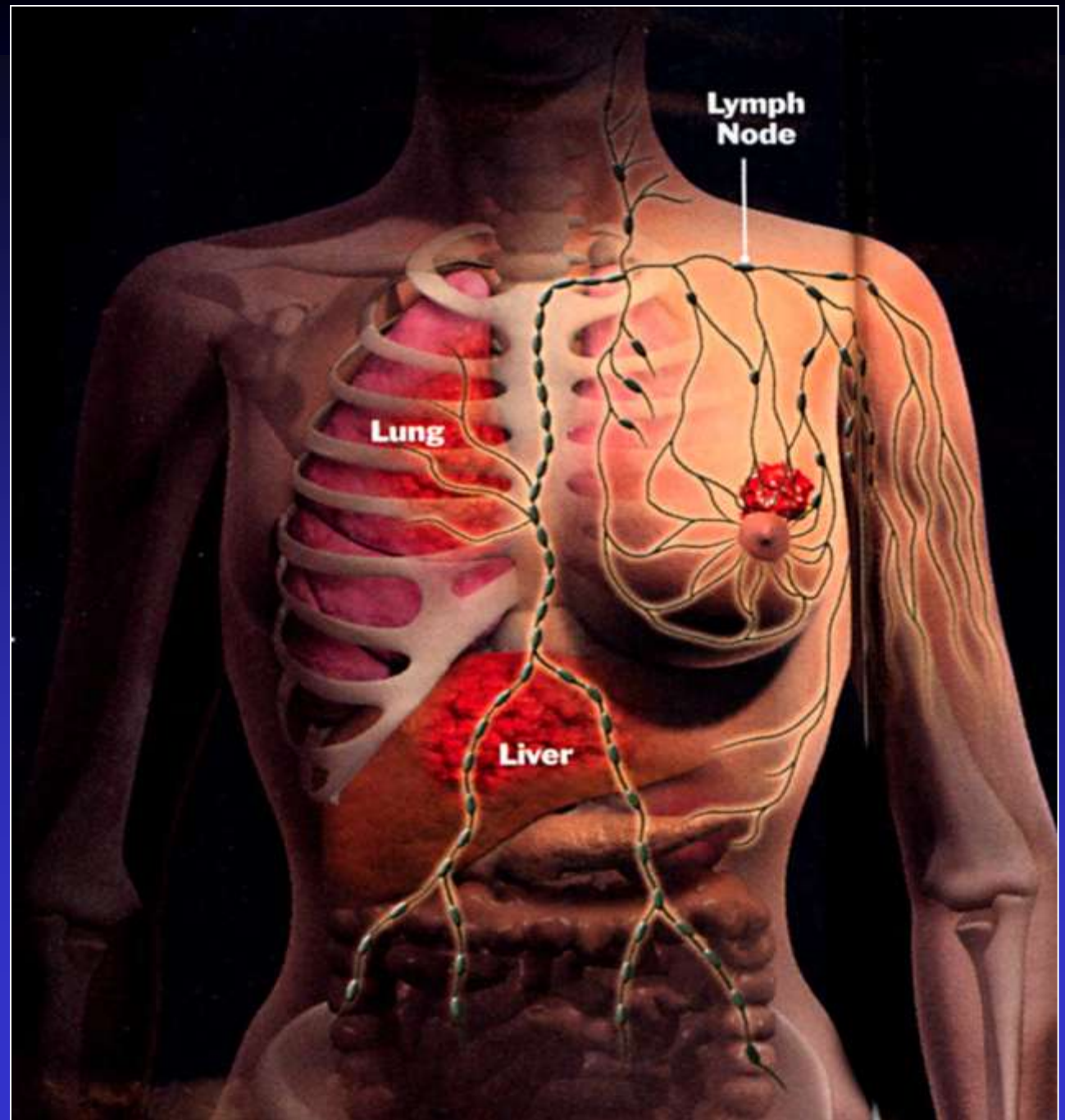
Total AKT
Ser 473

Control

MLT

MLT + S20928

**ENDOGENOUS
CIRCADIAN
MELATONIN AND
LINOLEIC ACID
SIGNALS IN
HUMAN BREAST
CANCER GROWTH
AND METABOLISM
- EFFECTS OF DIM
LIGHT AT NIGHT**

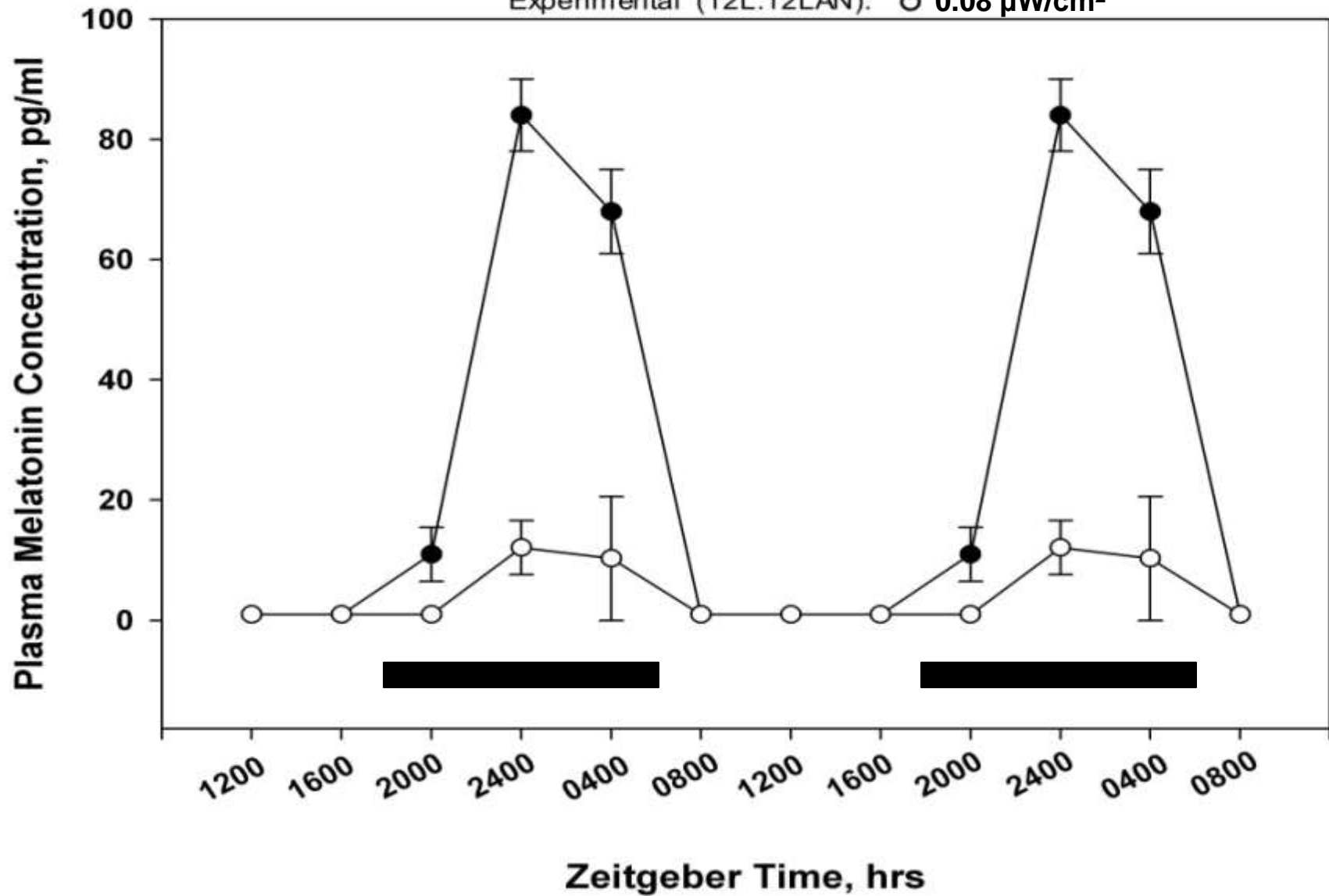


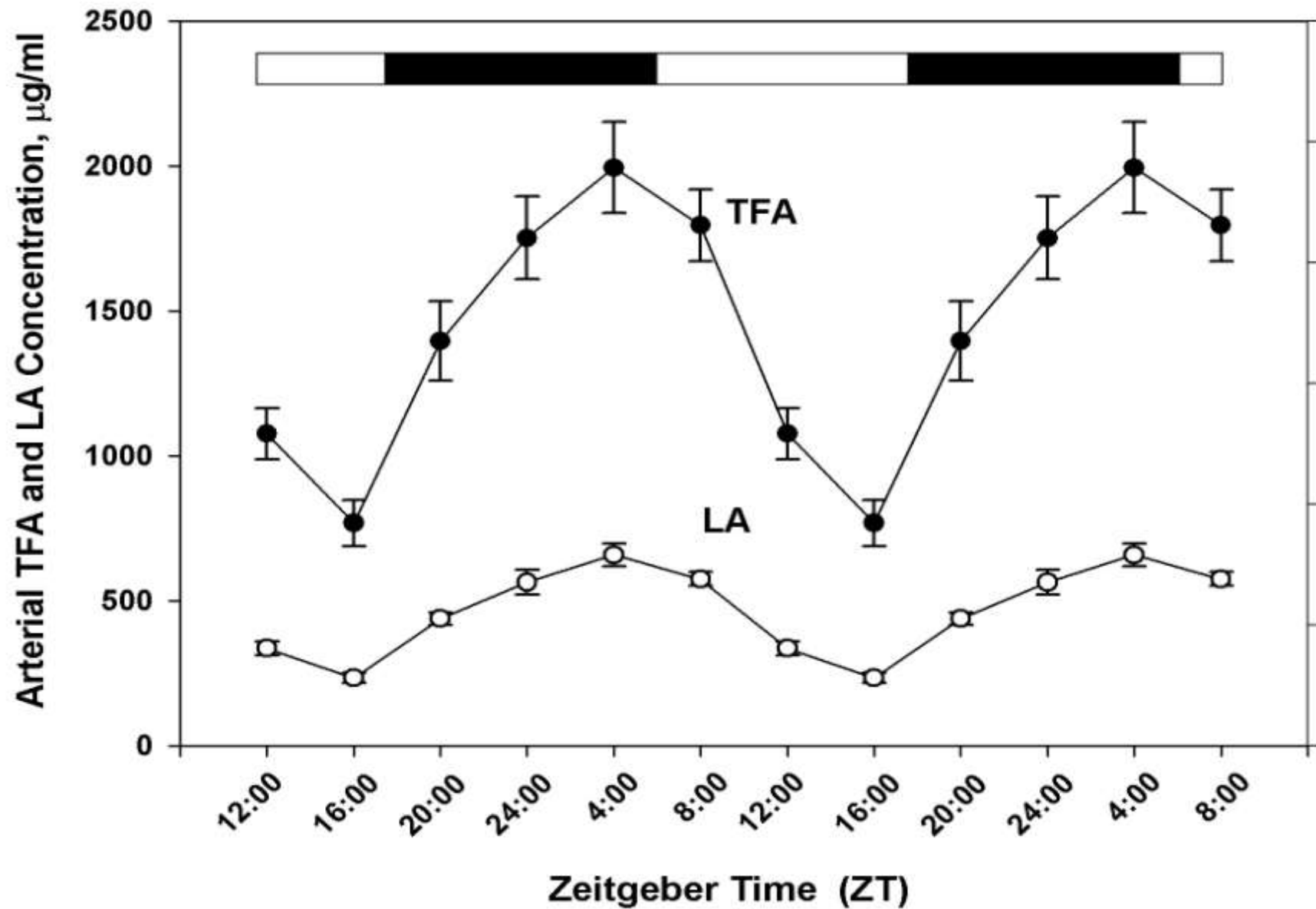
GLAS Investigation

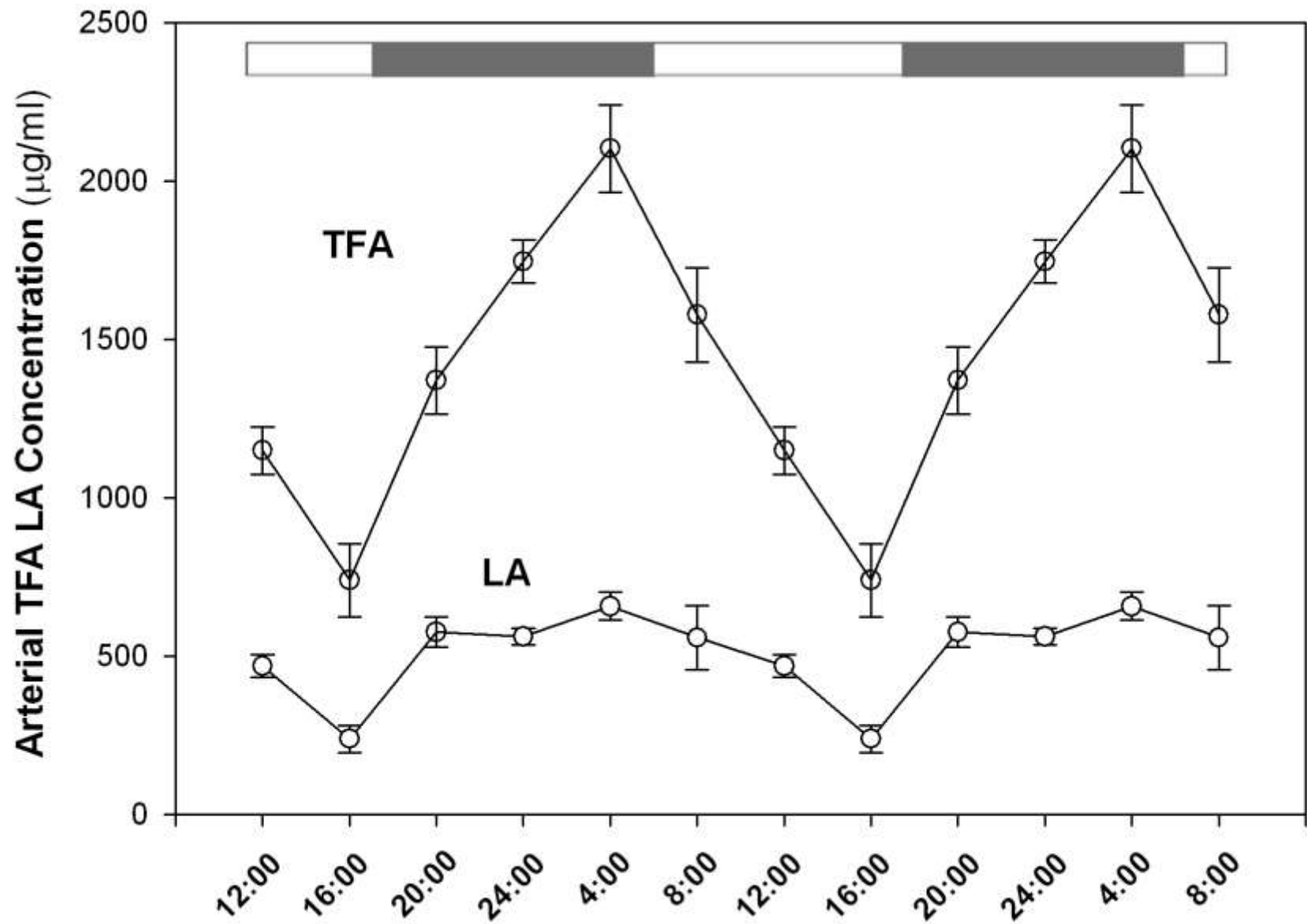
Nude females (non-tumor bearing)

CONTROLS (12L:12D): ●

Experimental (12L:12LAN): ○ 0.08 $\mu\text{W}/\text{cm}^2$





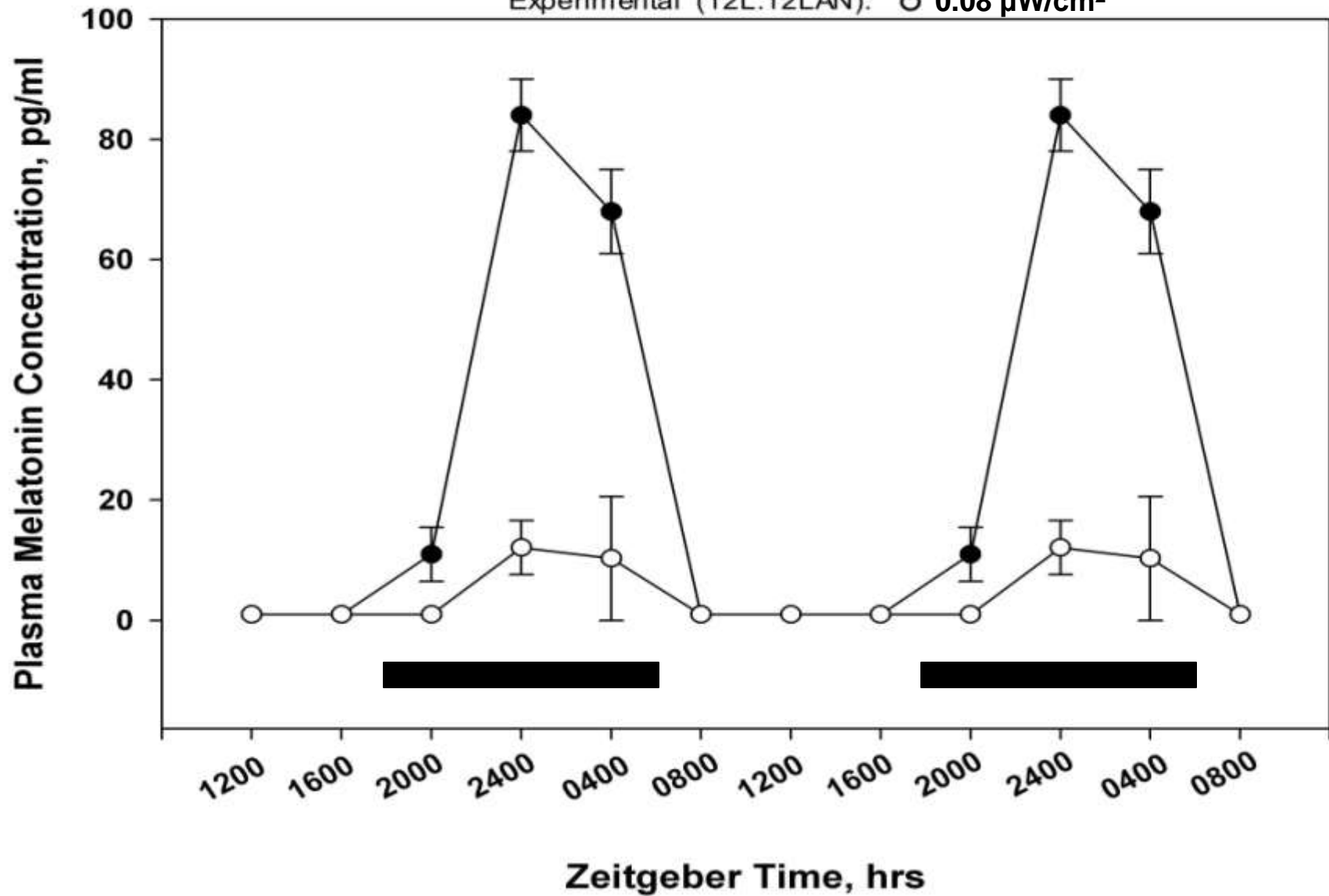


GLAS Investigation

Nude females (non-tumor bearing)

CONTROLS (12L:12D): ●

Experimental (12L:12LAN): ○ 0.08 $\mu\text{W}/\text{cm}^2$

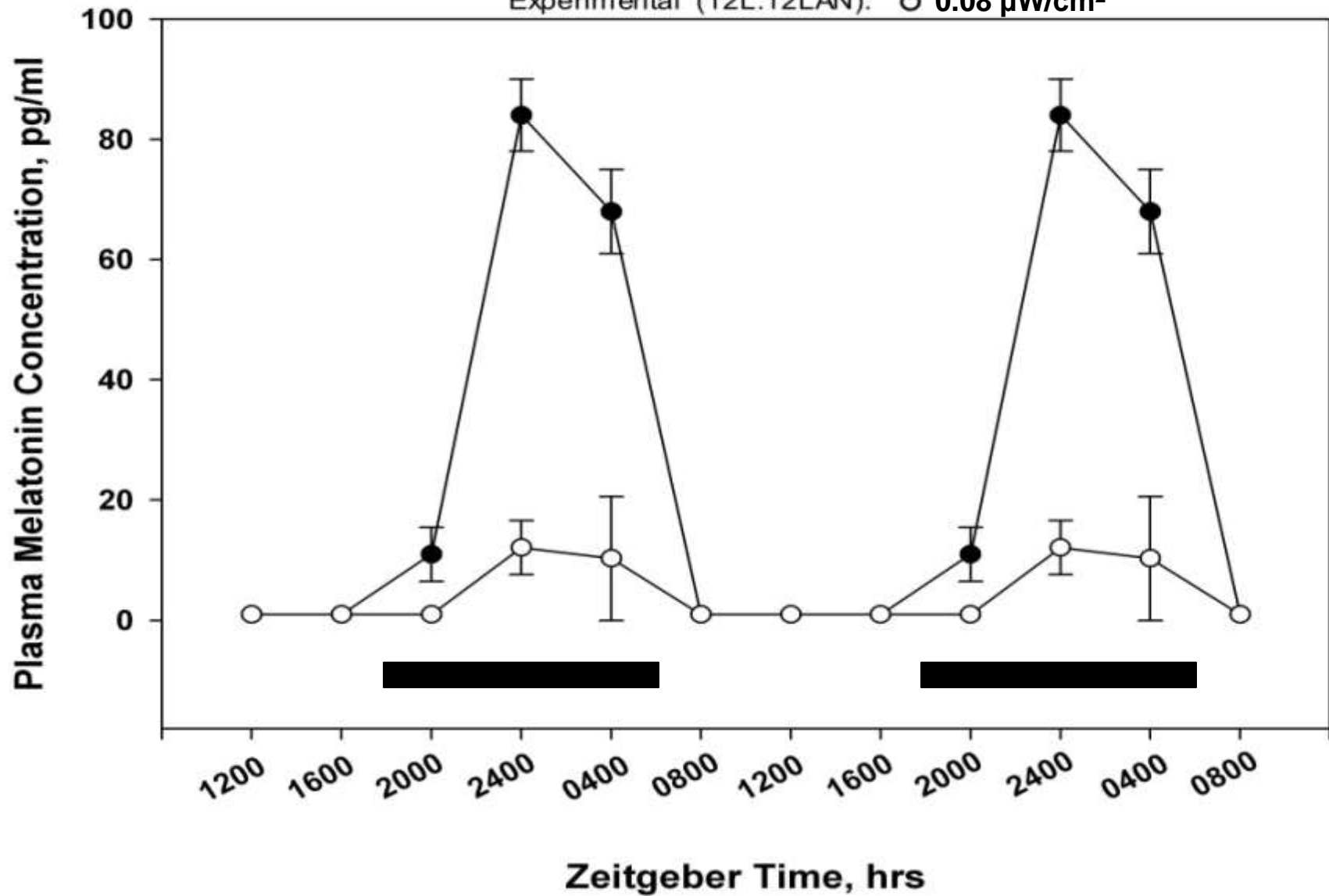


GLAS Investigation

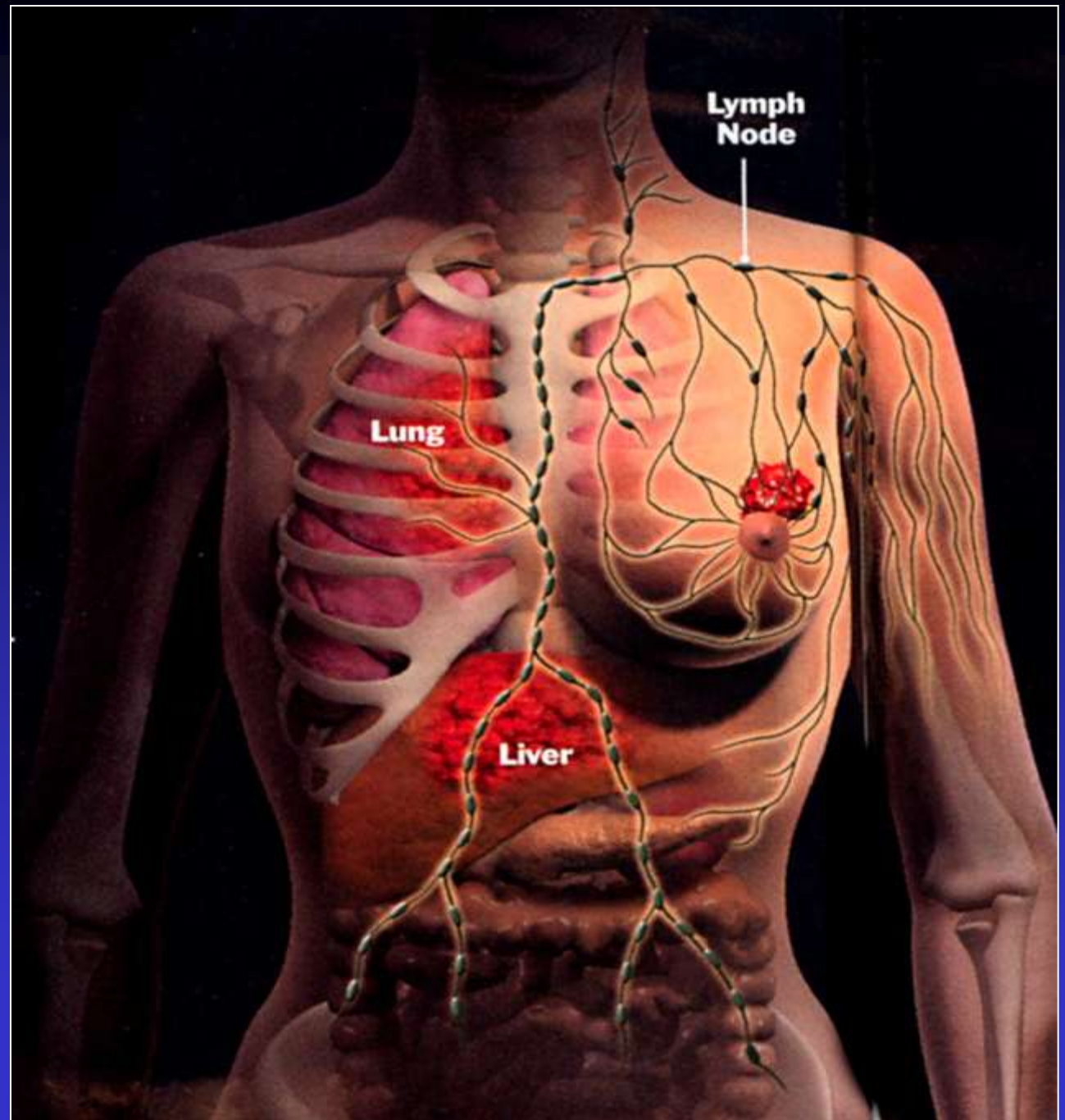
Nude females (non-tumor bearing)

CONTROLS (12L:12D): ●

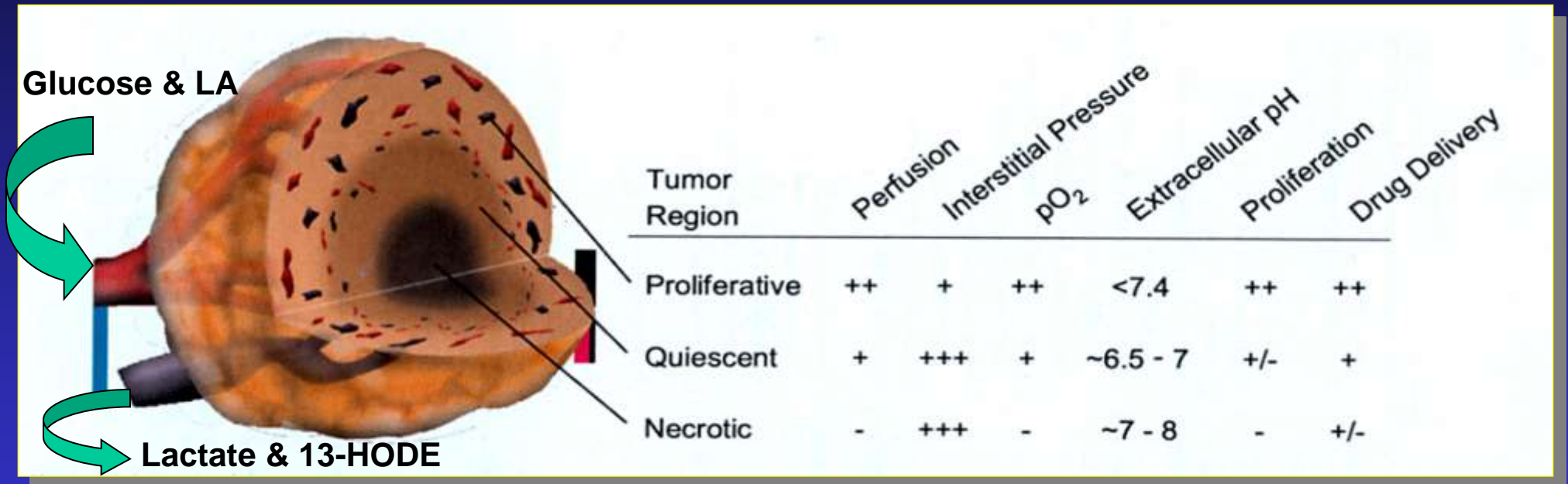
Experimental (12L:12LAN): ○ 0.08 $\mu\text{W}/\text{cm}^2$

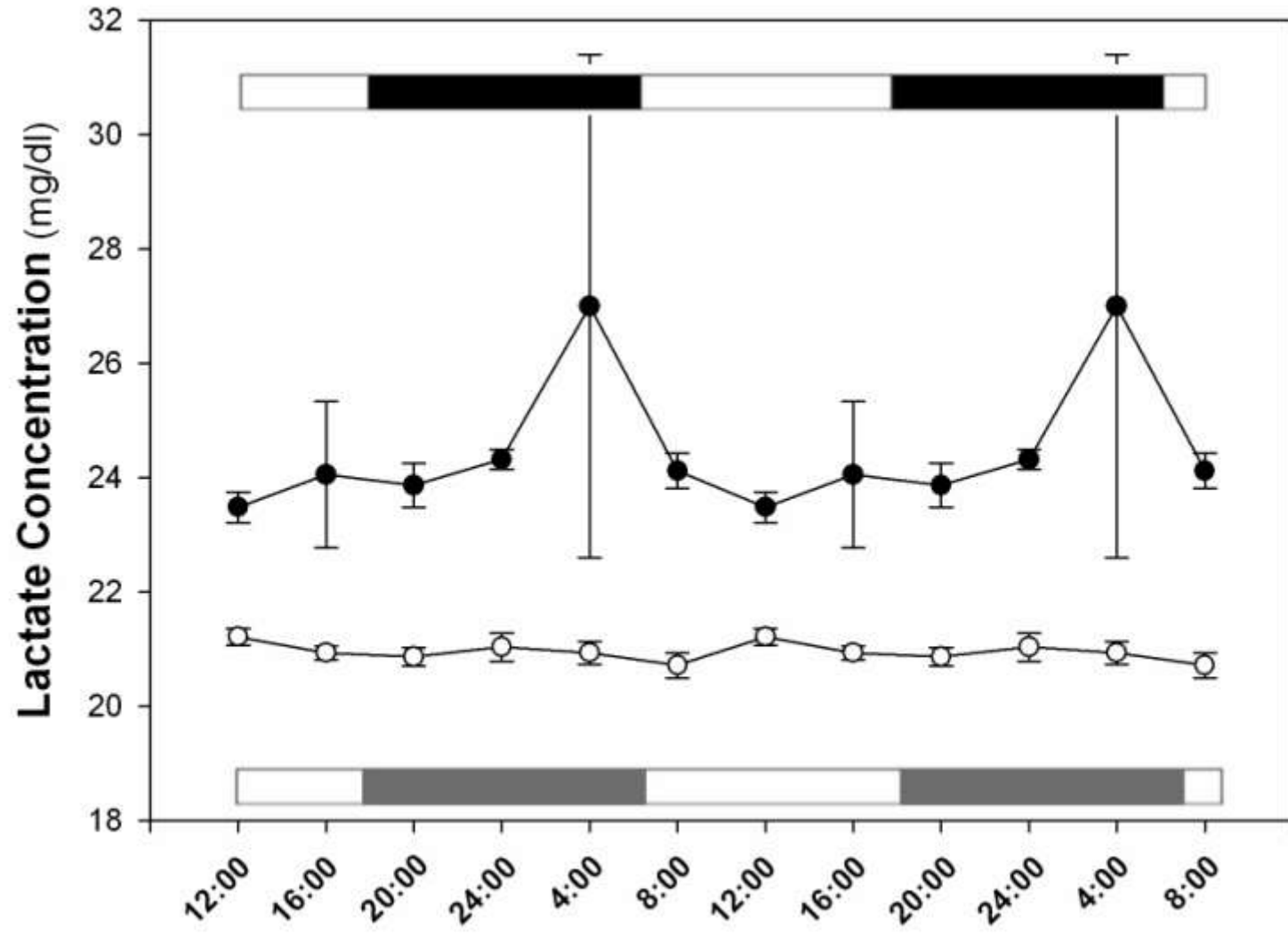


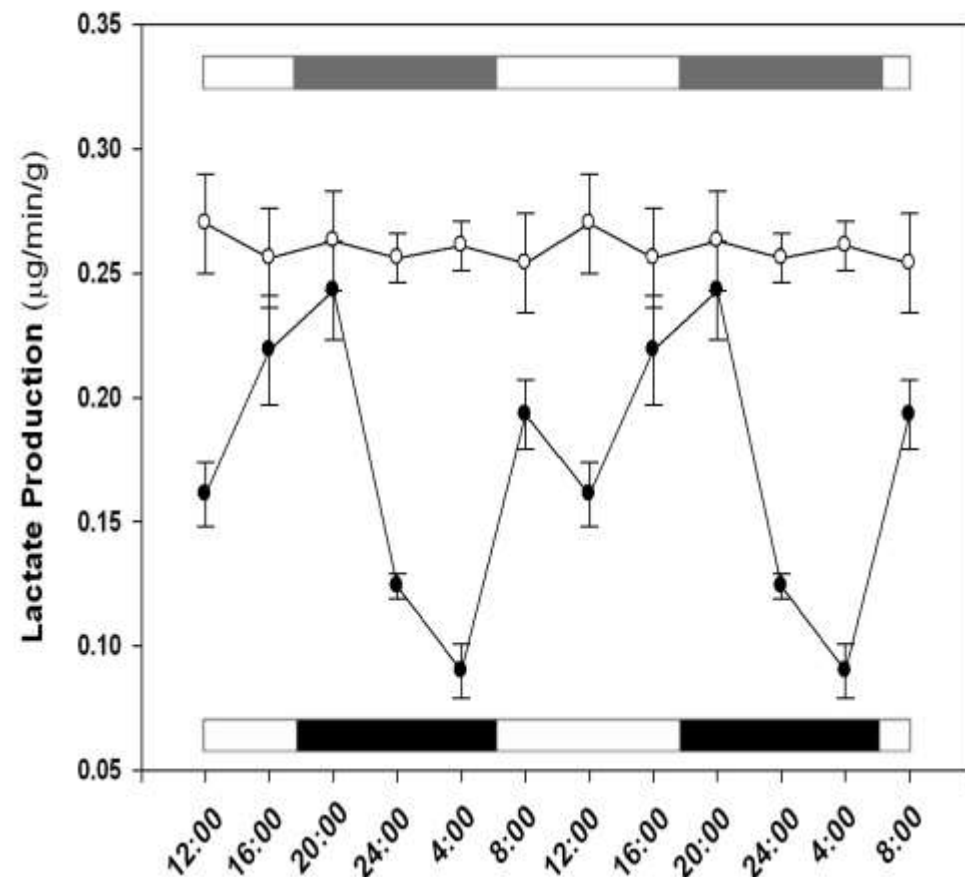
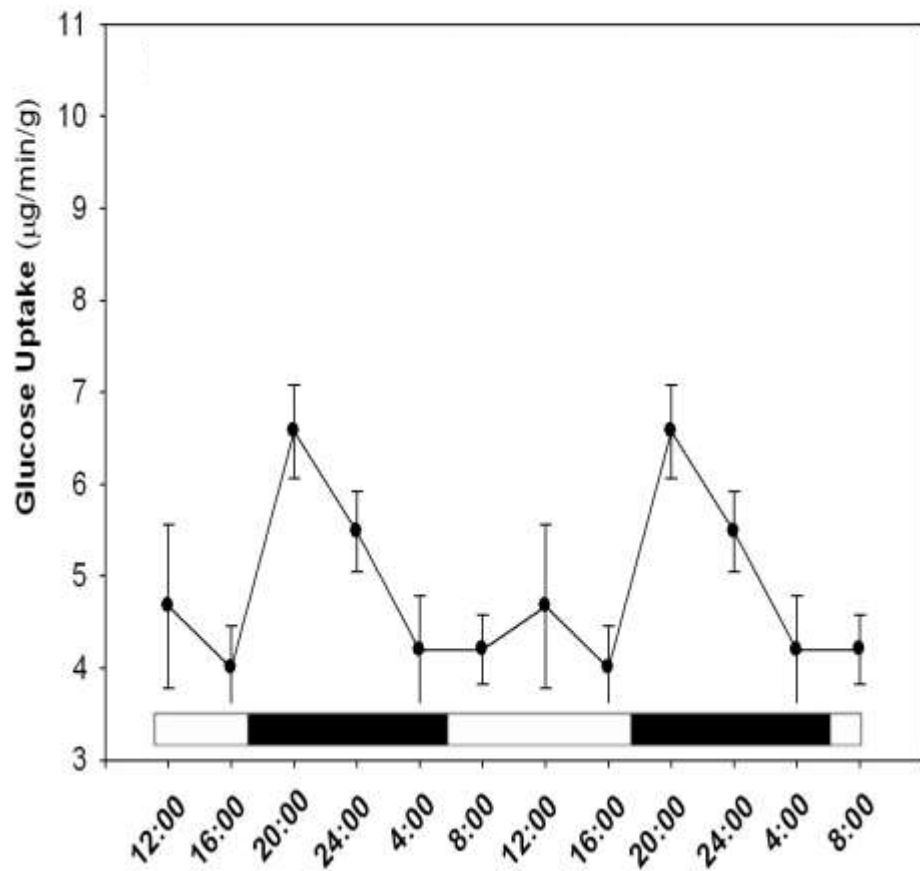
**ENDOGENOUS
CIRCADIAN
MELATONIN AND
GLUCOSE
SIGNALS IN
HUMAN BREAST
CANCER GROWTH
AND
METABOLISM:
EFFECTS OF DIM
LIGHT AT NIGHT**

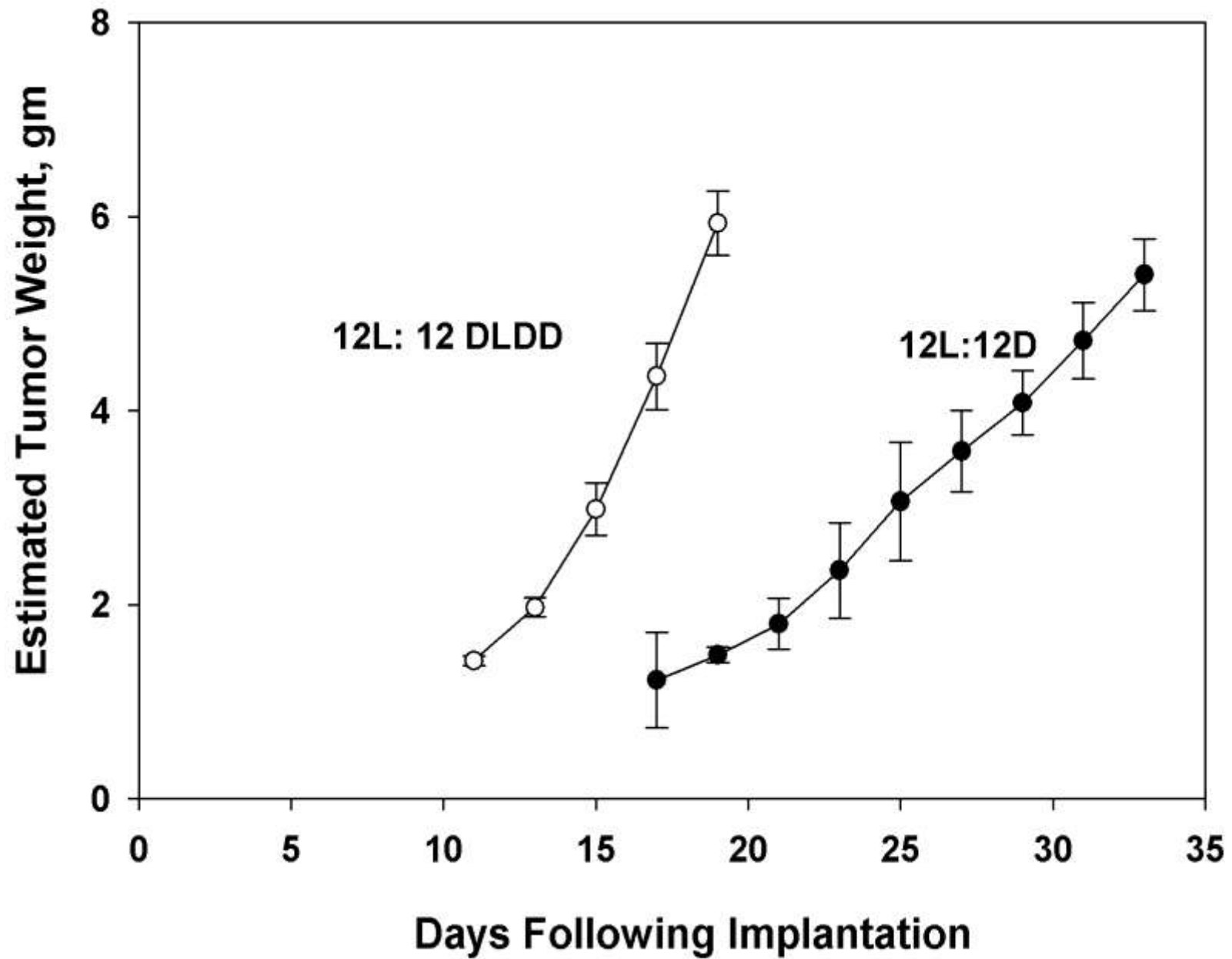


LA & GLUCOSE UPTAKE/UTILIZATION AND 13-HODE & LACTATE PRODUCTION/RELEASE BY TISSUE-ISOLATED TUMOR

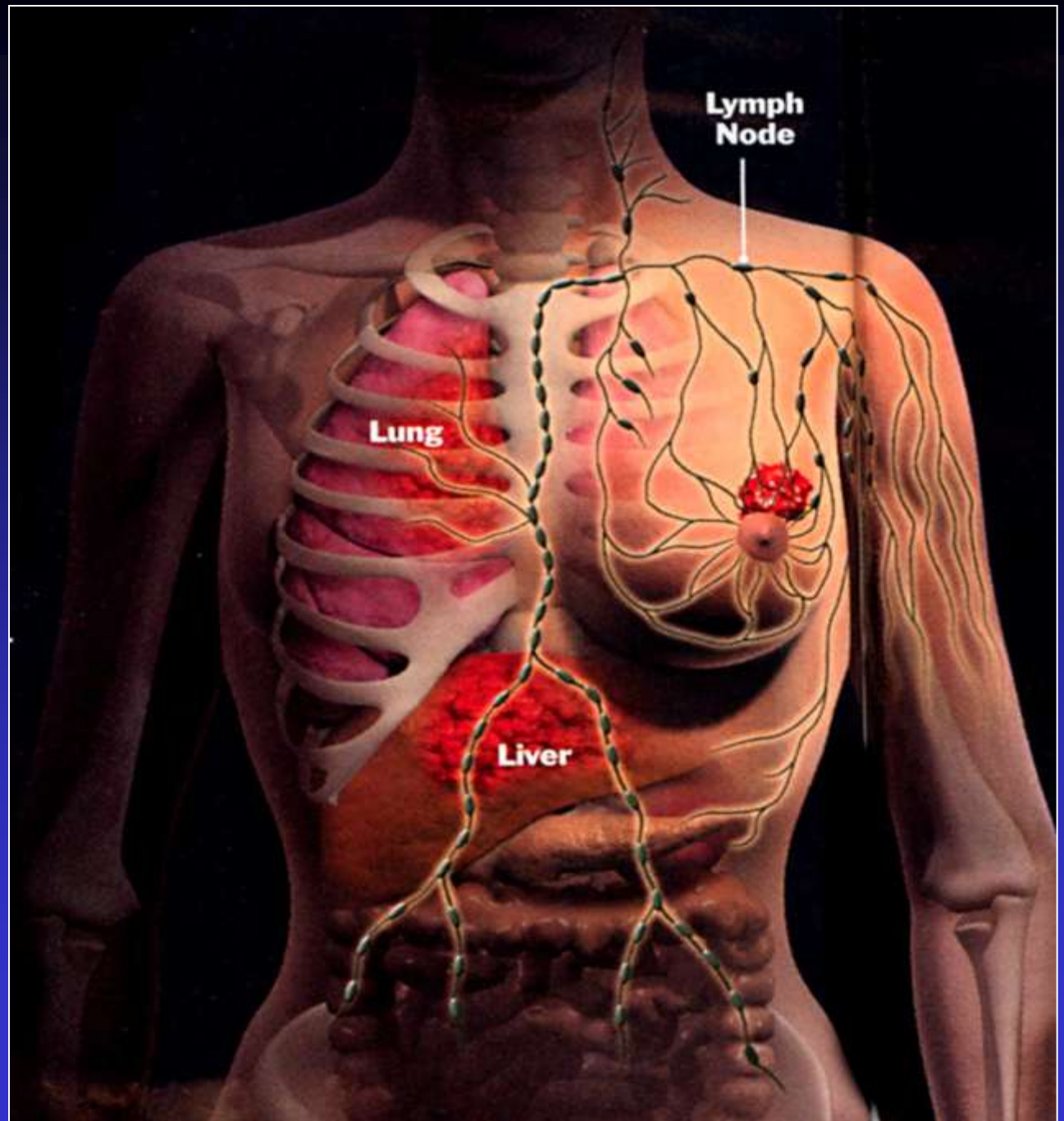








**CIRCADIAN
MELATONIN
SIGNAL
REINFORCEMENT/
REPLACEMENT BY
EXOGENOUS
MELATONIN
DURING DIM
LIGHT AT NIGHT
ON HUMAN
BREAST CANCER
GROWTH
PREVENTION**



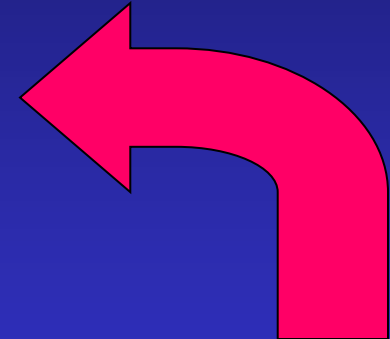
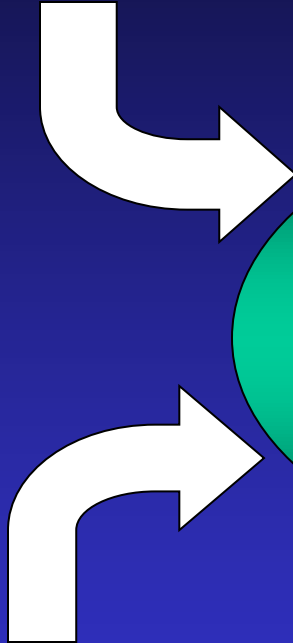
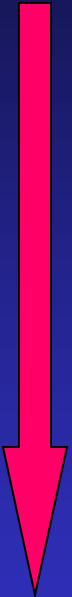
**CIRCADIAN
SYSTEM**
(BIOLOGICAL
CLOCK)

**NOCTURNAL
LIGHT
EXPOSURE**

**BREAST
CANCER**
(CLOCK GENES?)

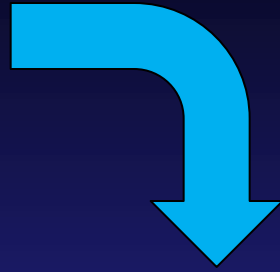
**NOCTURNAL
MELATONIN**

**FATTY ACIDS
(LA) &
GLUCOSE**

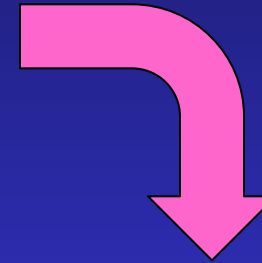




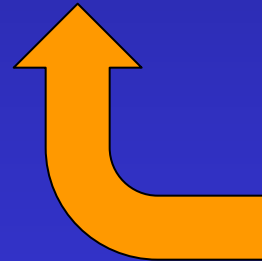
LIGHT AT NIGHT



BROKEN TIMING



CANCER



ACKNOWLEDGEMENTS

*Laboratory of Chrono-Neuroendocrine Oncology, Tulane University
SOM and *Bassett Research Institute, Cooperstown, NY*

Robert Dauchy, Leslie Davidson*, Michael Greene*

**Darin Lynch*, Leonard Sauer*, Paul Tirrell*, Erin Dauchy, Robert
Tirrell***

Thomas Jefferson University, Philadelphia, PA

George “Bud” Brainard

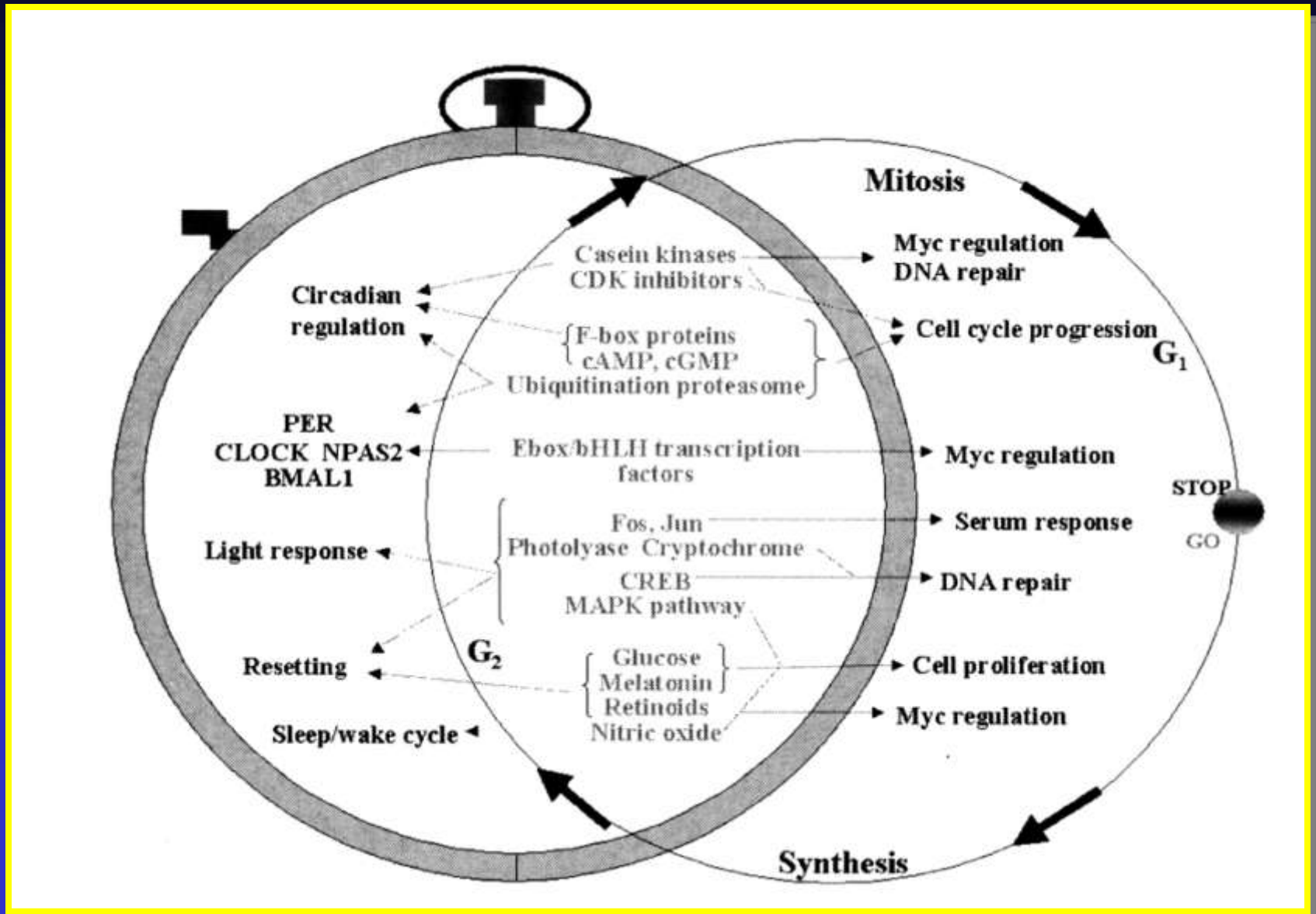
Northwestern University

Margarita Dubocovich

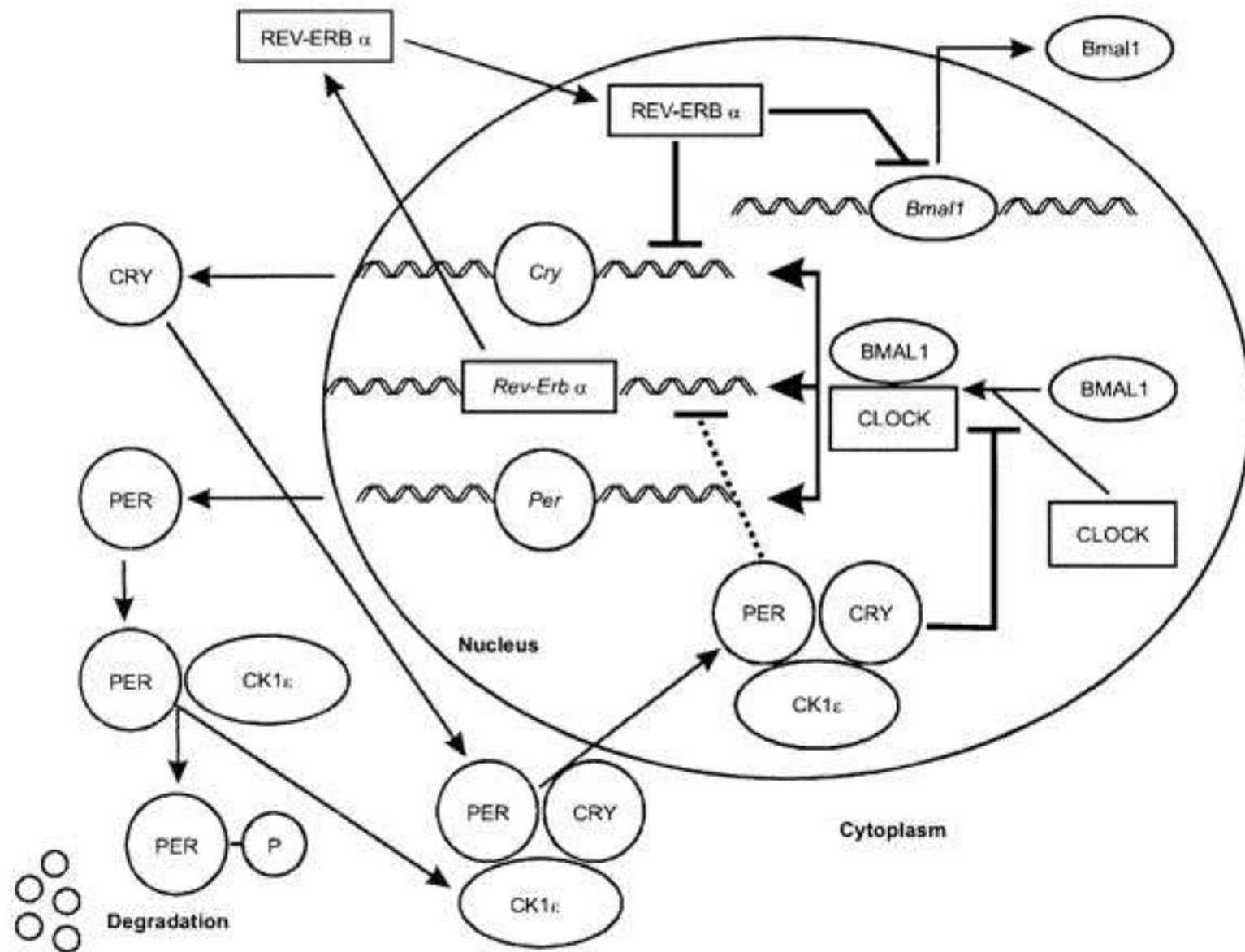
GRANT SUPPORT

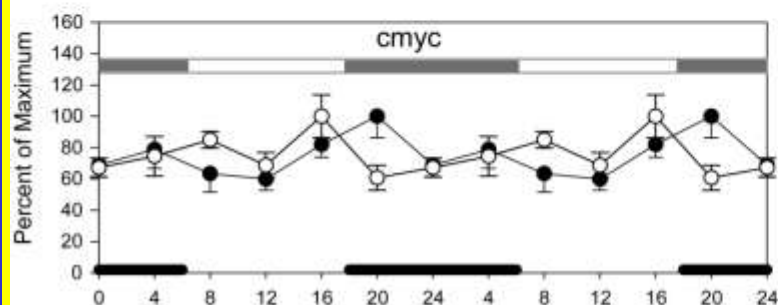
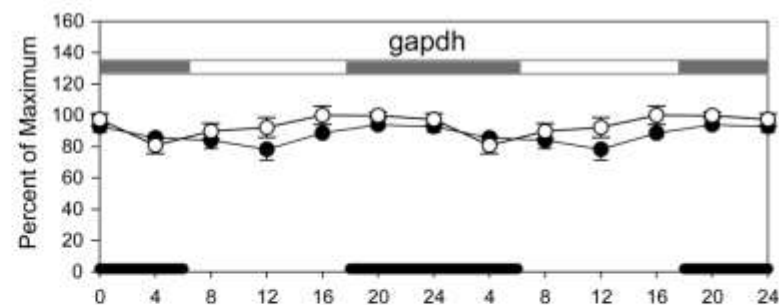
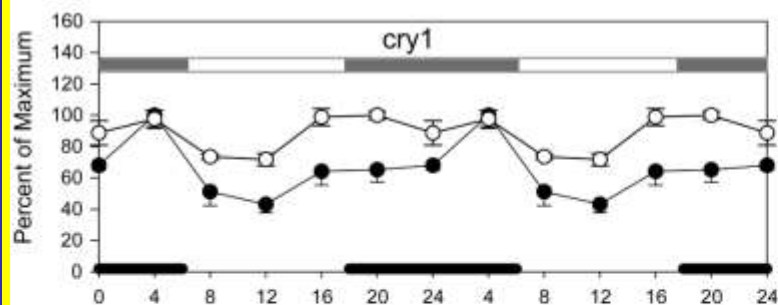
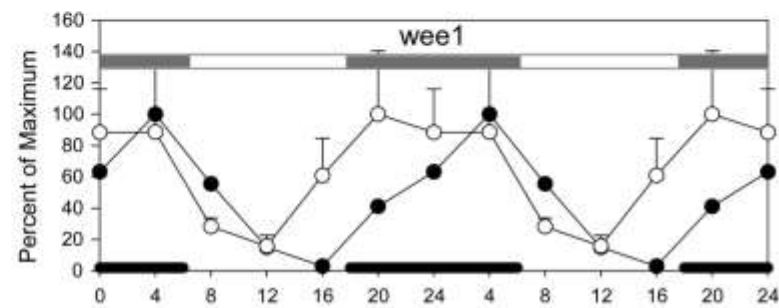
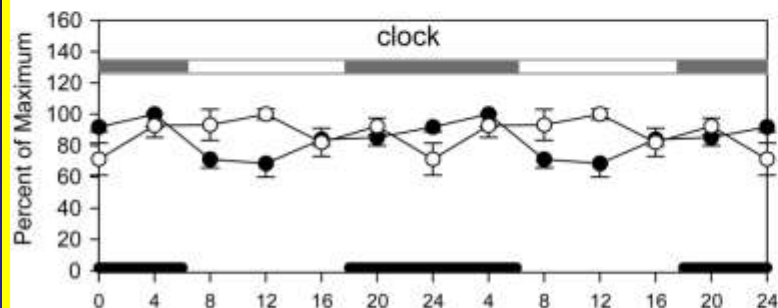
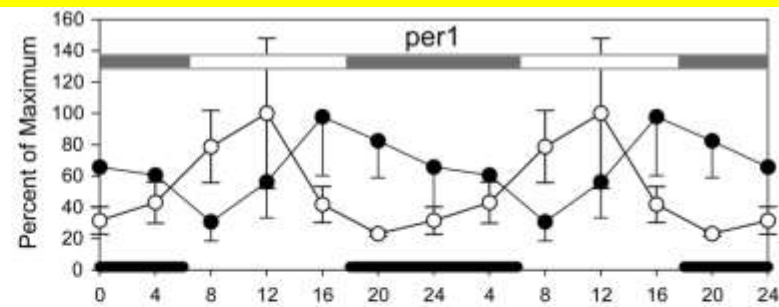
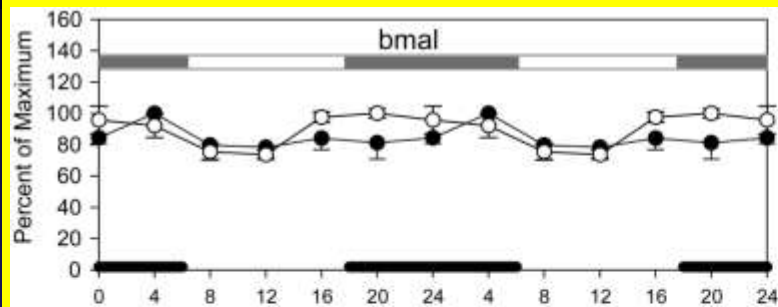
NCI, NIEHS, GLAS, Edwin Pauley Foundation, Clark Foundation

COMMON ELEMENTS SHARED BY BIOLOGICAL CLOCKS & CELL CYCLE

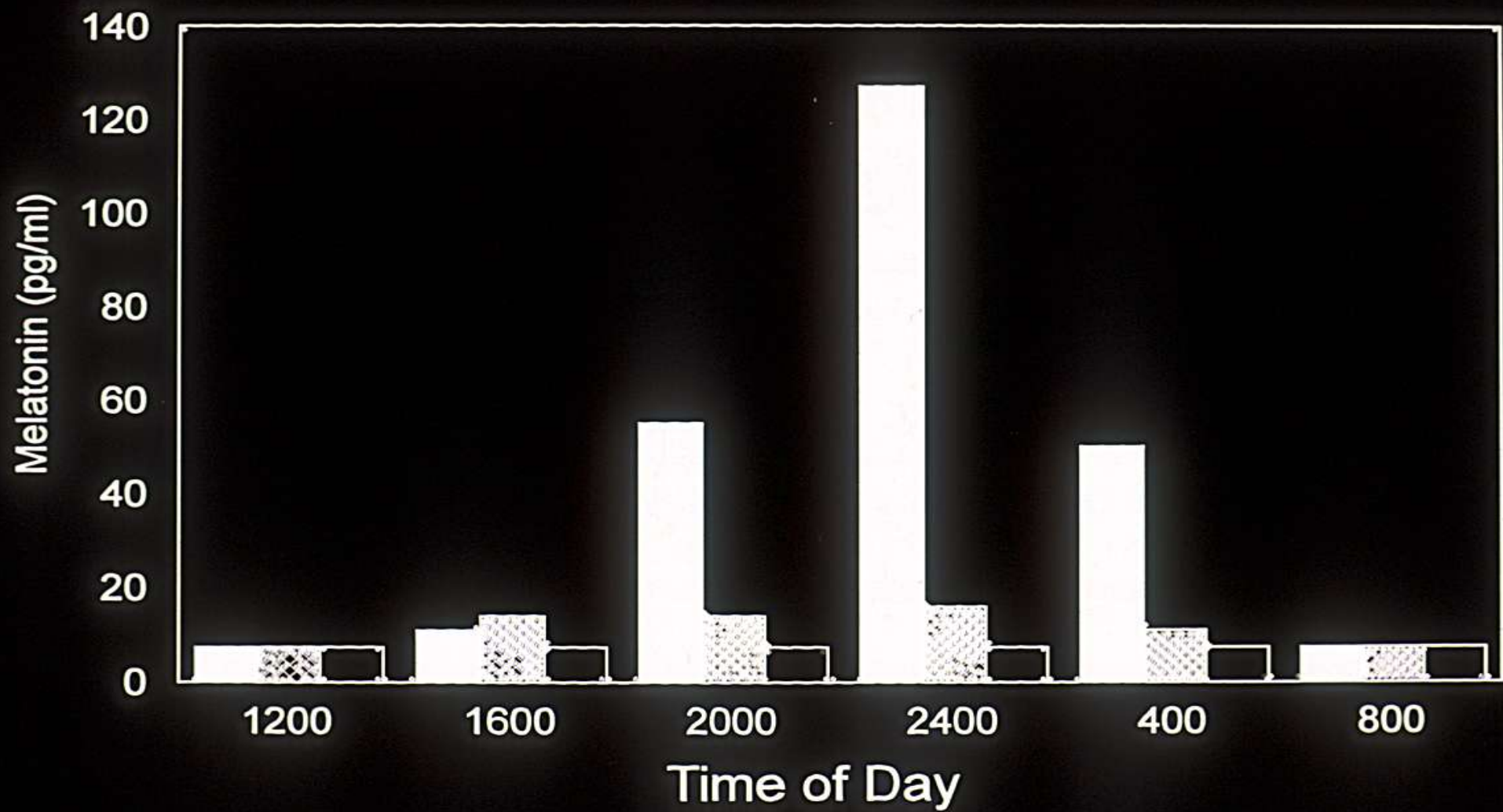


MOLECULAR CLOCKWORKS IN SCN, NORMAL CELLS AND CANCER CELLS

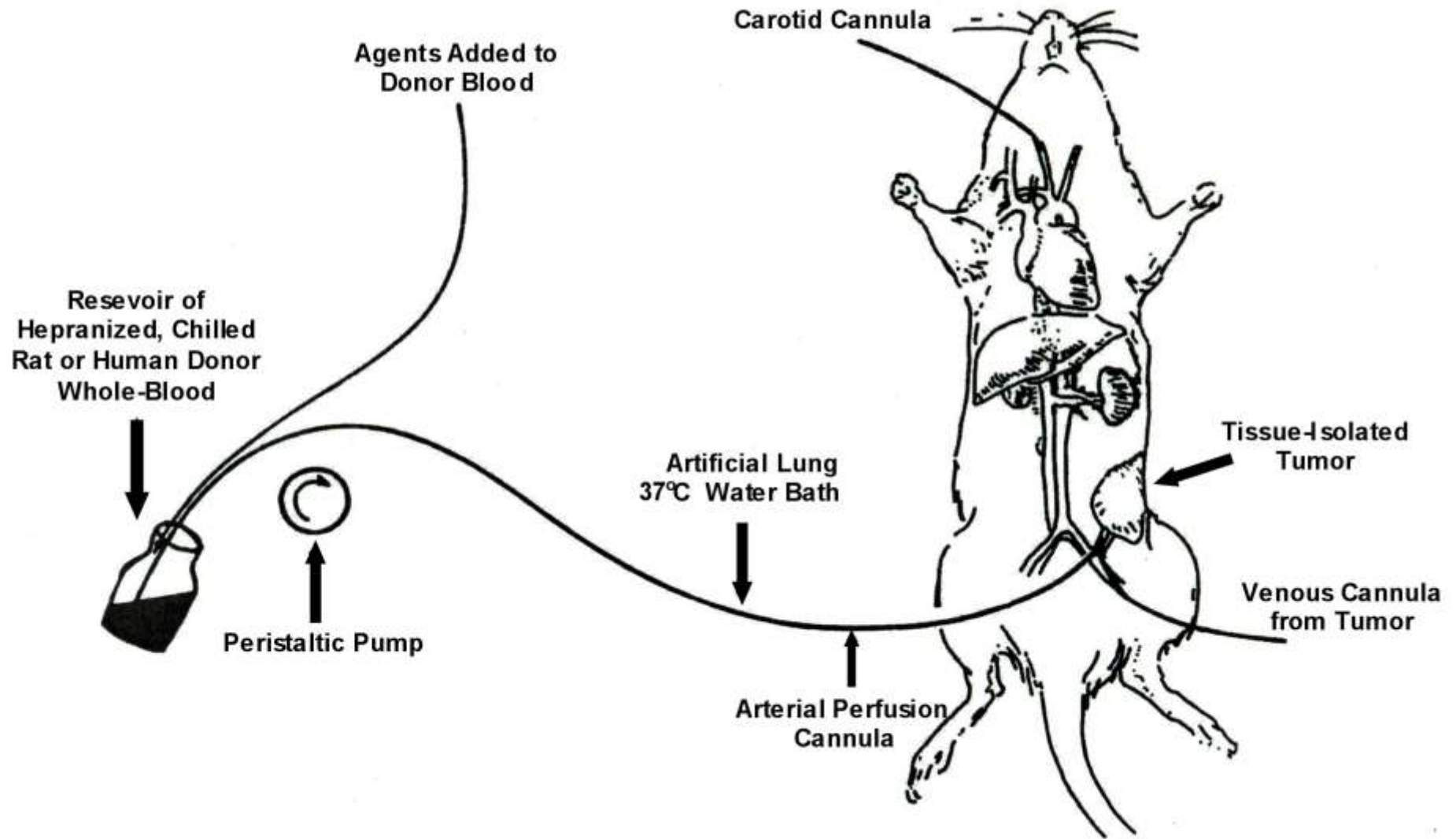




NOTE BENE: Per 2 mRNA IS NOT EXPRESSED IN TISSUE-ISOLATED (SR-) MCF-7 HUMAN BRREAST CANCER XENOGRAPTS



SYSTEM FOR PERFUSION OF TISSUE-ISOLATED TUMORS *IN SITU*



SUMMARY AND DELUSIONS

- **Dim light at night reduces the nocturnal amplitude of melatonin by approximately 85% in the nude female rat while leaving the circadian feeding/drinking rhythm intact**
- **Tissue-isolated SR- human breast cancer xenografts display prominent circadian rhythms in signal transduction, LA and glucose metabolism, apparent “rhythms”(?) in core clock-gene and clock gene-regulated cell cycle gene expression, and most importantly, tumor proliferative activity over a 24-hr day**
- **This rhythmicity is abolished or modulated by exposure of tumor-bearing animals to dim light at night in which rhythmic nocturnal melatonin production is suppressed while central circadian feeding/drinking rhythms persist**

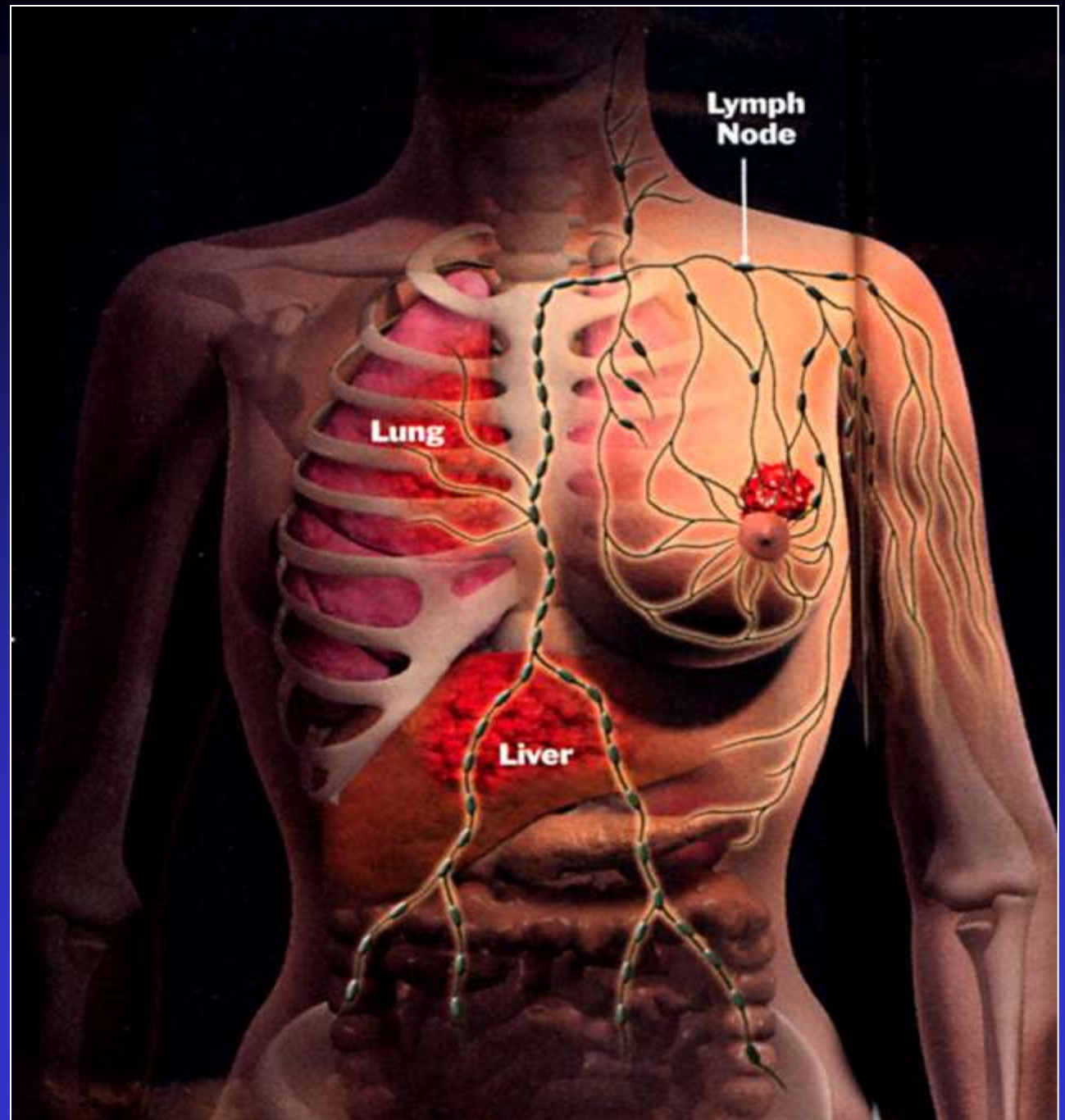
CONCLUSION

As chronobiotic anticancer agents, melatonin or melatonin agonists, administered orally in a circadian-optimized manner, may play an important role in cancer risk, growth prevention and therapy while at the same time correcting circadian rhythm/sleep dysregulation in certain types of circadian-disrupted individuals (i.e., suppressed melatonin) due to light at night (i.e., most of us?) as well in those with a totally intact circadian system and melatonin signal (i.e., those living under a rock!)

SUMMARY AND DELUSIONS

- **Suppression of the physiological nocturnal melatonin signal by dim light at night or blocking melatonin's action at the receptor level (MT_1) are effective in accelerating human breast cancer xenograft growth**
- **Re-establishing a quasi-physiological, circadian-like program of melatonin reinforcement/replacement totally prevents the “tumor-take” of breast cancer xenografts via an MT_1 melatonin receptor-mediated mechanism in animals with an intact melatonin signal or in those with a suppressed MLT signal due to dim light at night exposure**

ENDOGENOUS CIRCADIAN MELATONIN AND CANCER CLOCK GENES



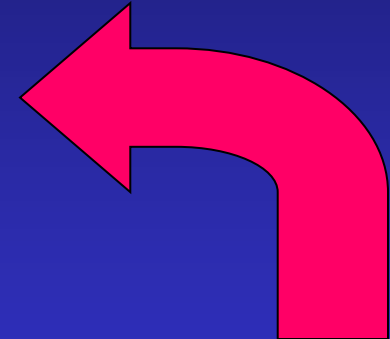
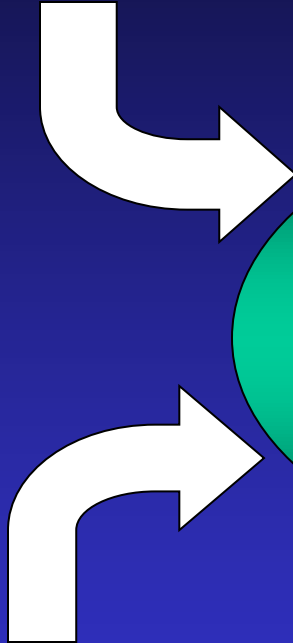
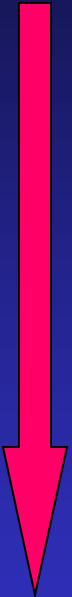
**CIRCADIAN
SYSTEM**
(BIOLOGICAL
CLOCK)

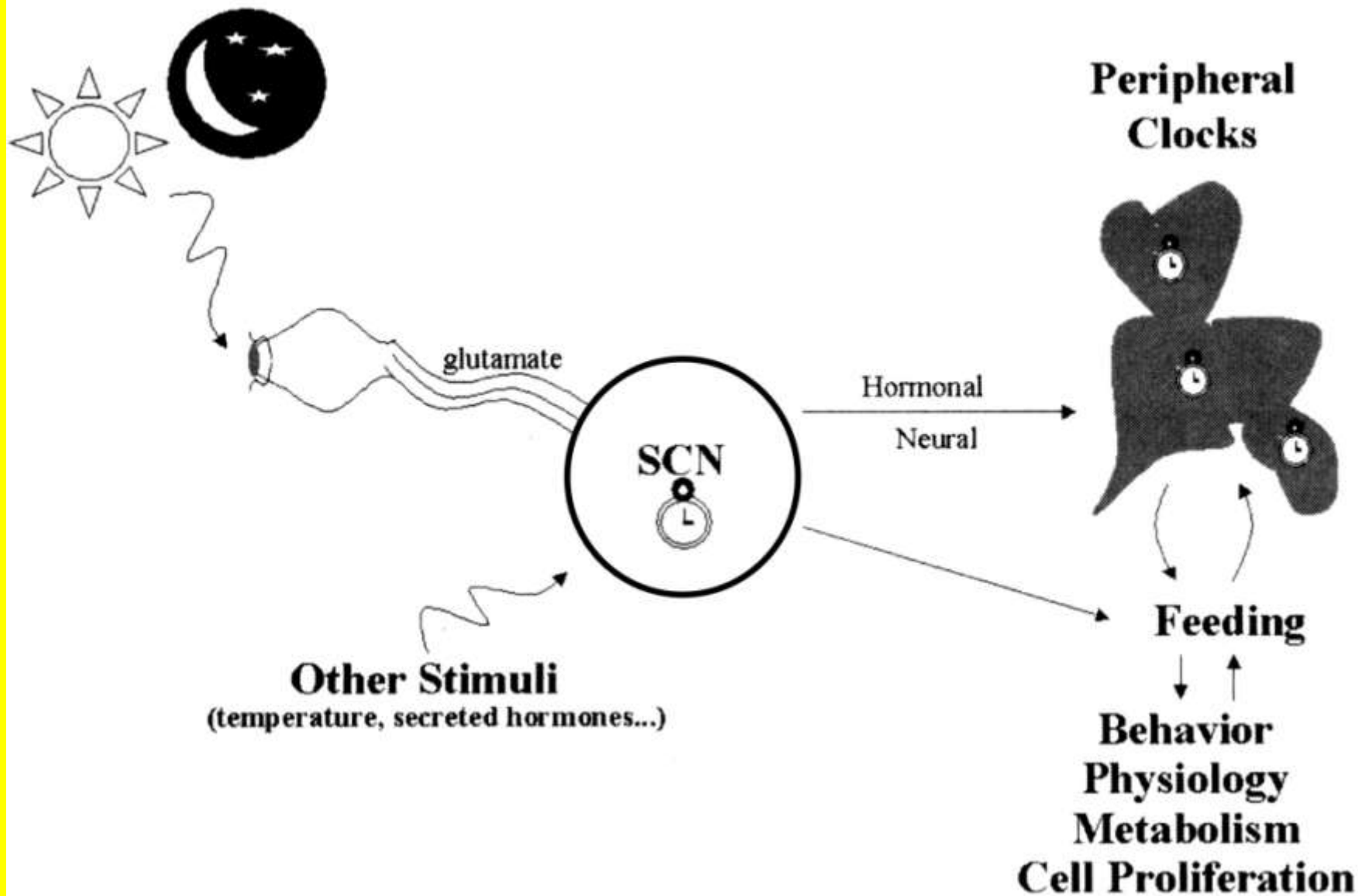
**NOCTURNAL
LIGHT
EXPOSURE**

**BREAST
CANCER**
(CLOCK GENES?)

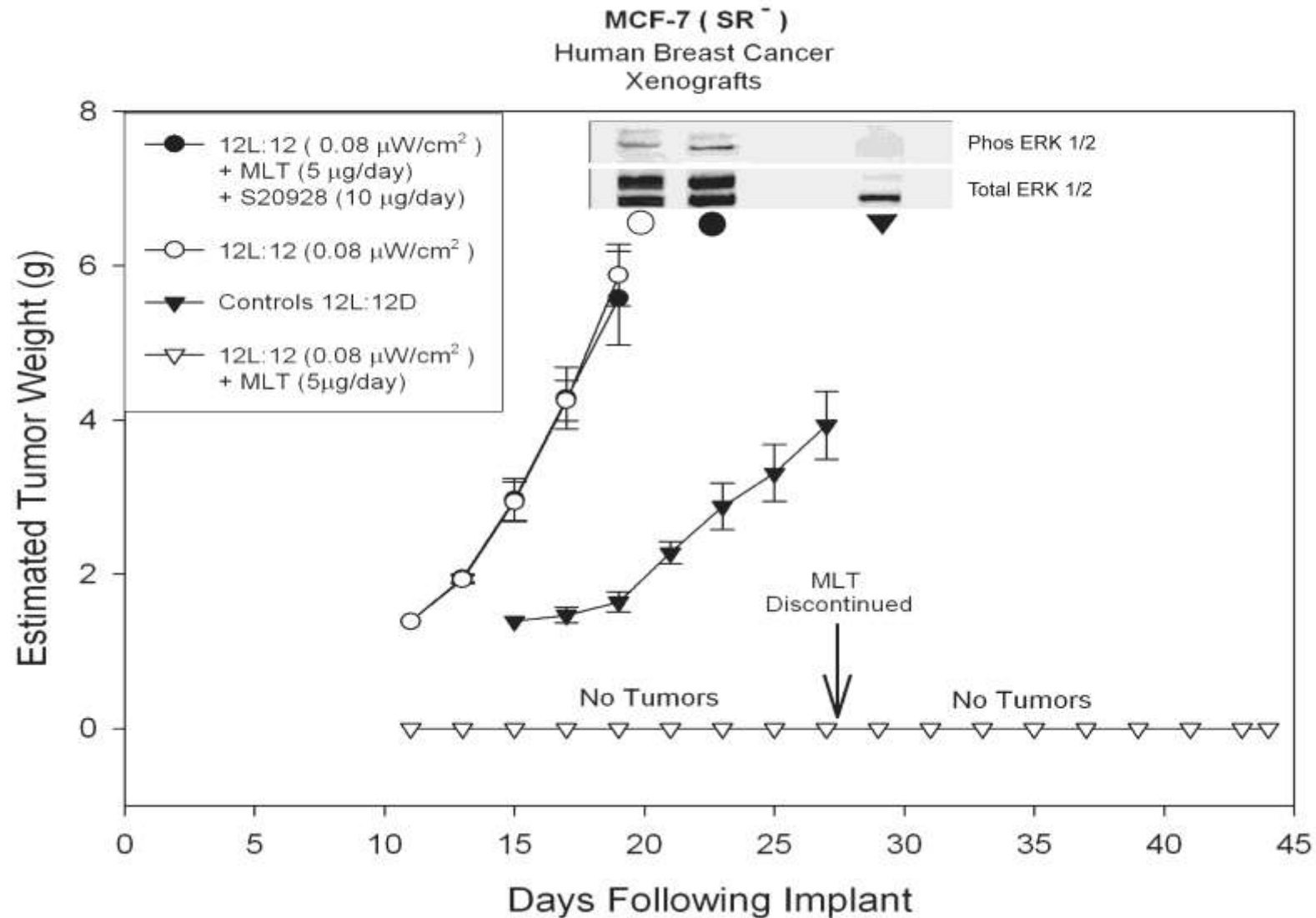
**NOCTURNAL
MELATONIN**

**FATTY ACIDS
(LA) &
GLUCOSE**





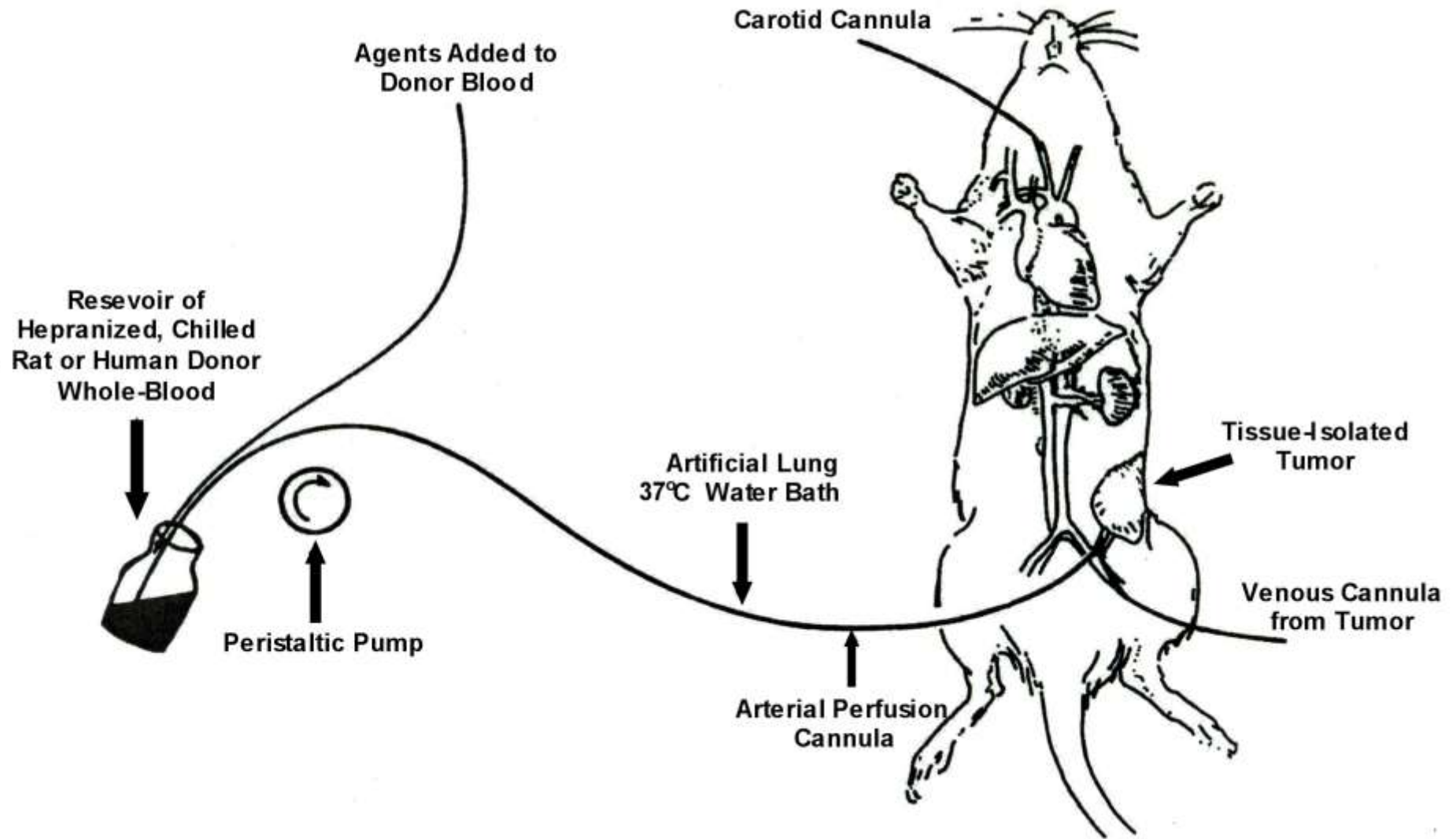
EFFECTS OF CIRCADIAN-BASED MELATONIN SUPPLEMENTATION ON GROWTH AND ERK1/2 ACTIVATION IN TISSUE-ISOLATED MCF-7 (SR-) HUMAN BREAST CANCER XENOGRAFTS DURING DIM LIGHT DURING DARKNESS

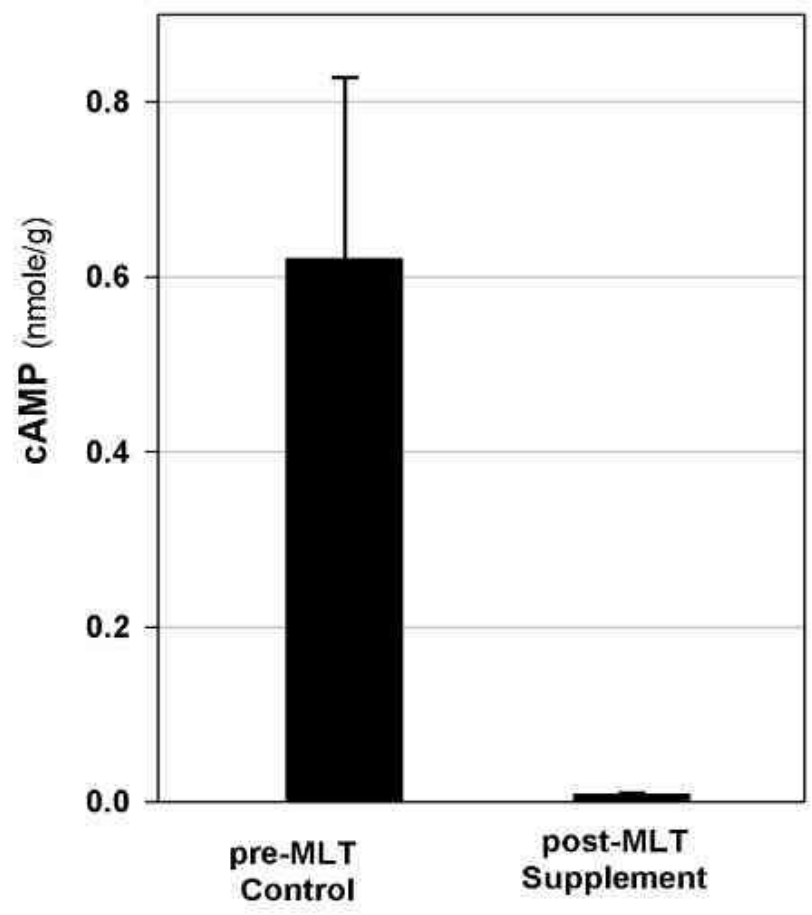
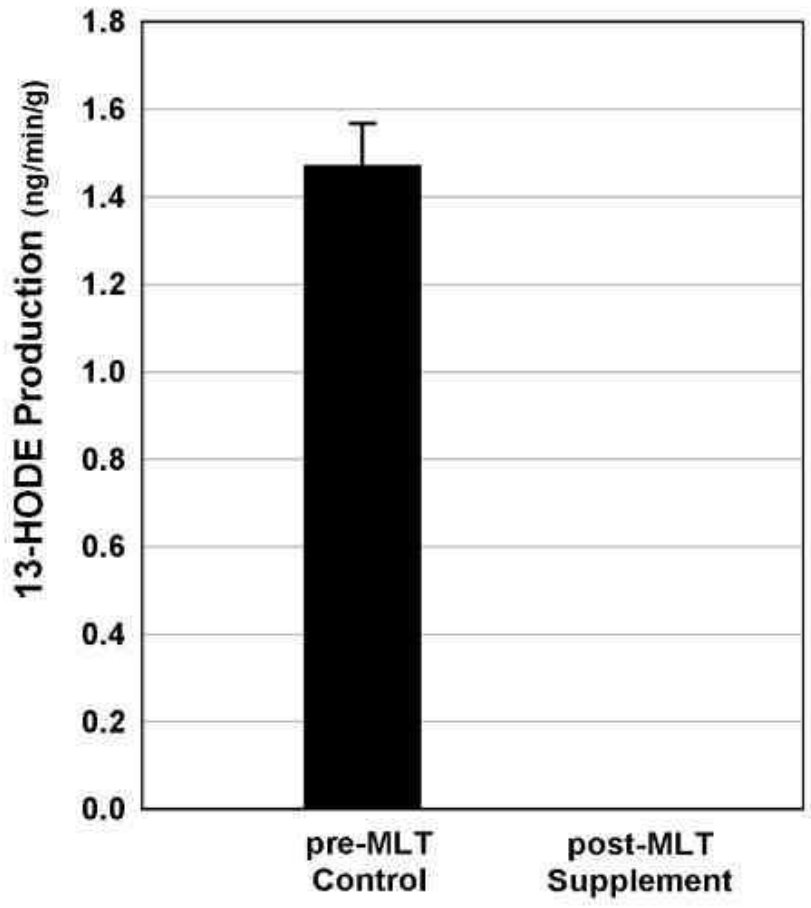
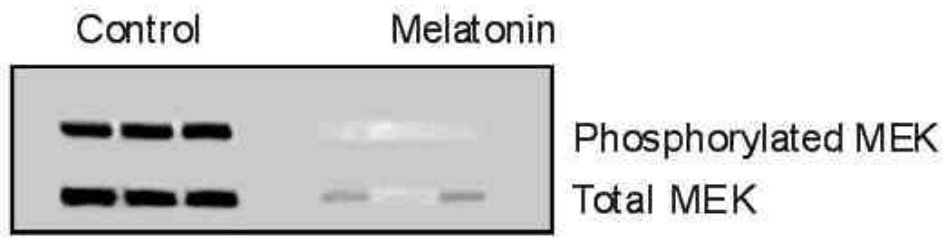


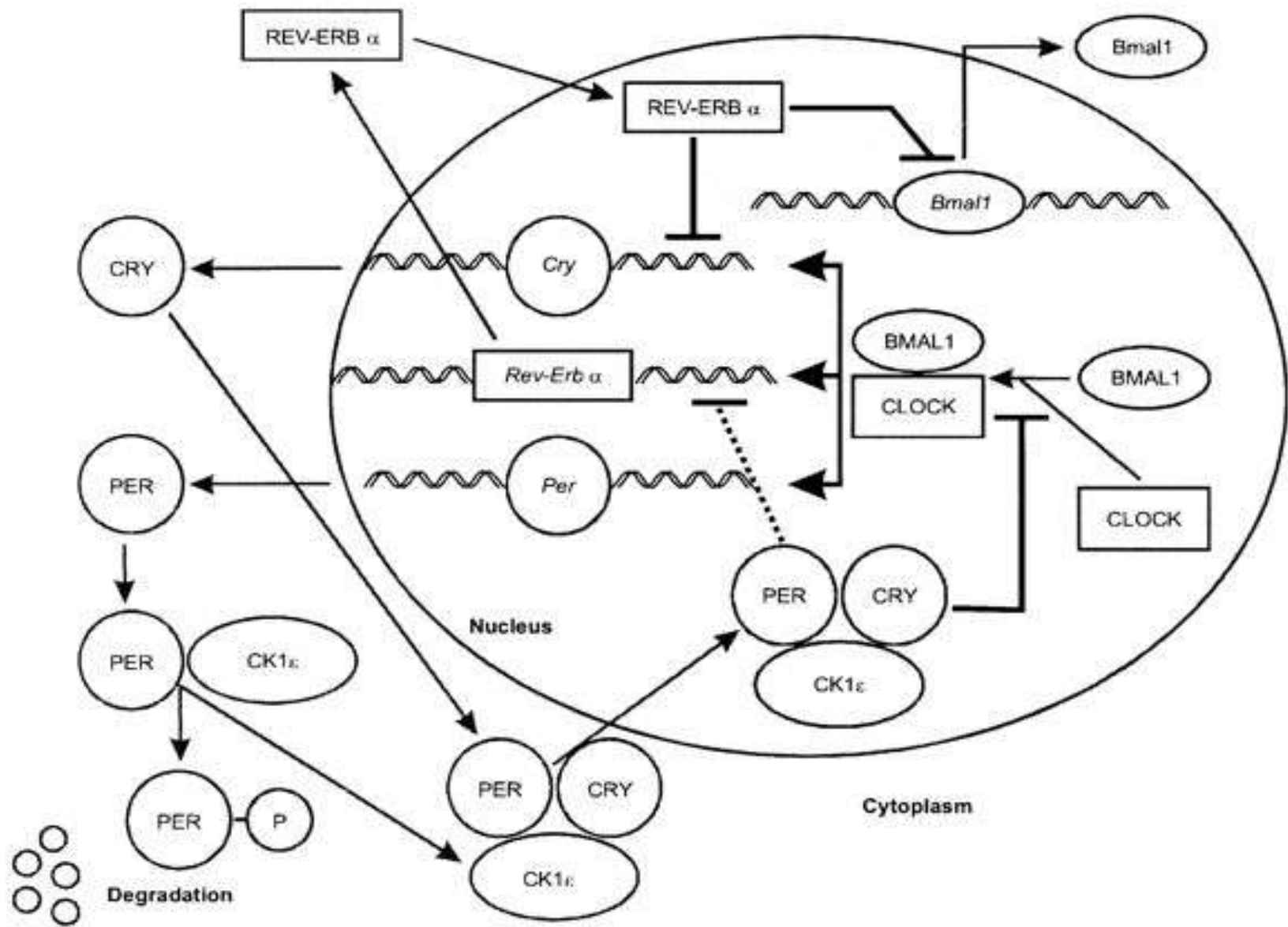
EFFECTS OF MELATONIN SUPPLEMENTATION IN A HUMAN SUBJECT

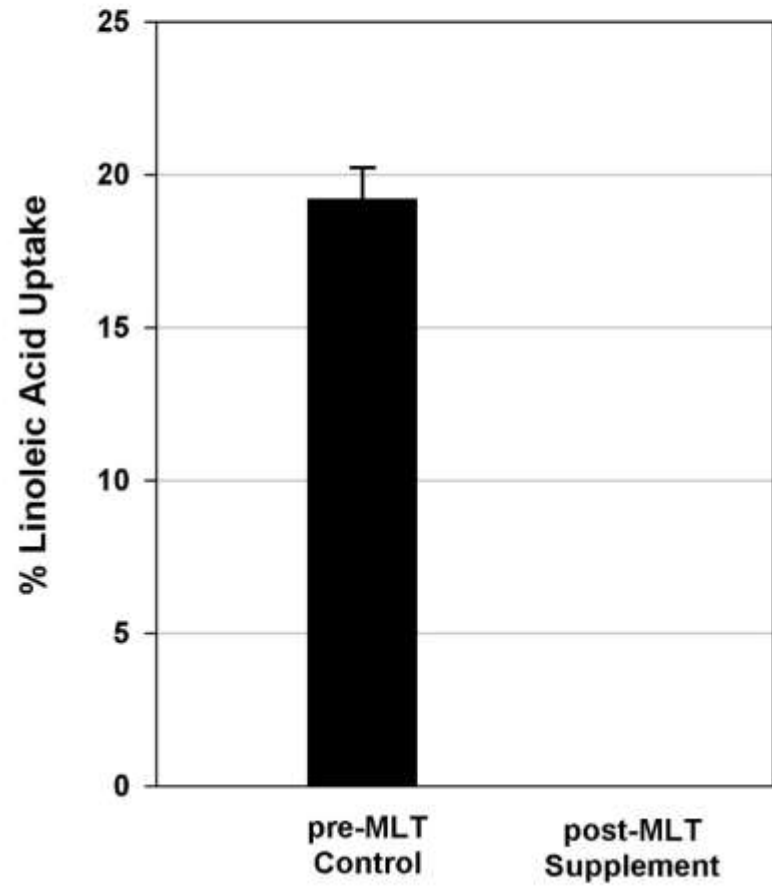
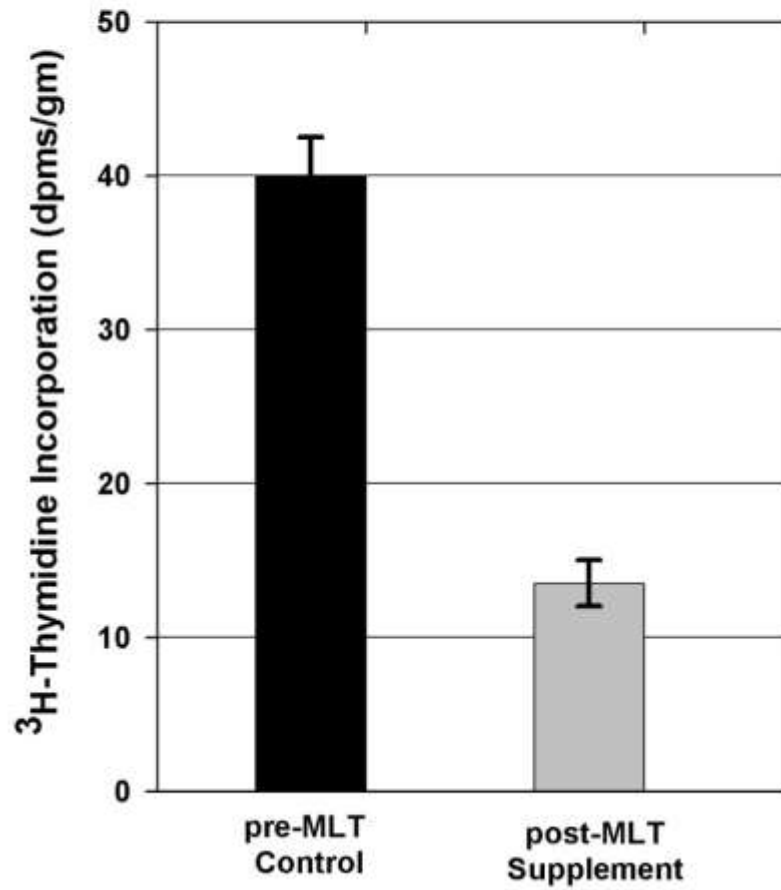
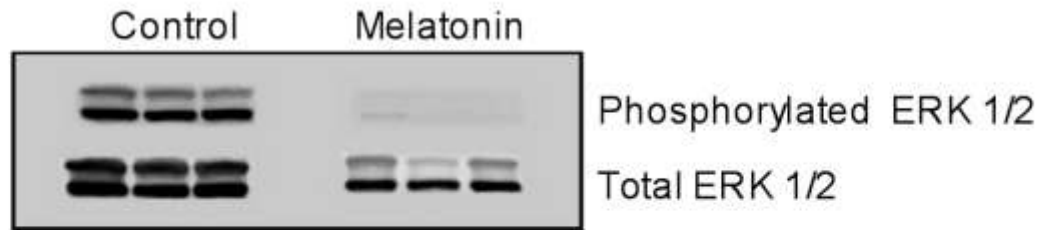


SYSTEM FOR PERFUSION OF TISSUE-ISOLATED TUMORS *IN SITU*









Summary, Conclusions and Speculations

- Tissue-isolated human breast cancer xenografts exhibit daily rhythms of signal transduction, LA uptake/metabolism and proliferative activity that are temporally coordinated with and by circadian rhythms of plasma melatonin (SCN/pineal-driven) and LA (SCN/feeding-driven).
- Plasma LA is a light phase oncogenic signal whose uptake drives tumor 13-HODE - mediated tumor growth whereas melatonin is a dark phase oncostatic signal that, via MT_1 melatonin receptor-mediated signaling, counteracts LA uptake/metabolism-driven tumor growth . Dim light during darkness dampens the mid-dark phase oncostatic circadian melatonin signal while ramping-up the late-dark phase amplitude of the plasma oncogenic LA signal.
- This form of circadian disruption induces a loss of circadian organization manifested by constantly (24/7) up-regulated tumor signal transduction, LA uptake/metabolism to 13-HODE and proliferative activity culminating in accelerated tumor growth rates. Thus, an occult slow-growing breast cancer (i.e., DCIS) that might ordinarily remain clinically undetectable may, in fact, become clinically detectable advanced breast cancer under these circumstances.
- Reinforcement/replacement of the endogenous melatonin signal with exogenous melatonin may help prevent this scenario by “reorganizing” a balance of stimulatory and inhibitory tumor growth mechanisms within circadian time structure.

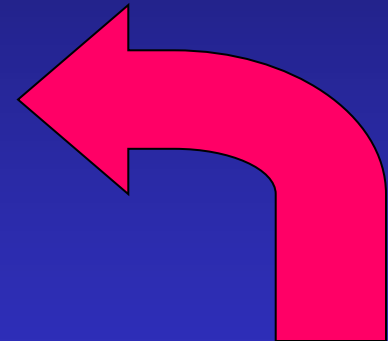
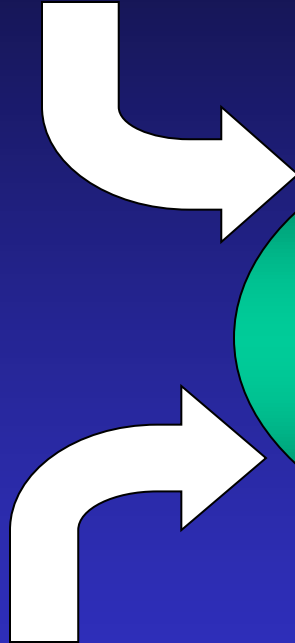
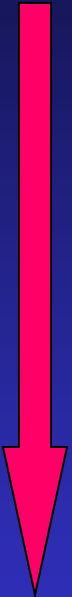
**CIRCADIAN
SYSTEM
(BIOLOGICAL
CLOCK)**

**NOCTURNAL
LIGHT
EXPOSURE**

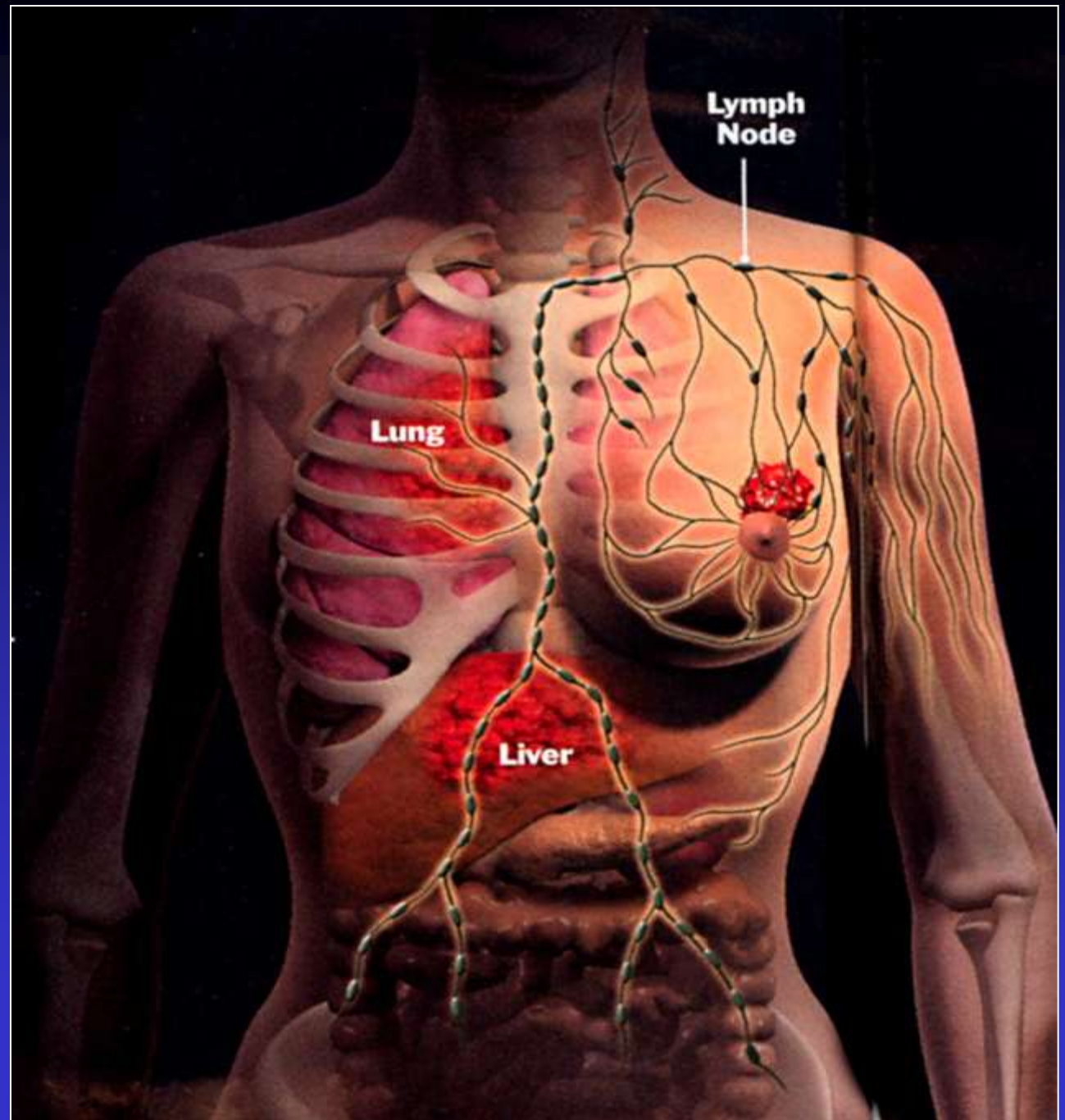
**BREAST
CANCER**

**NOCTURNAL
MELATONIN**

**DIETARY &
ENDOGENOUS
FAT (LA)**



**ENDOGENOUS
CIRCADIAN
MELATONIN
AND LINOLEIC
ACID SIGNALS
IN HUMAN
BREAST
CANCER
GROWTH
PREVENTION**



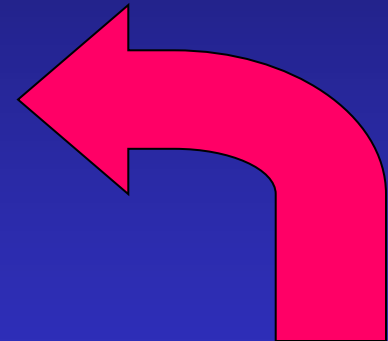
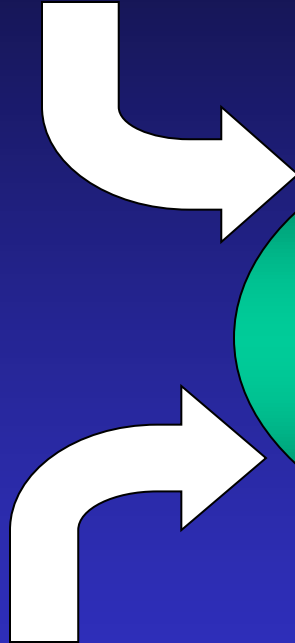
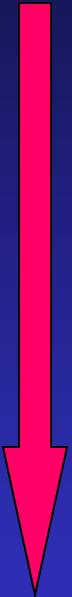
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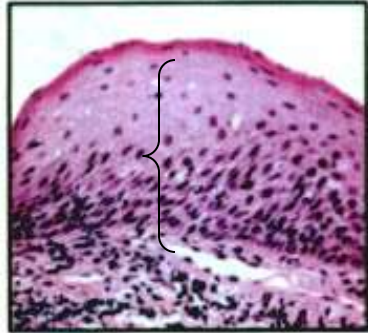
**BREAST
CANCER**

**NOCTURNAL
MELATONIN**

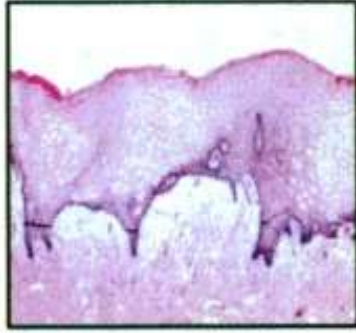
**DIETARY &
ENDOGENOUS
FAT (LA)**



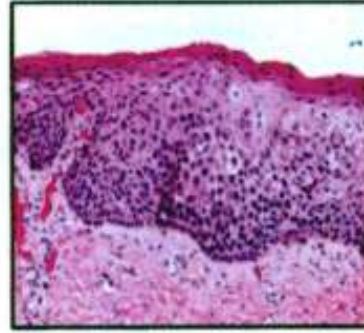
ROLE OF MELATONIN IN CIRCADIAN CANCER PREVENTION



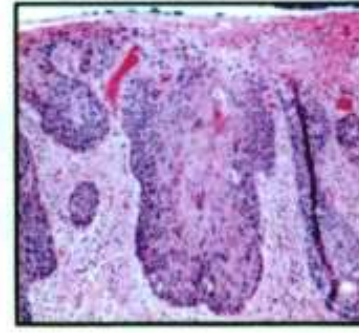
Normal
mucosa



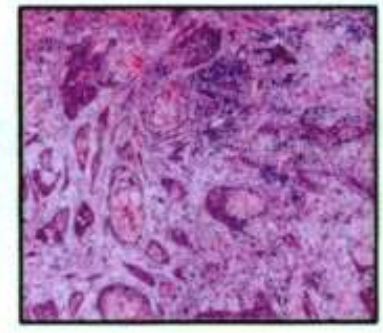
Hyper-
plasia



Dysplasia



CIS

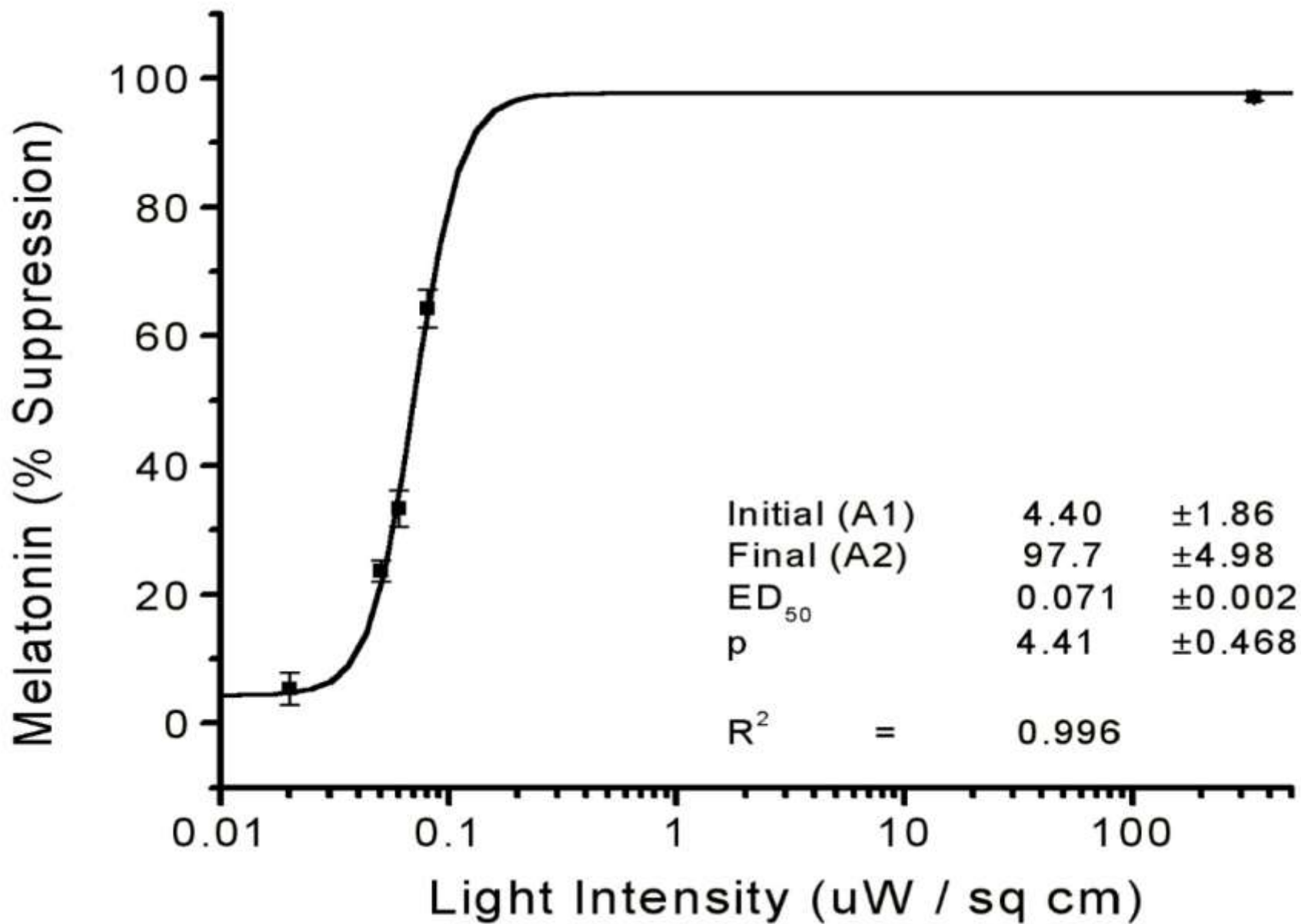


Carcinoma

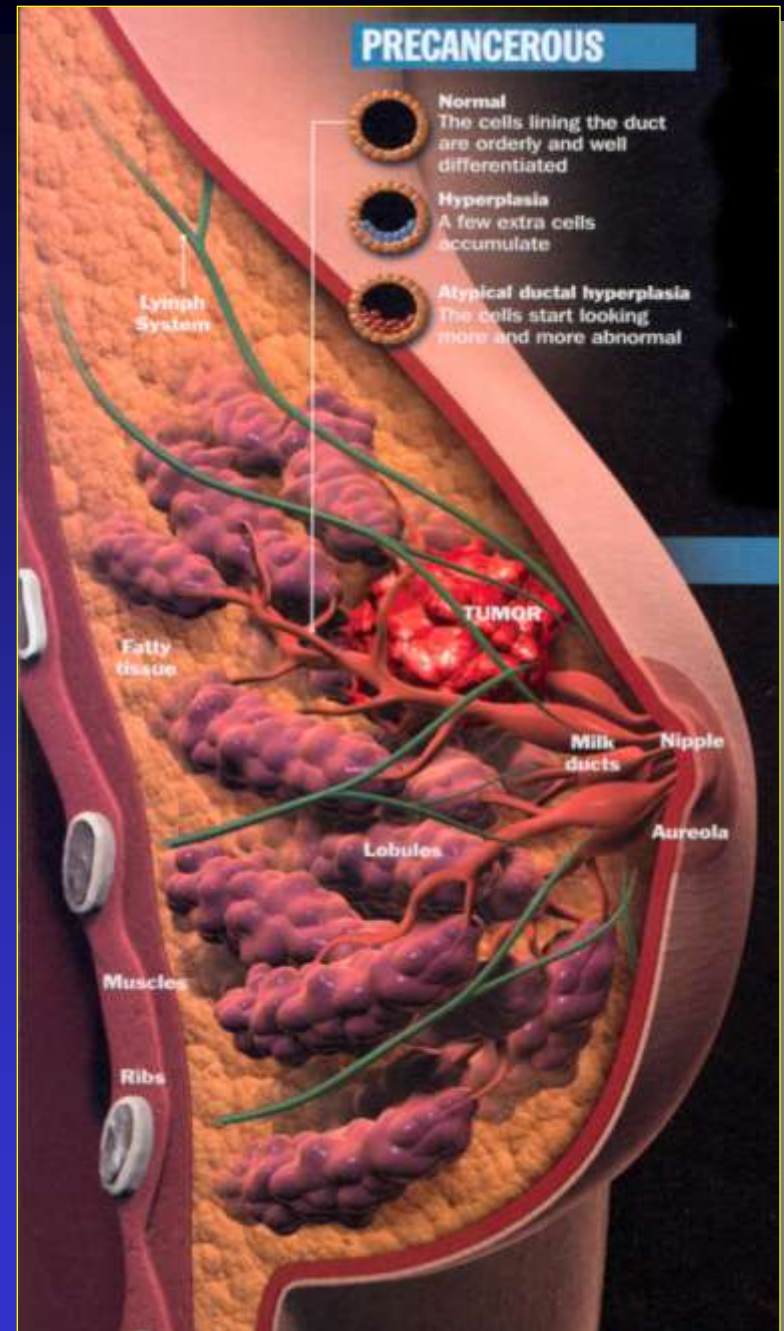
Initiation → Promotion/Progression → Invasion/Metastasis

Melatonin

Initiation (DNA Damage/Repair), Differentiation, Proliferation/
Survival (Apoptosis), Signal Transduction, Metabolism,
Invasion/Metastasis



MELATONIN SUPPLEMENTATION IN THE PREVENTION OF BREAST CANCER GROWTH



Contents

- **Definition of Breast Cancer Growth Prevention, Background and Model System (Tissue-Isolated Human Breast Cancer Xenograft)**
- **Endogenous Circadian Melatonin and Linoleic Acid Signals in Human Breast Cancer Growth Prevention**
- **Circadian Disruption by Dim Light During Darkness (DLDD) of the Endogenous Melatonin Signal**
- **Endogenous Melatonin Signal Reinforcement /Replacement by Exogenous Melatonin in DLDD Conditions in Human Breast Cancer Prevention**
- **Summary, Conclusions and Speculations**

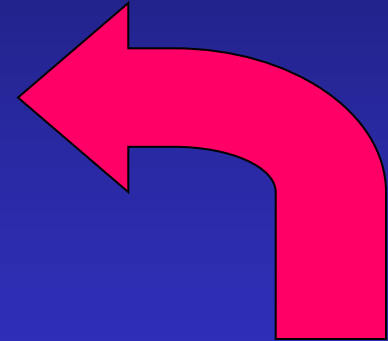
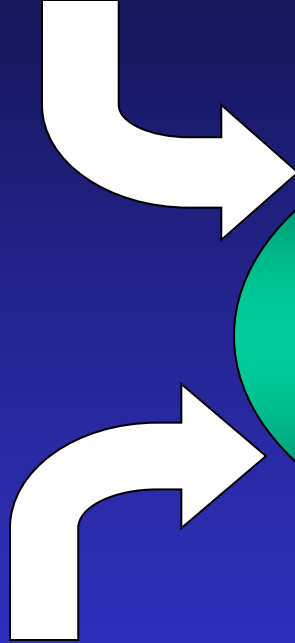
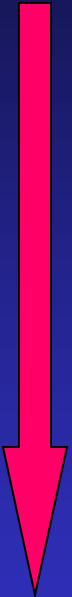
**CIRCADIAN
SYSTEM
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**NOCTURNAL
LIGHT
EXPOSURE**

**BREAST
CANCER**

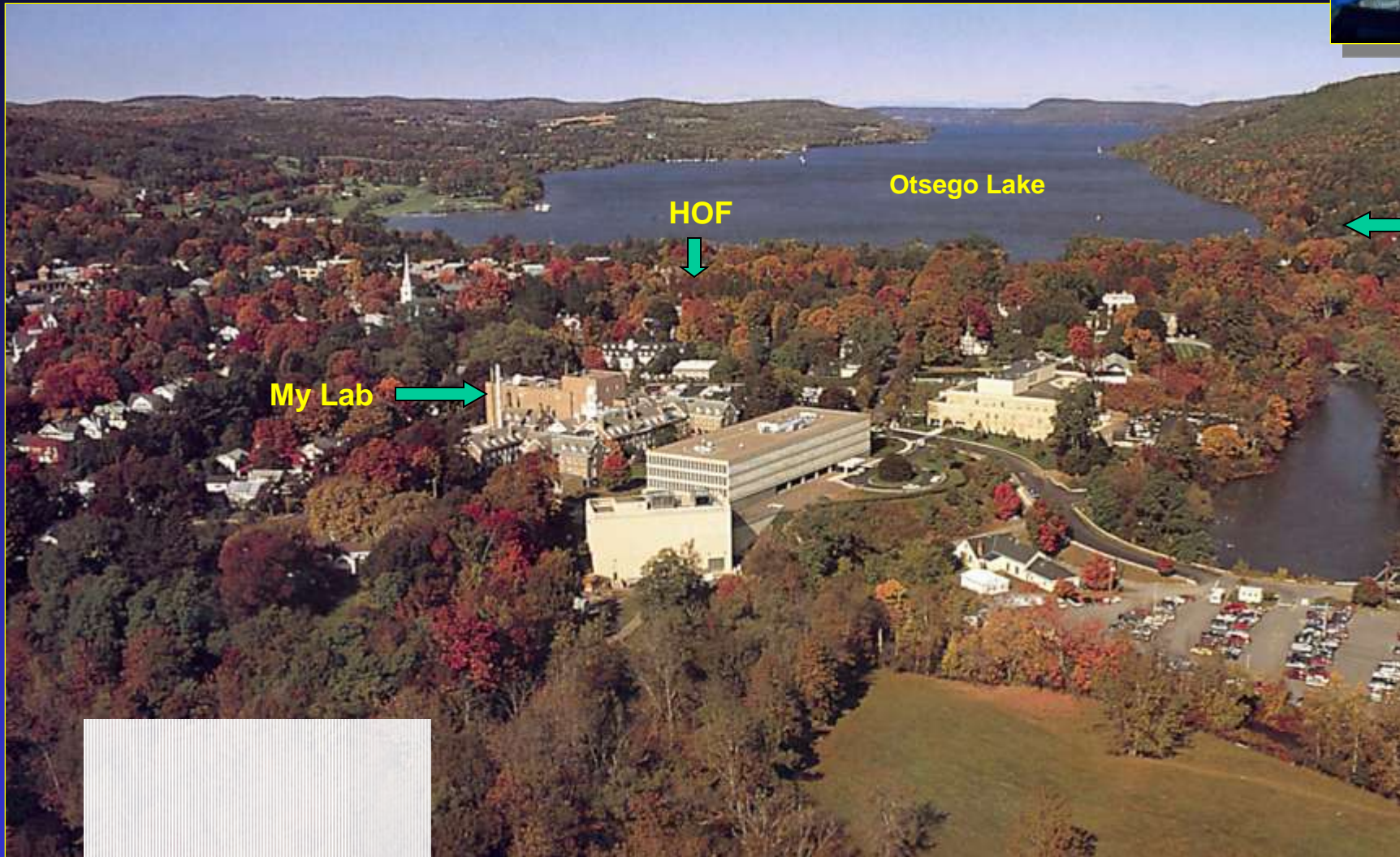
**NOCTURNAL
MELATONIN**

**DIETARY &
ENDOGENOUS
FAT (LA)**



BASSETT RESEARCH INSTITUTE

Cooperstown, New York



Otsego Lake

HOF

My Lab

My House

ENVIRONMENTAL LIGHT/DIETARY INTERACTIONS AND CANCER

- **BIOLOGICAL TIME (CIRCADIAN RHYTHMS)**
- **LIGHT/DARK CYCLES (MELATONIN)**
- **LIGHT AT NIGHT (MELATONIN SUPPRESSION; CIRCADIAN DISRUPTION)**
- **DIETARY FAT (LINOLEIC ACID)**
- **CANCER GROWTH**

LIGHT DURING THE DAY

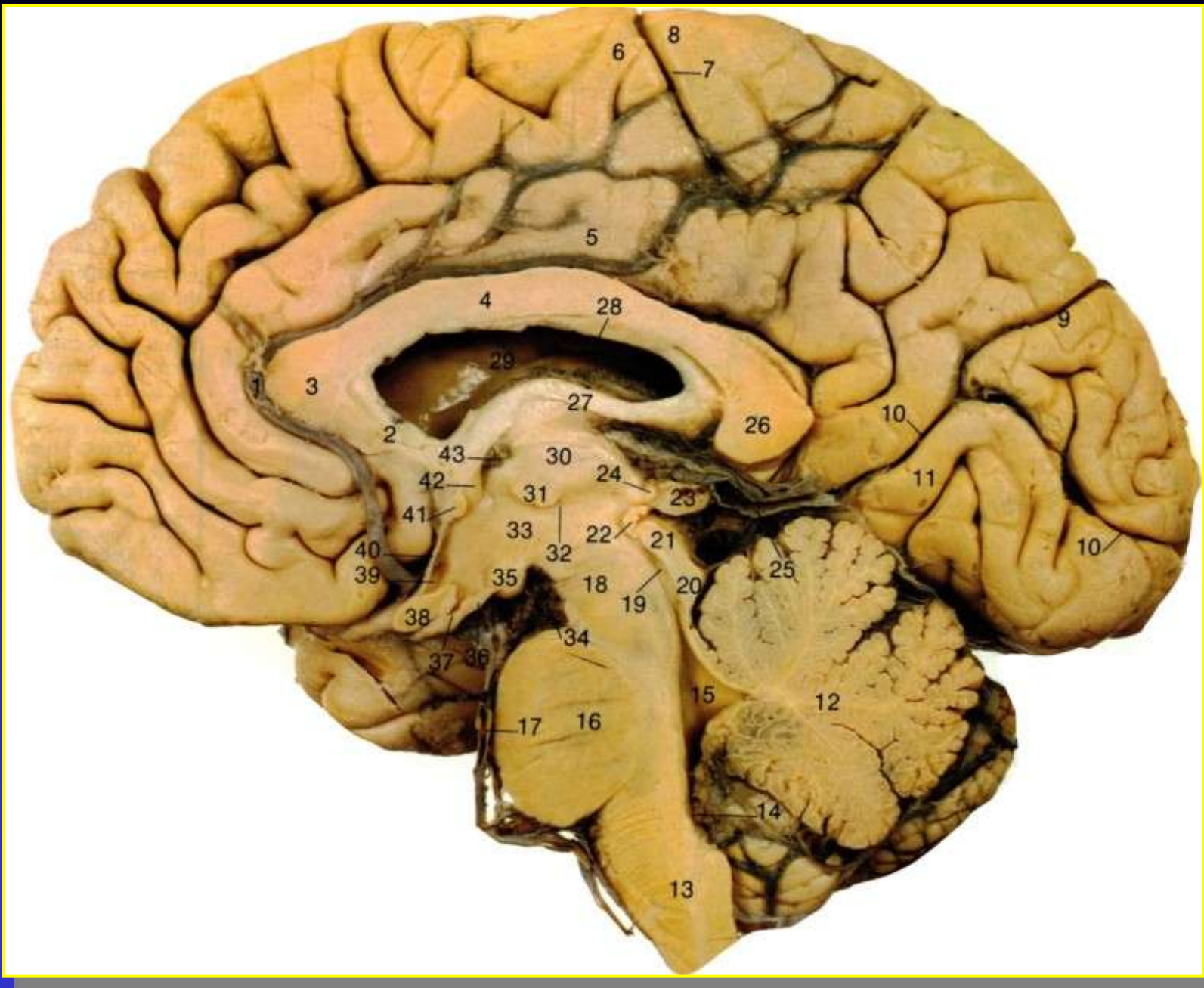
GOOD!!!



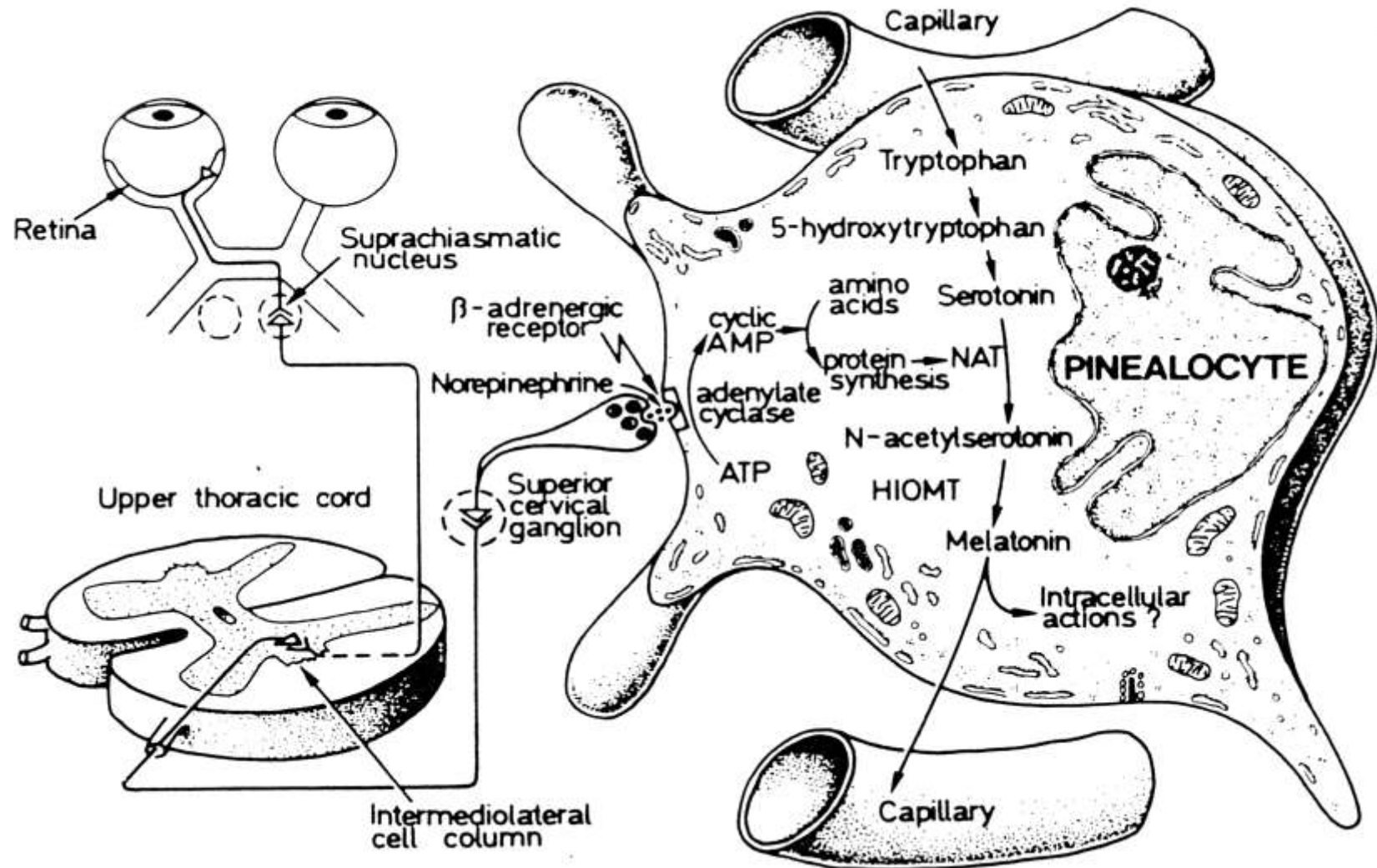
LIGHT DURING THE NIGHT

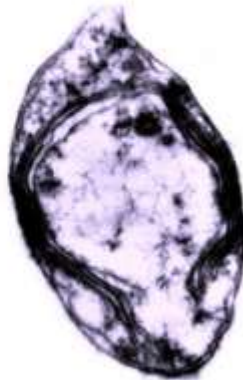
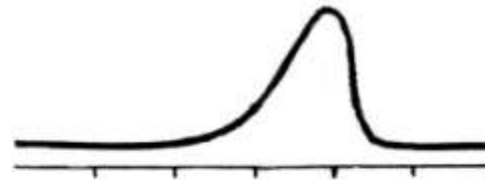
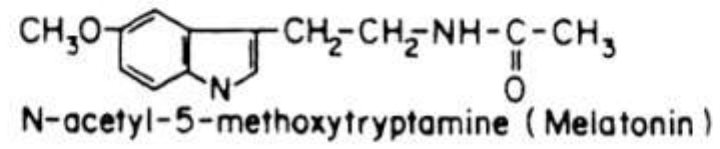
BAD!!!





PINEAL MELATONIN SYNTHESIS

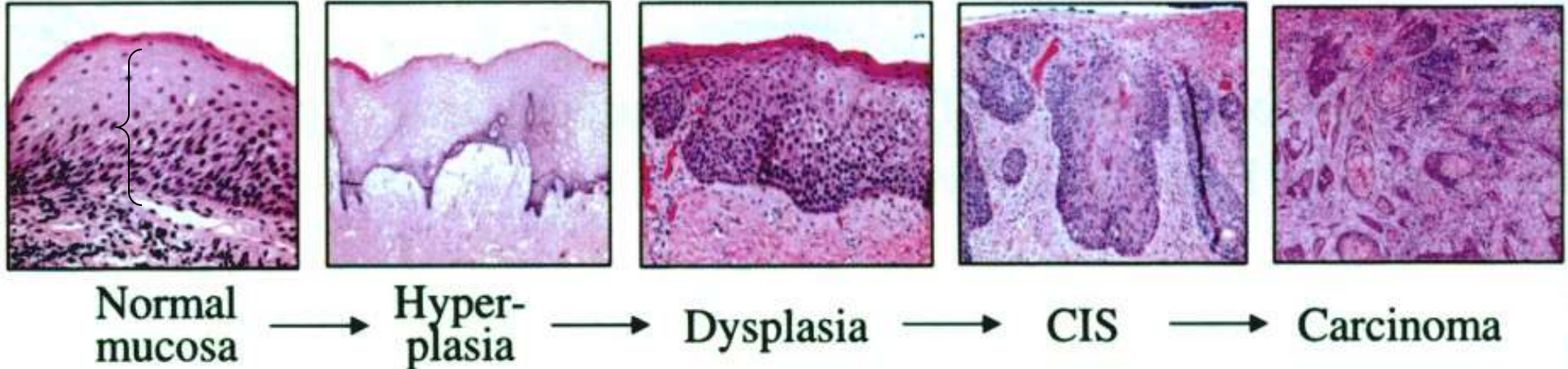




MELATONIN ACTIONS

- **Circadian rhythm regulation**
- **Sleep**
- **Chemical Expression of Darkness**
- **Seasonal reproduction**
- **Retinal physiology**
- **Antioxidant/free radical scavenger**
- **Cardiovascular regulation**
- **Immune activity**
- **Cancer control**
- **Lipid and glucose metabolism**
- **Mitochondrial function**

CARCINOGENESIS: DEVELOPMENT, GROWTH AND SPREAD OF CANCER



Initiation → Promotion/Progression → Invasion/Metastasis

MELATONIN (+ or -)

Initiation (DNA Damage/Repair), Differentiation, Proliferation/
Survival (Apoptosis), Signal Transduction, Metabolism,
Invasion/Metastasis

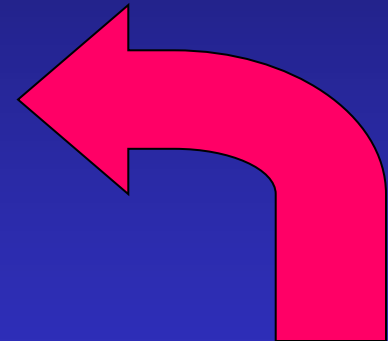
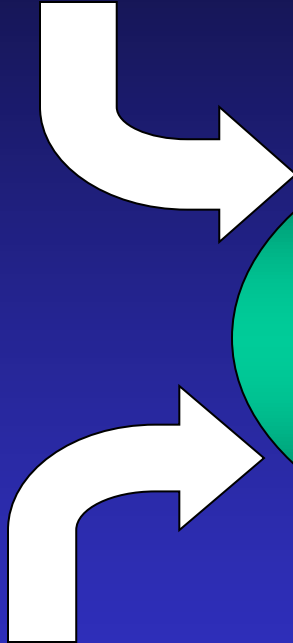
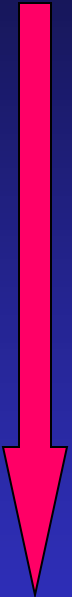
**CIRCADIAN
SYSTEM
(BIOLOGICAL
CLOCK)**

**NOCTURNAL
LIGHT
EXPOSURE**

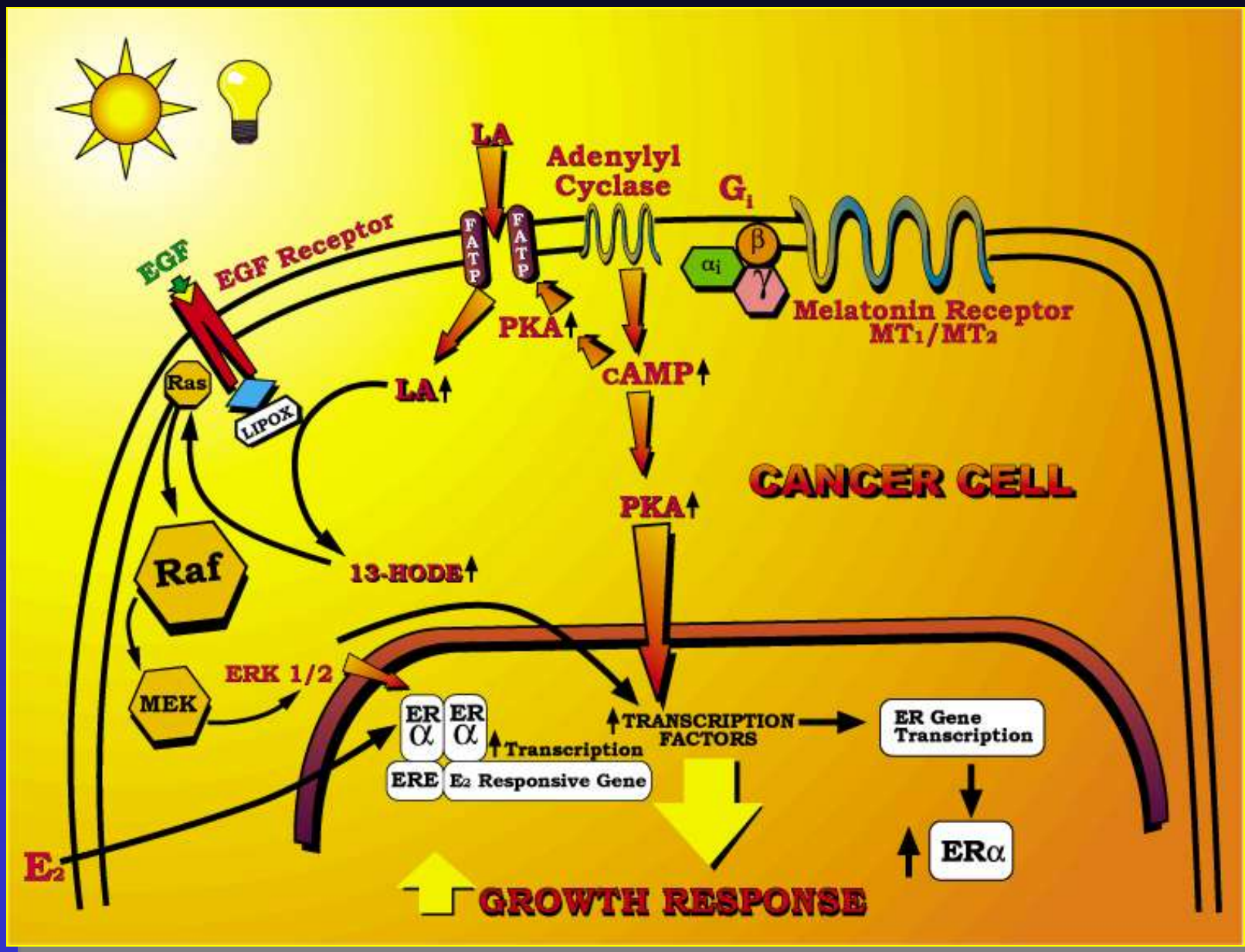
**BREAST
CANCER**

**NOCTURNAL
MELATONIN**

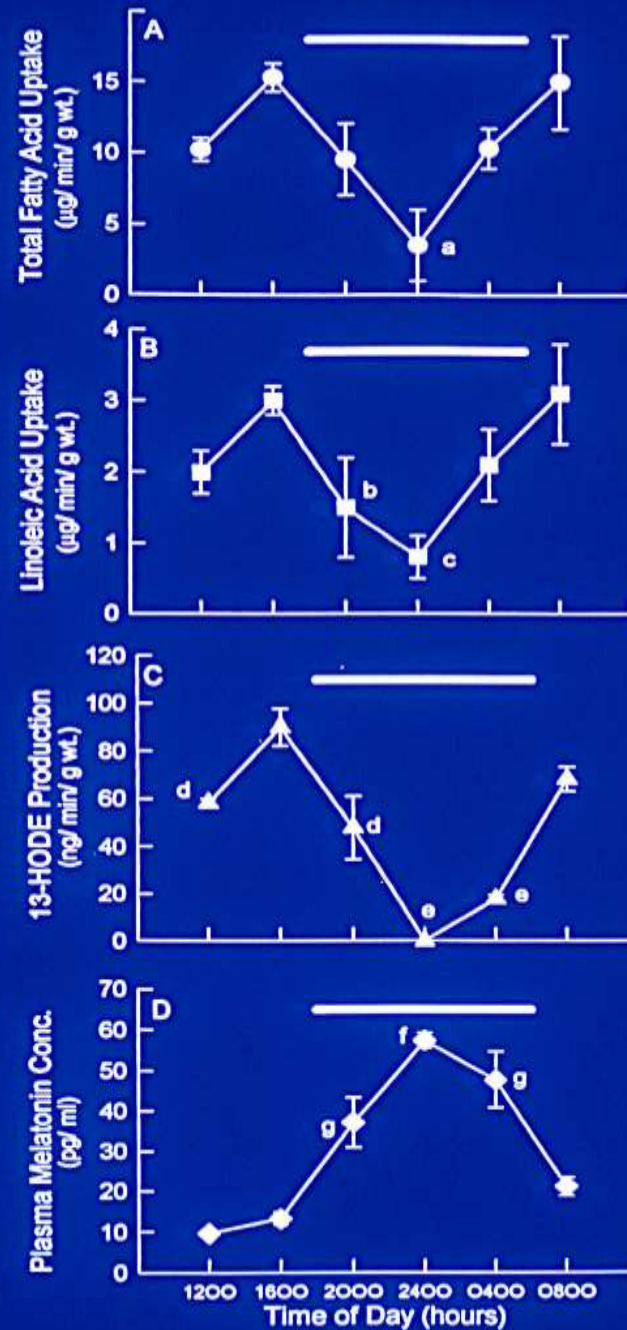
**DIETARY &
ENDOGENOUS
FAT (LA)**

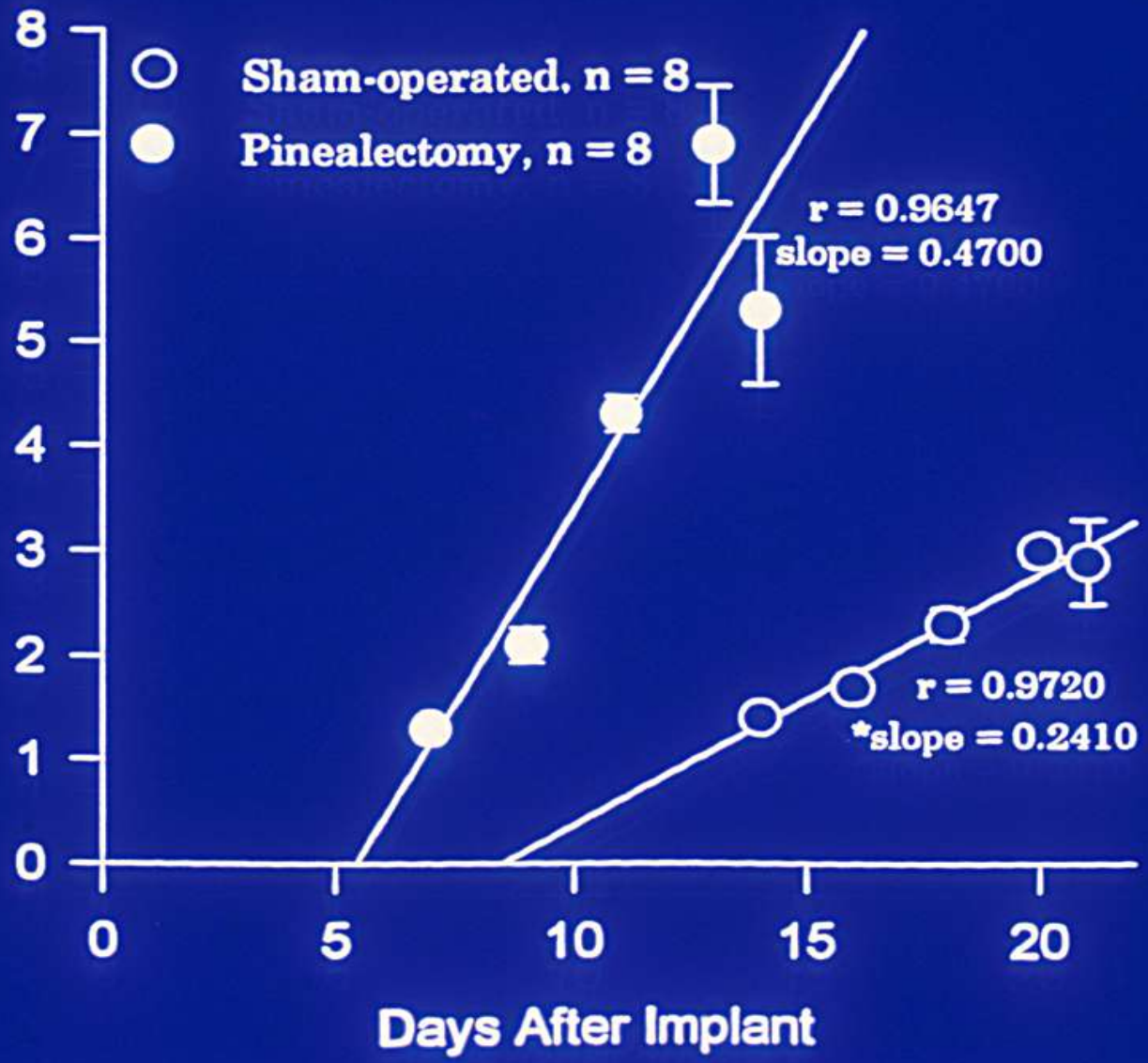


SIGNAL TRANSDUCTION IN TISSUE-ISOLATED TUMORS

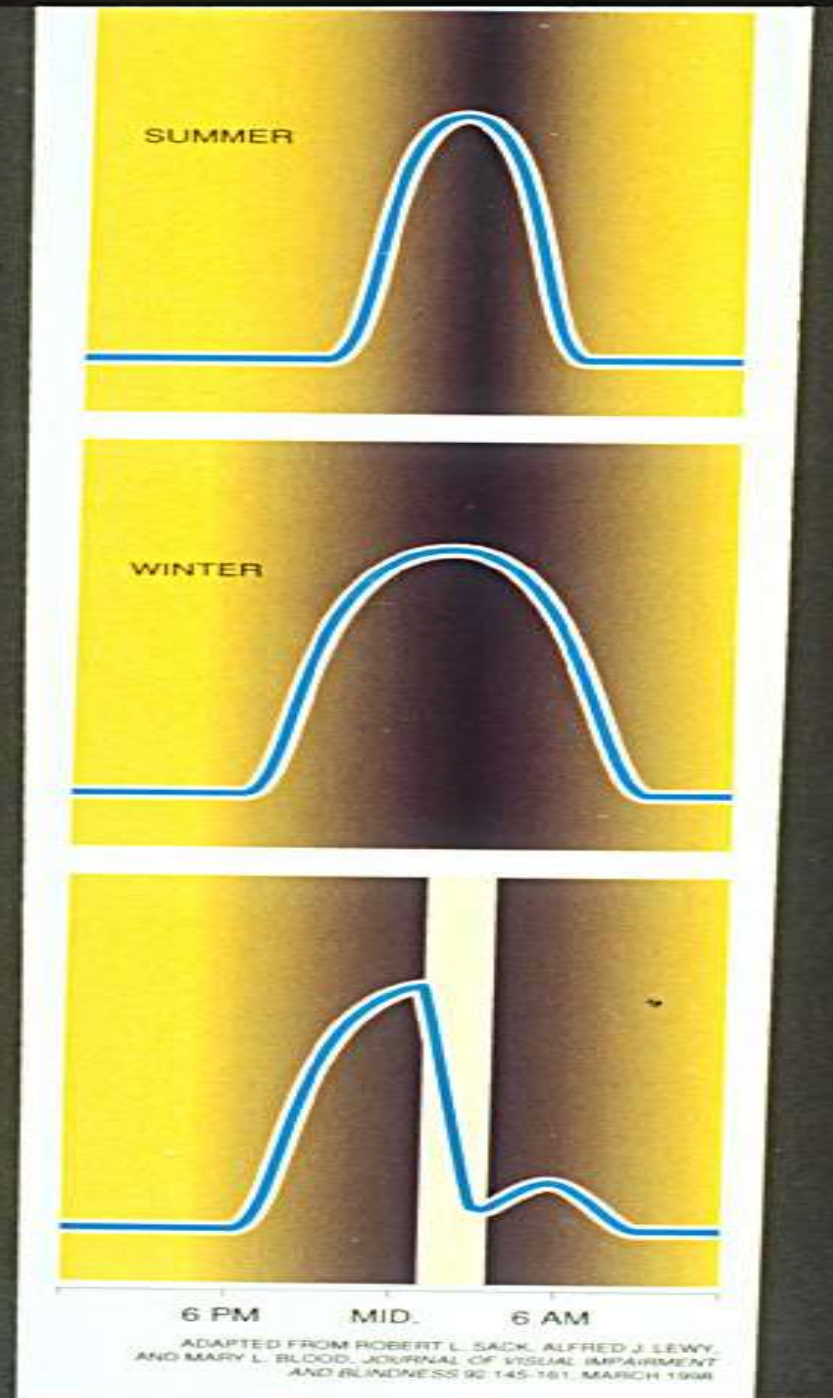


CIRCADIAN MELATONIN-DRIVEN RHYTHM OF TUMOR FATTY ACID UPTAKE AND METABOLISM

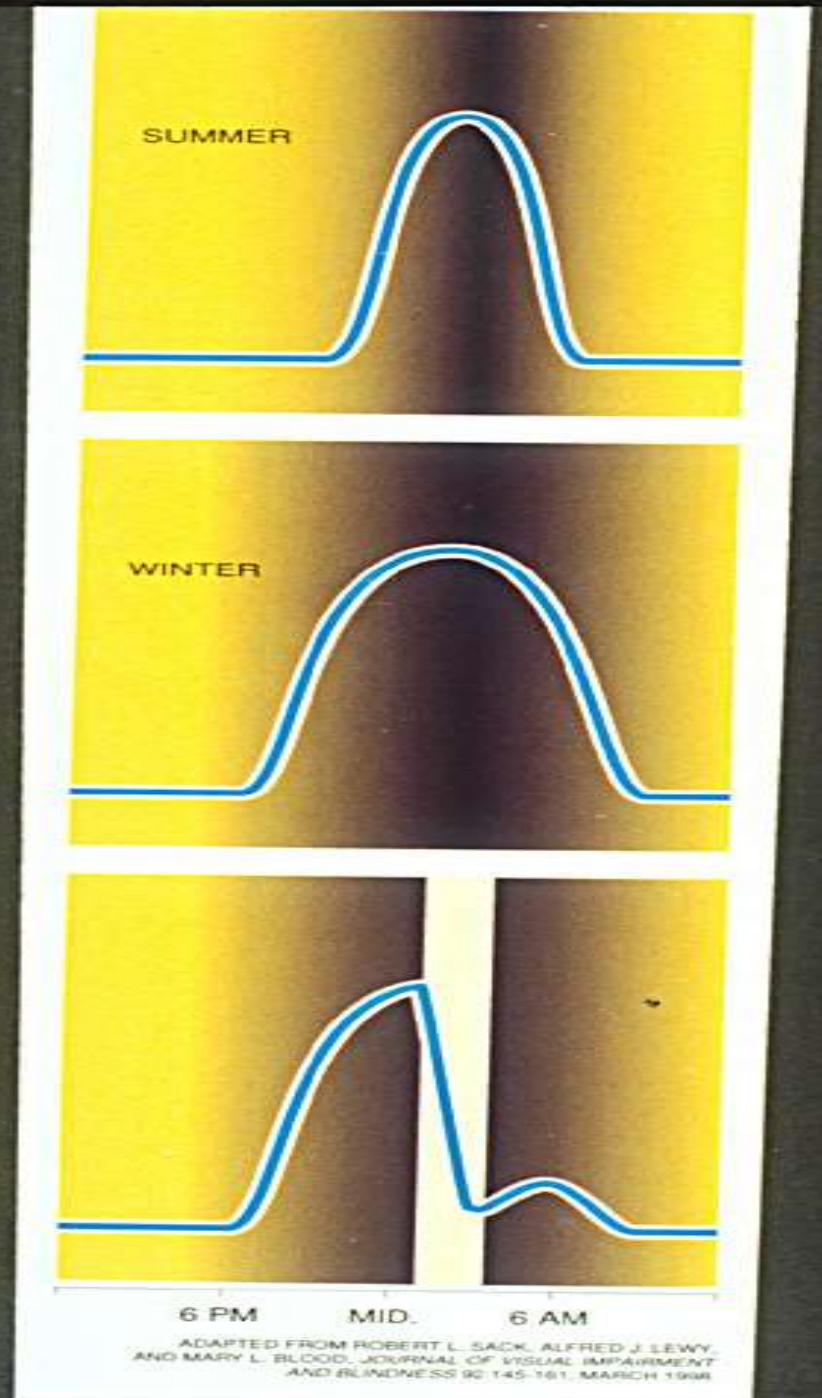


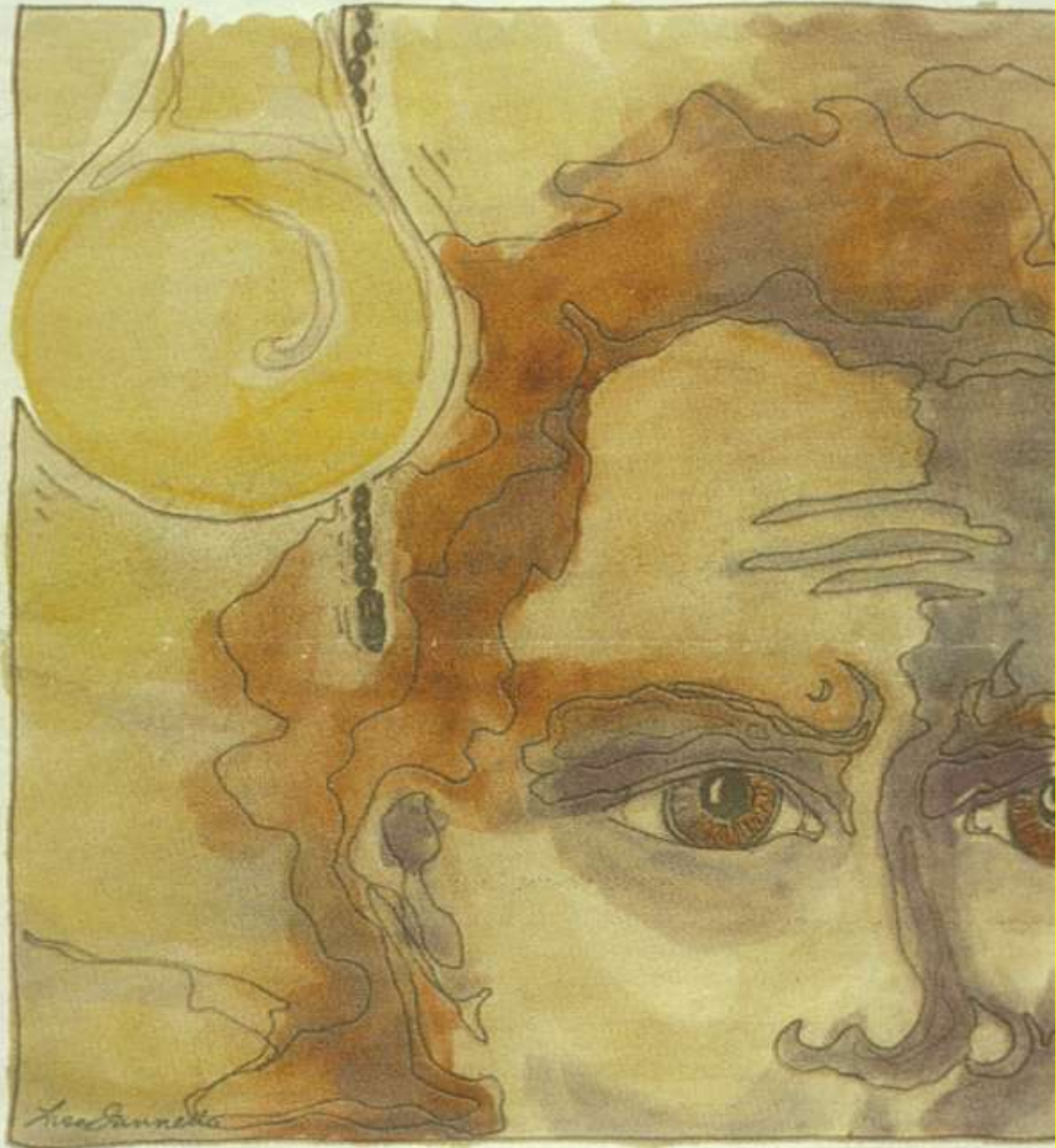


**IMPACT OF
PHOTOPERIODIC
DAYLENGTH ON THE
DURATION OF THE
NOCTURNAL
MELATONIN SIGNAL**



**IMPACT OF
PHOTOPERIODIC
DAYLENGTH ON THE
DURATION OF THE
NOCTURNAL
MELATONIN SIGNAL**





Lisa Iannello

illustration by Lisa Iannello, L.M. Sunrise

THE BREAST CANCER EPIDEMIC

- **Breast cancer risk is five times higher in industrialized nations**
- **Risk increases as societies industrialize**
- **Nearly 50% of breast cancer is not explained by known risk factors**
- **What is it about industrialization that explains this increased risk?**

LIGHT AT NIGHT HYPOTHESIS

Stevens, R.G., Amer. J. Epidemiol. 125:556-561, 1987

**EXPOSURE TO LIGHT AT NIGHT
SUPPRESSES
PINEAL MELATONIN PRODUCTION
WHICH MAY EXPLAIN SOME OF THE
HIGH AND UNACCOUNTED FOR RISK OF
BREAST CANCER IN
INDUSTRIALIZED 24-HR/DAY SOCIETIES
(shift work and fat at night)**

Basic Science Evidence Driving the Light at Night Hypothesis and Subsequent Epidemiology in Night Shift Workers

- **Melatonin inhibits experimental breast cancer development and growth in animal models**
- **Pinealectomy or constant light exposure stimulates experimental breast cancer development and growth in animal models**
- **Physiological nocturnal circulating concentrations of melatonin are directly oncostatic to human breast cancer cell proliferation in the culture dish**

Epidemiology of Night Shift Work and Cancer

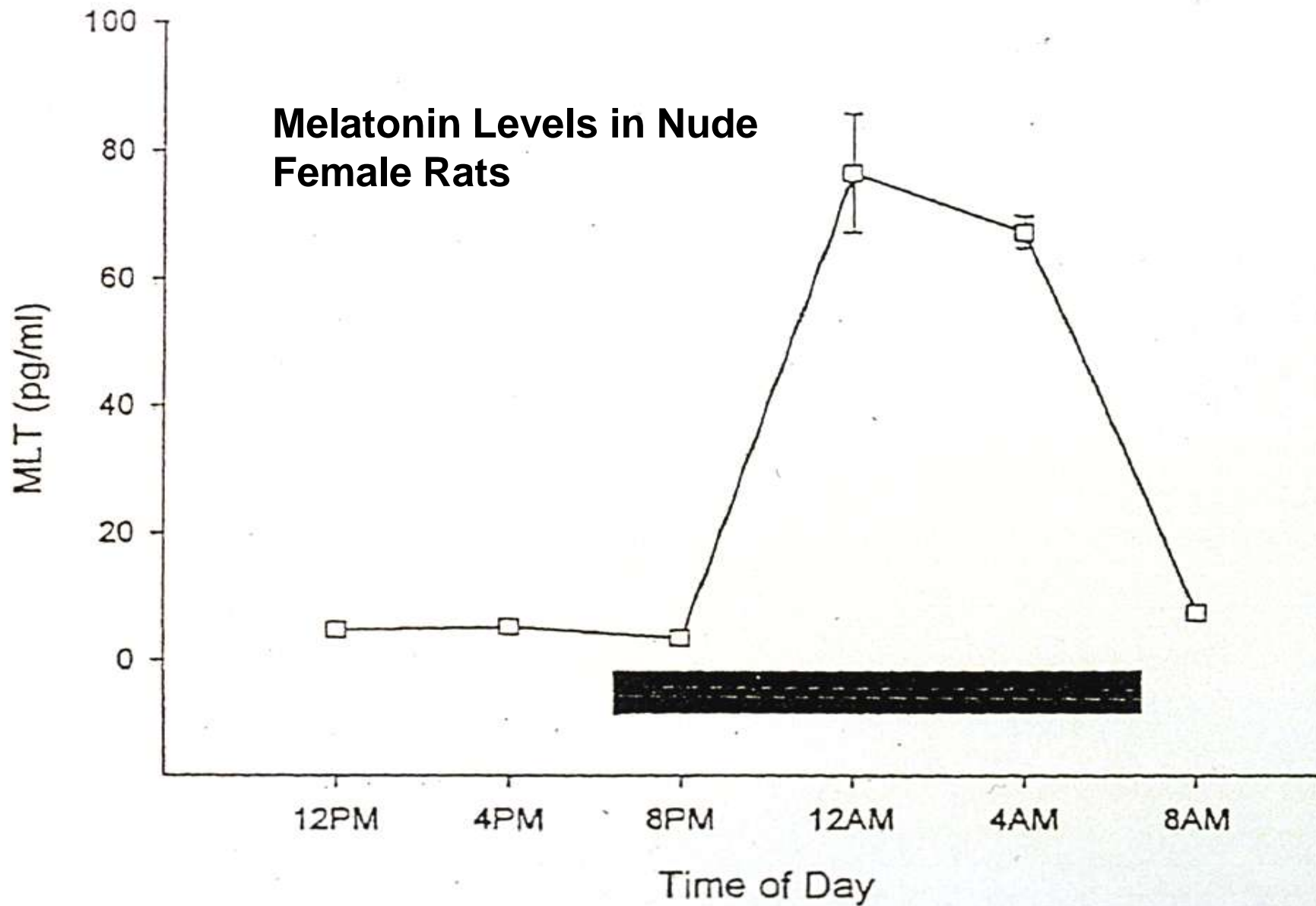
- **Breast Cancer** (mostly postmenopausal - modest increase in risk for long-term rotating night shift workers - 20+ years)
- **2 Prospective cohort studies** (nested in NHS I and II) - (2 +)
- **3 Nested case control studies** - (3+)
- **2 Case control studies** - (1+/1-)
- **1 Nationwide census-based cohort study** - (1-)
- **Prospective** - OR or RR 1.36 - 1.79
- **Retrospective** - OR or RR - 1.2 - 4.0

Epidemiology of Night Shift Work and Cancer

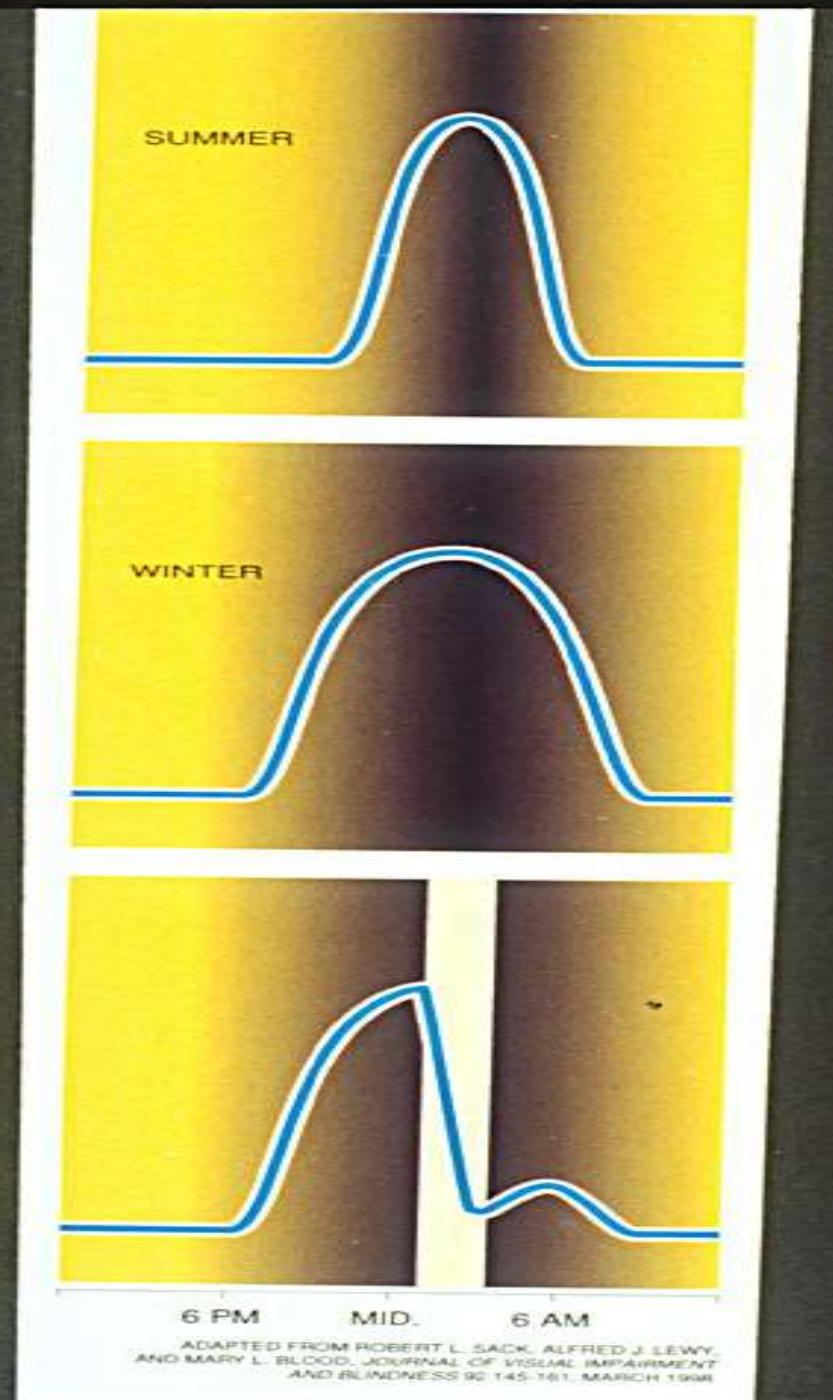
- **Prostate Cancer (fixed and rotating night shifts vs day shift)**
 - 1 Prospective nested in Japan collaborative cohort study evaluating cancer risk (1+) - OR or RR 2.3 (fixed) and 3.0 (rotating)
 - 1 Cancer registry based case control study (1-)
- **Colorectal Cancer (postmenopausal women - modest increase in risk for long-term rotating night shift work - 15+ years)**
 - 1 Prospective cohort study (NHS I) (1+) - OR or RR 1.36
- **Endometrial Cancer (postmenopausal women - modest increase in risk for long-term rotating night shift work - 20+ years)**
 - 1 Prospective cohort study (nested in NHS I) (1+) - OR or RR 1.47

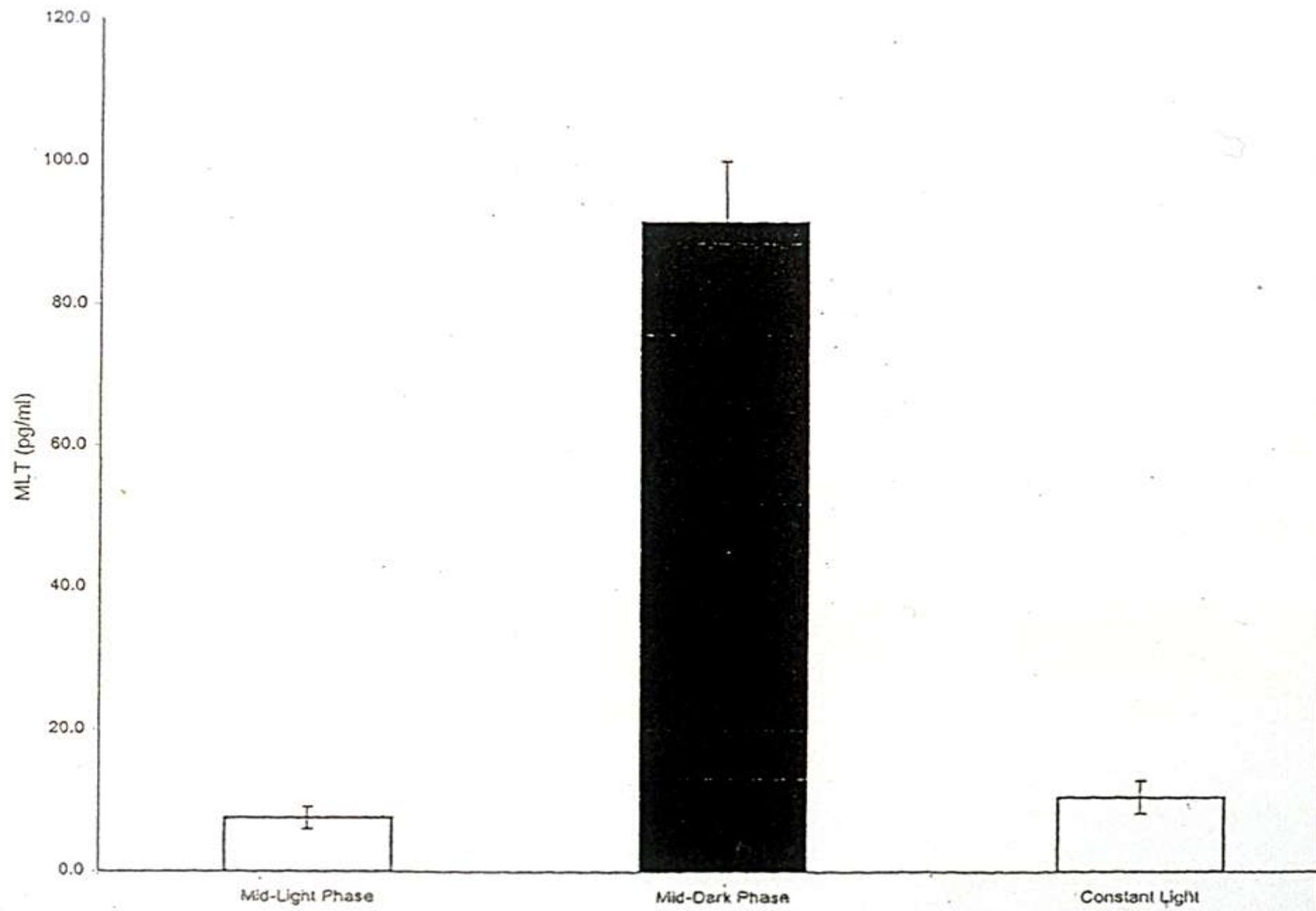
0.08 $\mu\text{W}/\text{cm}^2$ or 0.2 lux, or 0.02 ft.-candles

Melatonin Levels in Nude Female Rats

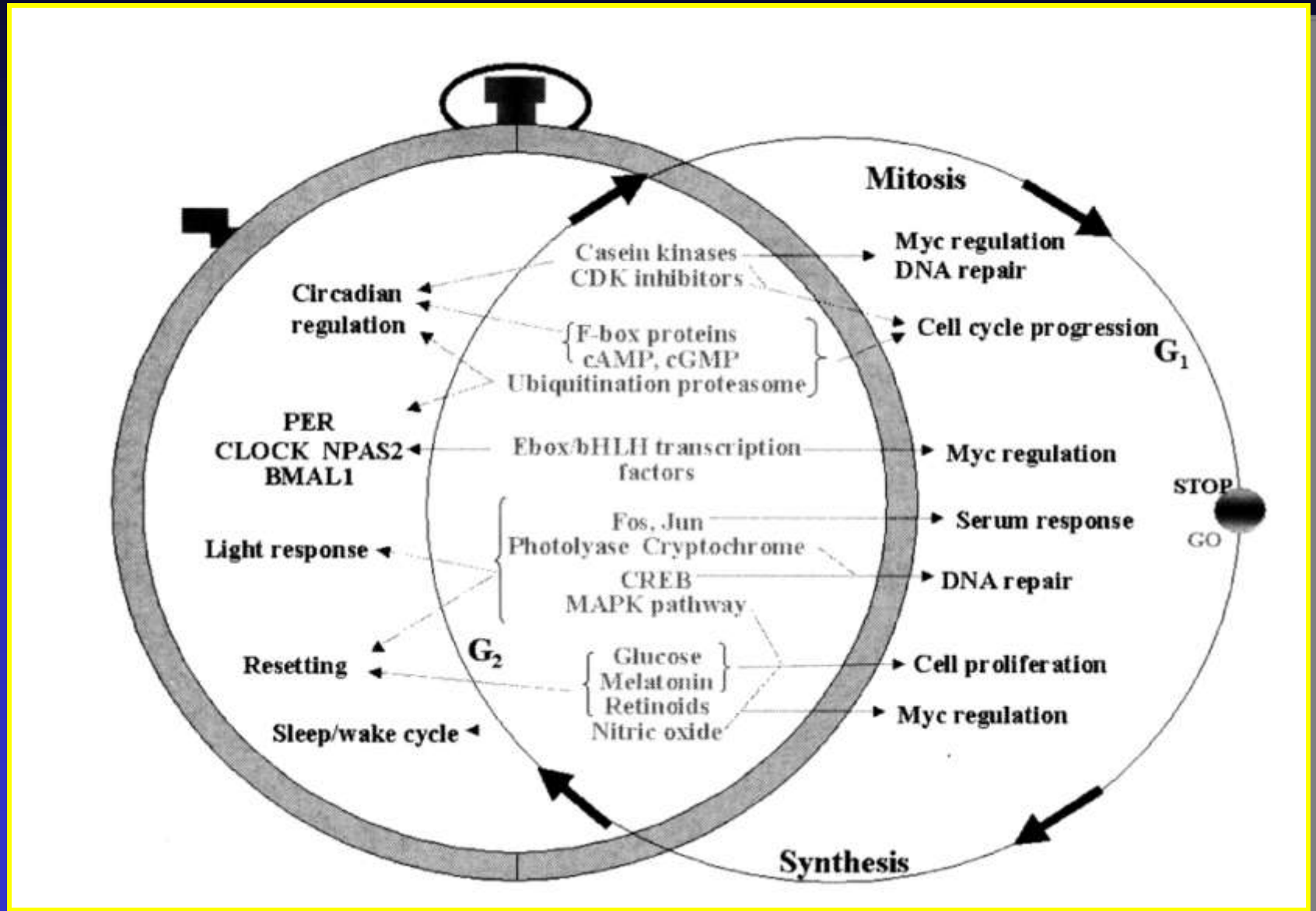


IMPACT OF LIGHT AT NIGHT ON THE NOCTURNAL MELATONIN SIGNAL

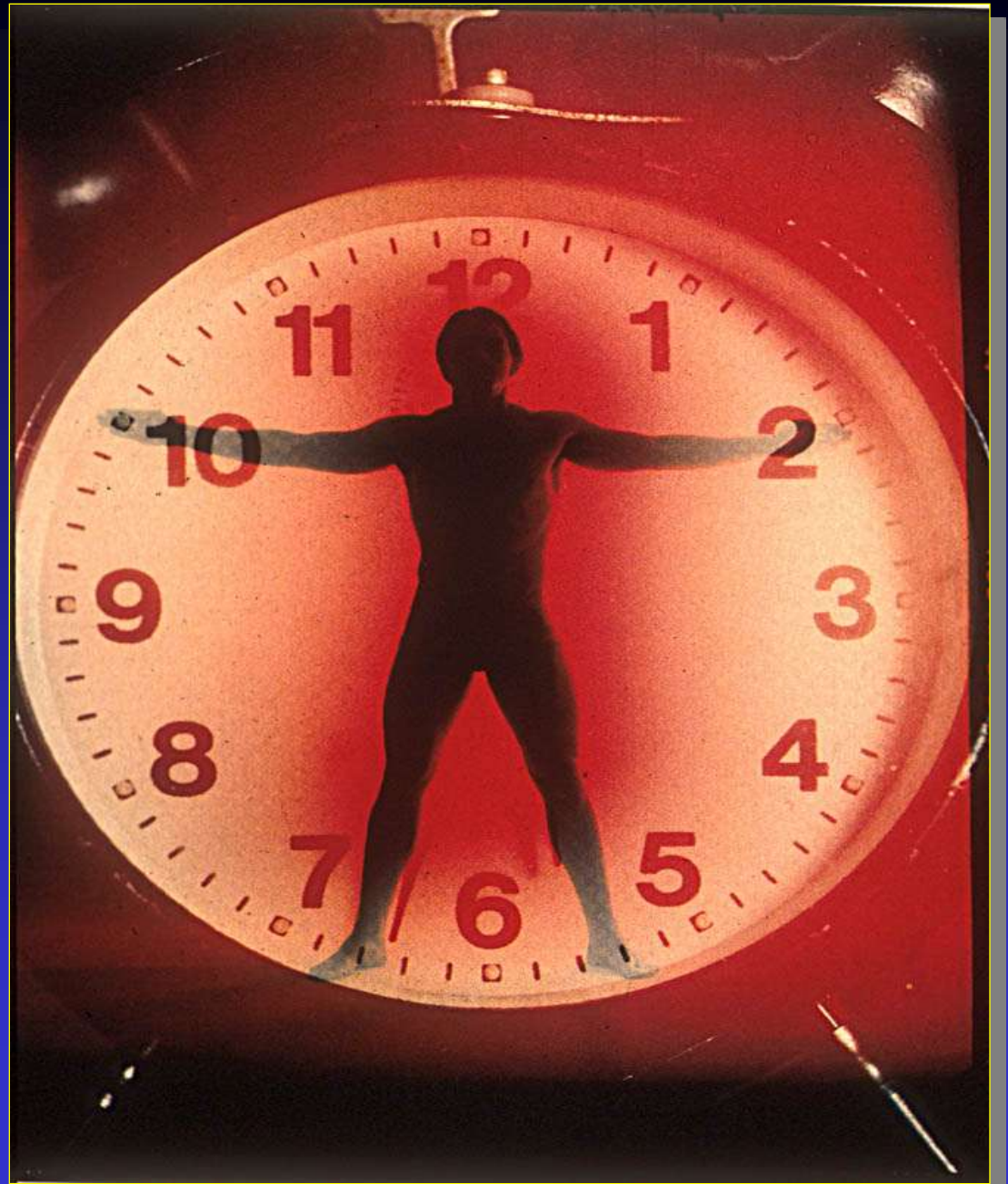


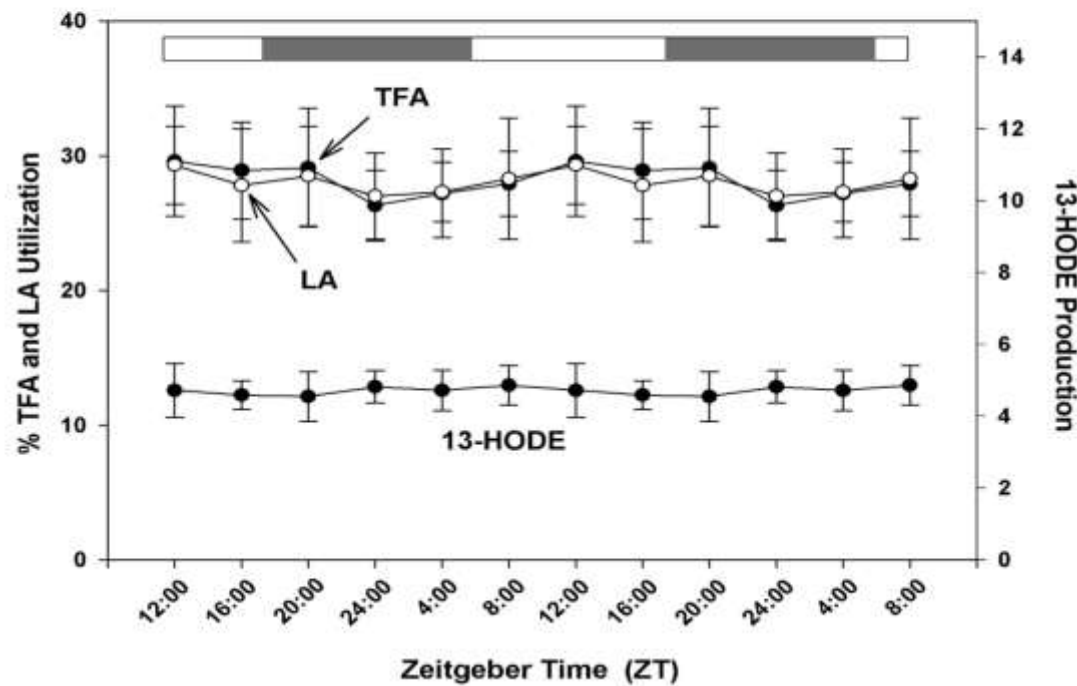
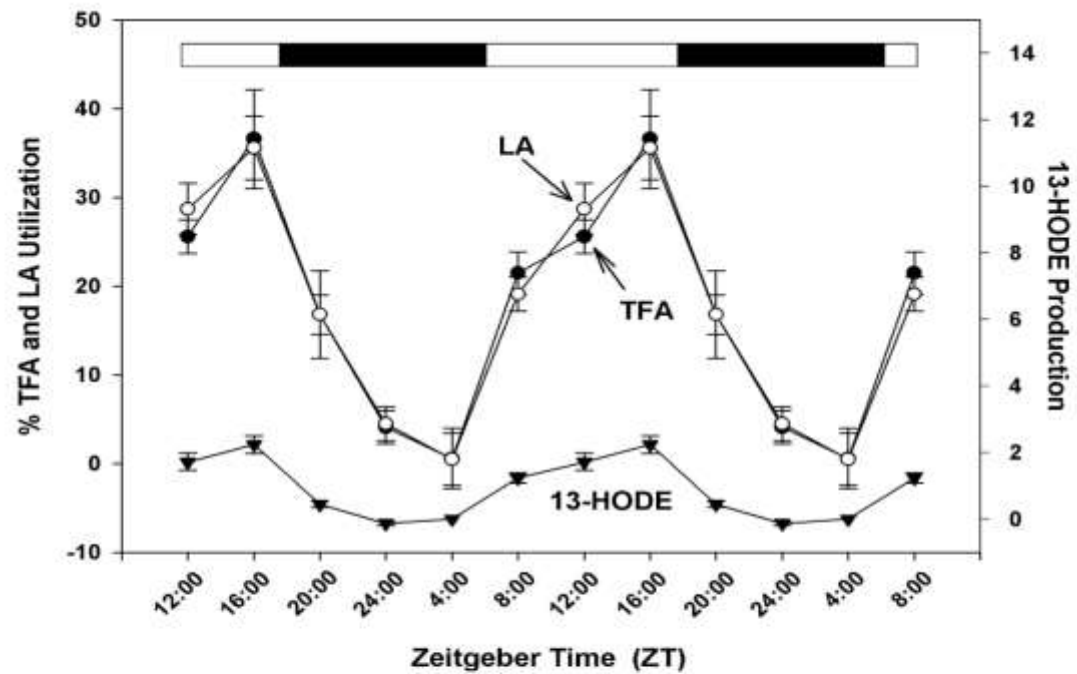


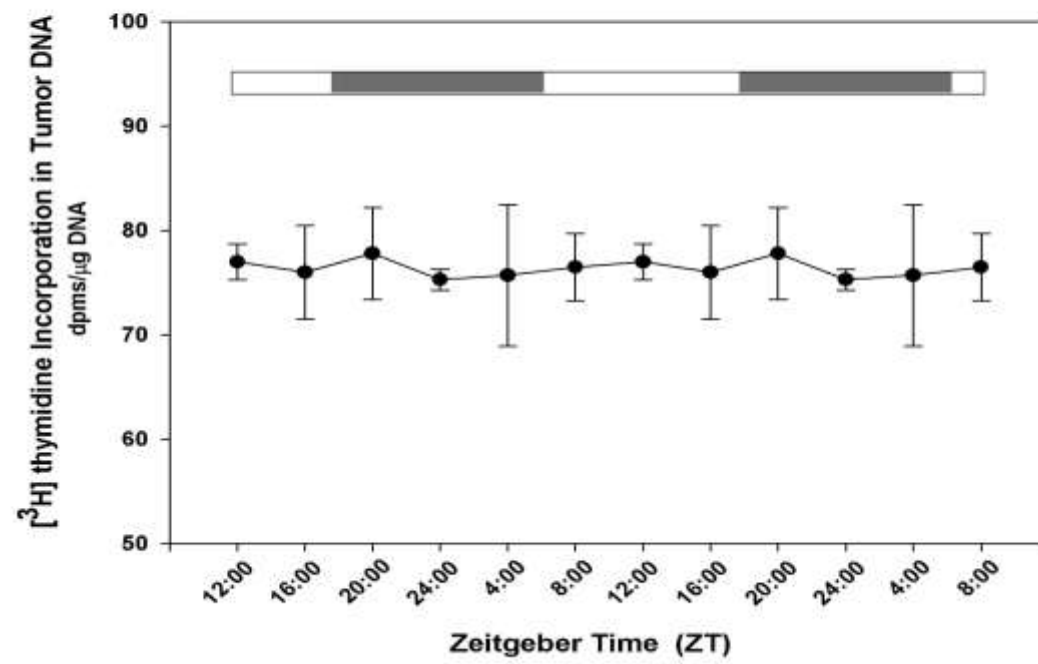
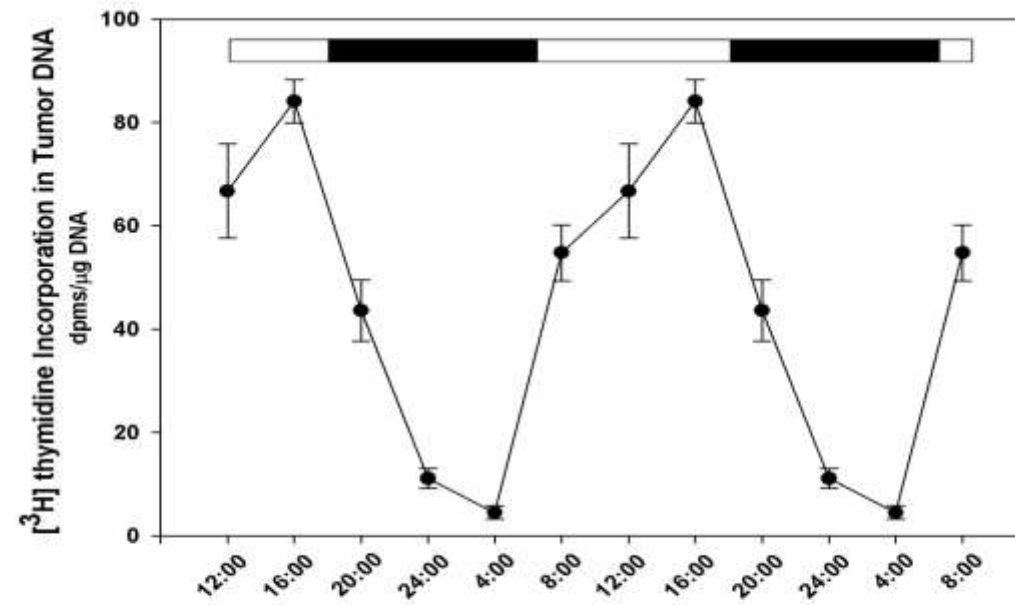
COMMON ELEMENTS SHARED BY BIOLOGICAL CLOCKS & CELL CYCLE



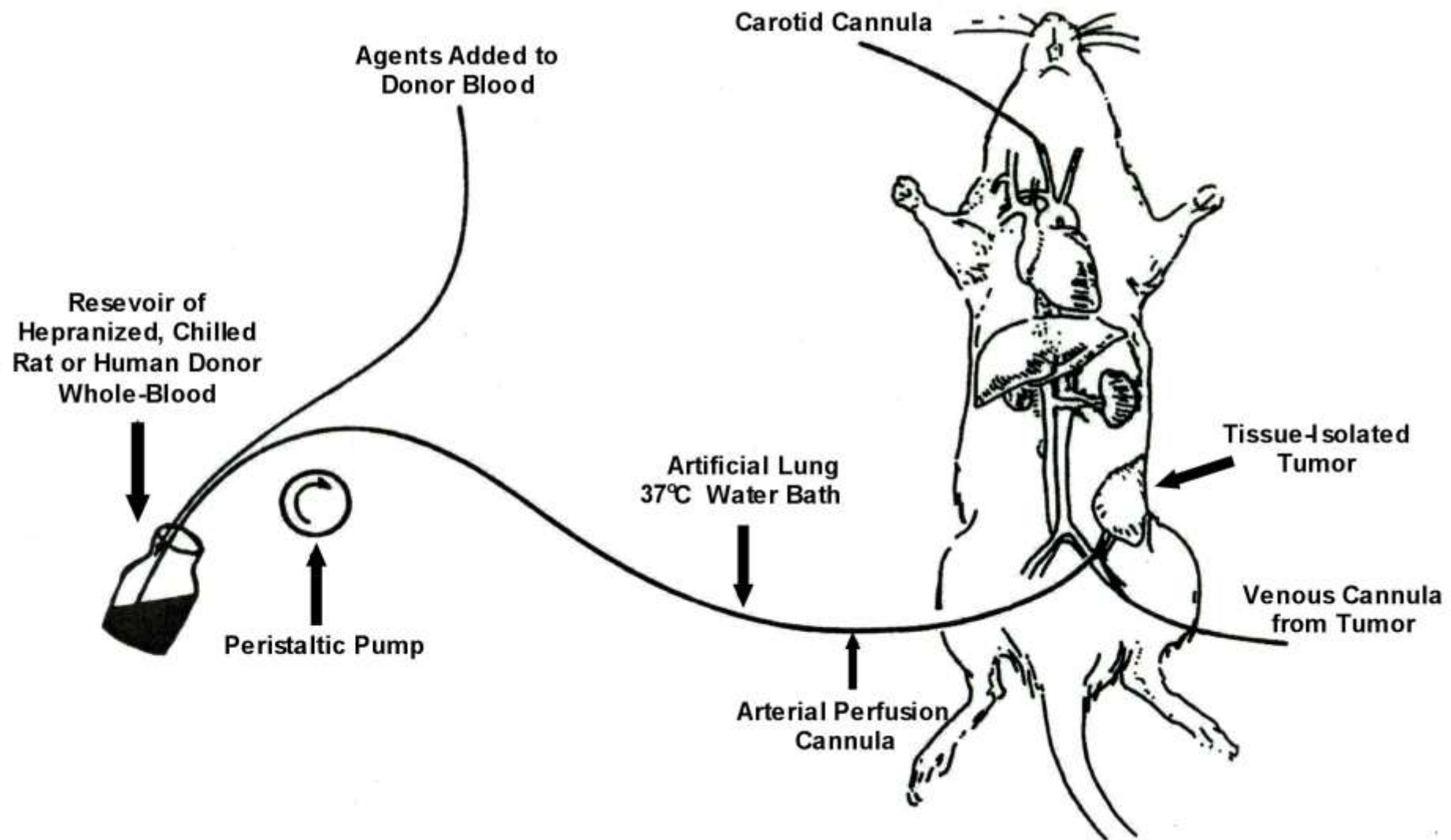
**Your body is
full of clocks**



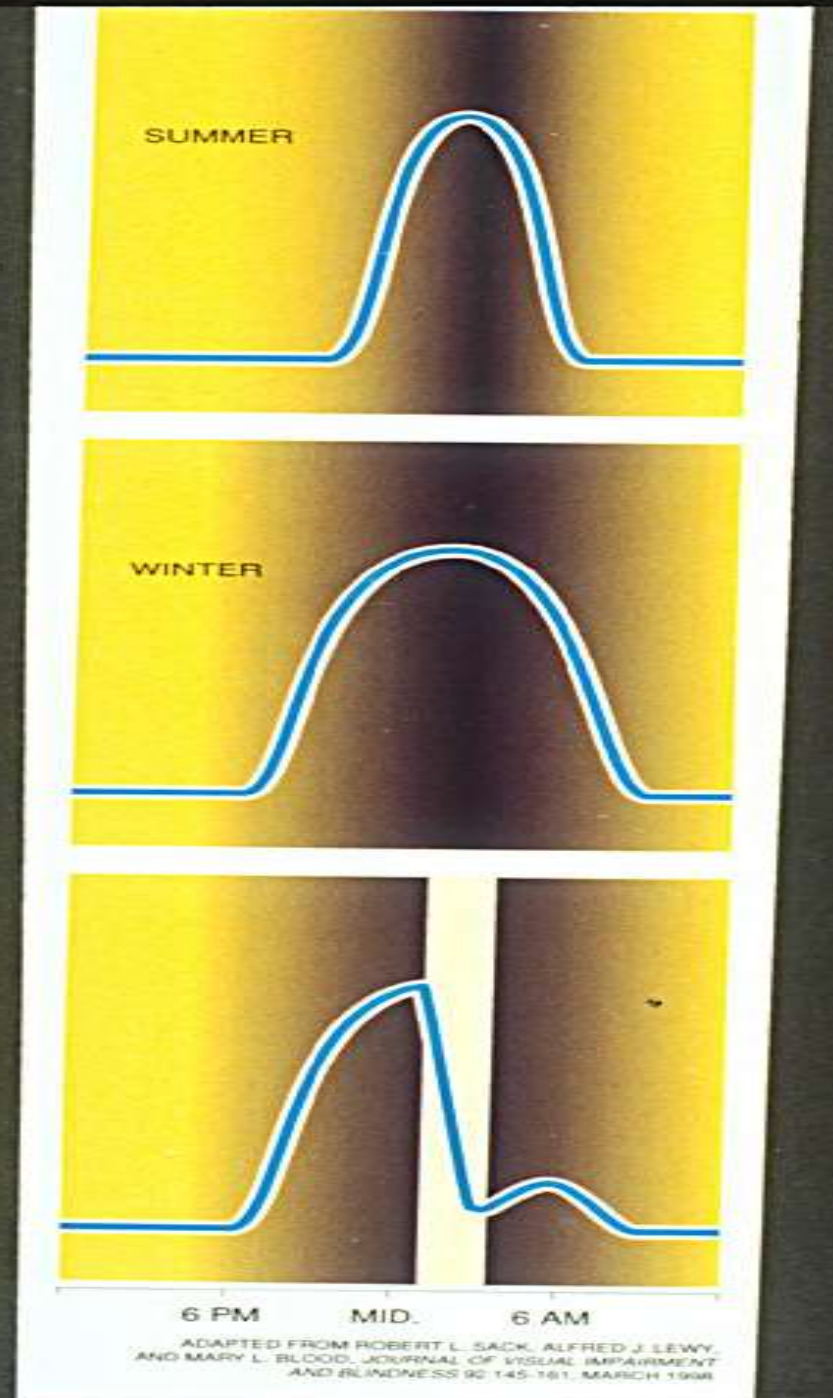




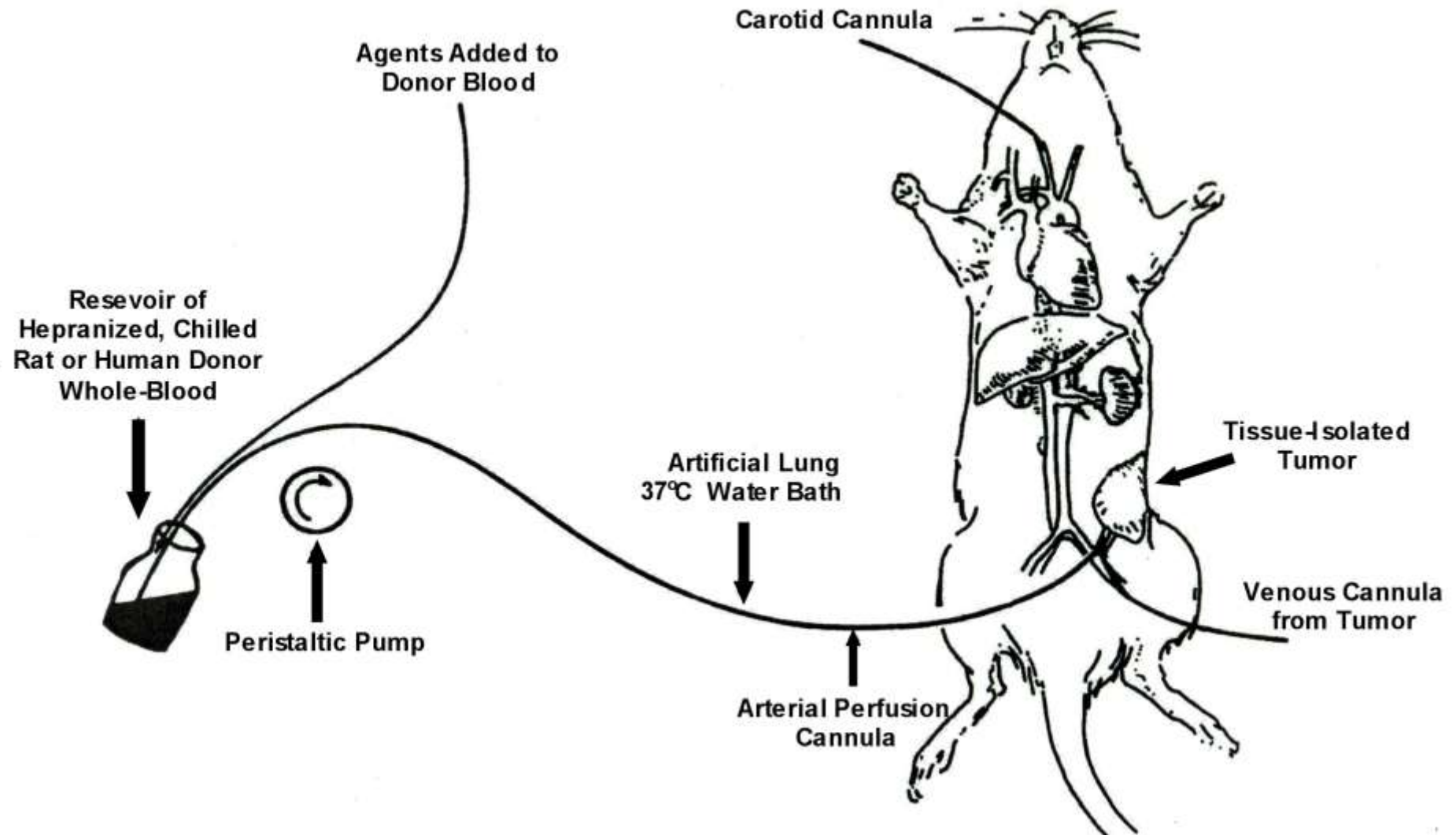
SYSTEM FOR PERFUSION OF TISSUE-ISOLATED TUMORS *IN SITU*



**IMPACT OF
PHOTOPERIODIC
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DURATION OF THE
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MELATONIN SIGNAL**

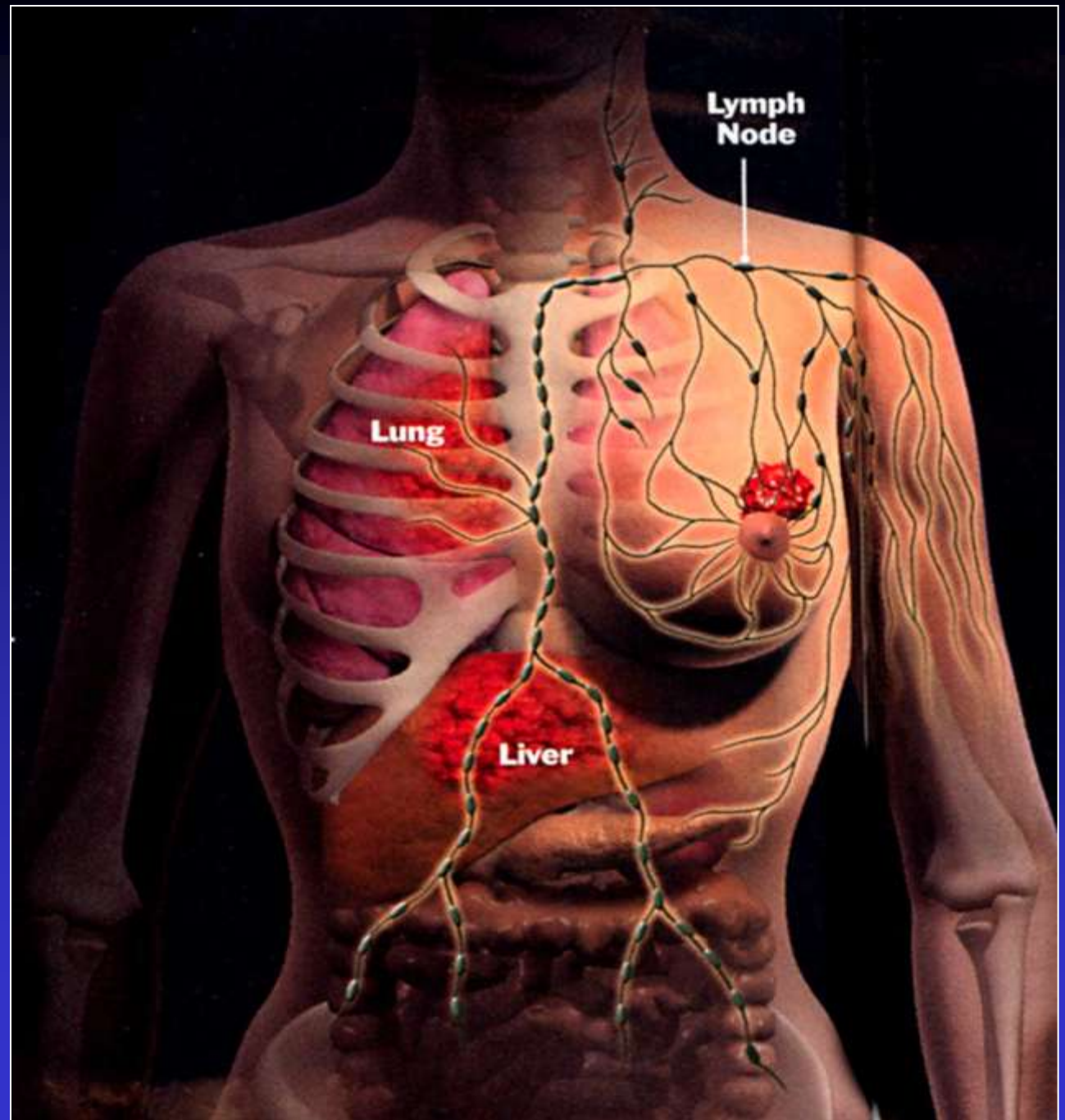


PERFUSION OF TISSUE-ISOLATED (ER-) MCF-7 HUMAN BREAST CANCER XENOGRAFTS *IN SITU* WITH MELATONIN -RICH BLOOD FOLLOWING ORAL MELATONIN SUPPLEMENTATION (3 mg)



**EXPOSURE TO LIGHT
AT NIGHT,
MELATONIN
SUPPLEMENTATION,
AND BREAST
CANCER**

**SUMMARY AND
CONCLUSIONS**



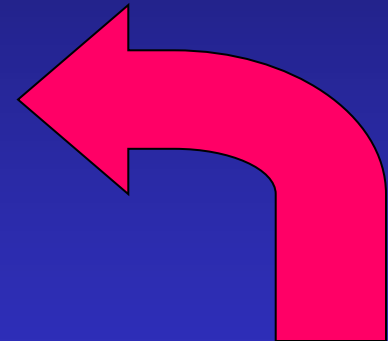
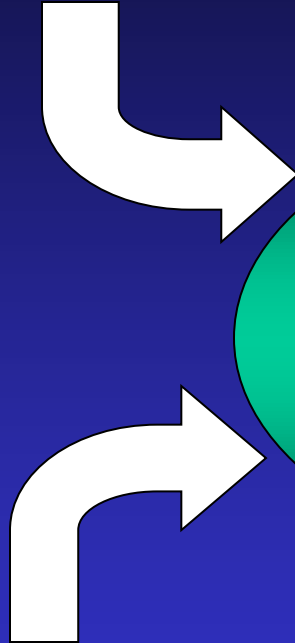
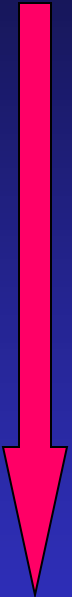
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**DIETARY &
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FAT (LA)**



LIGHT DURING THE DAY

GOOD!!!

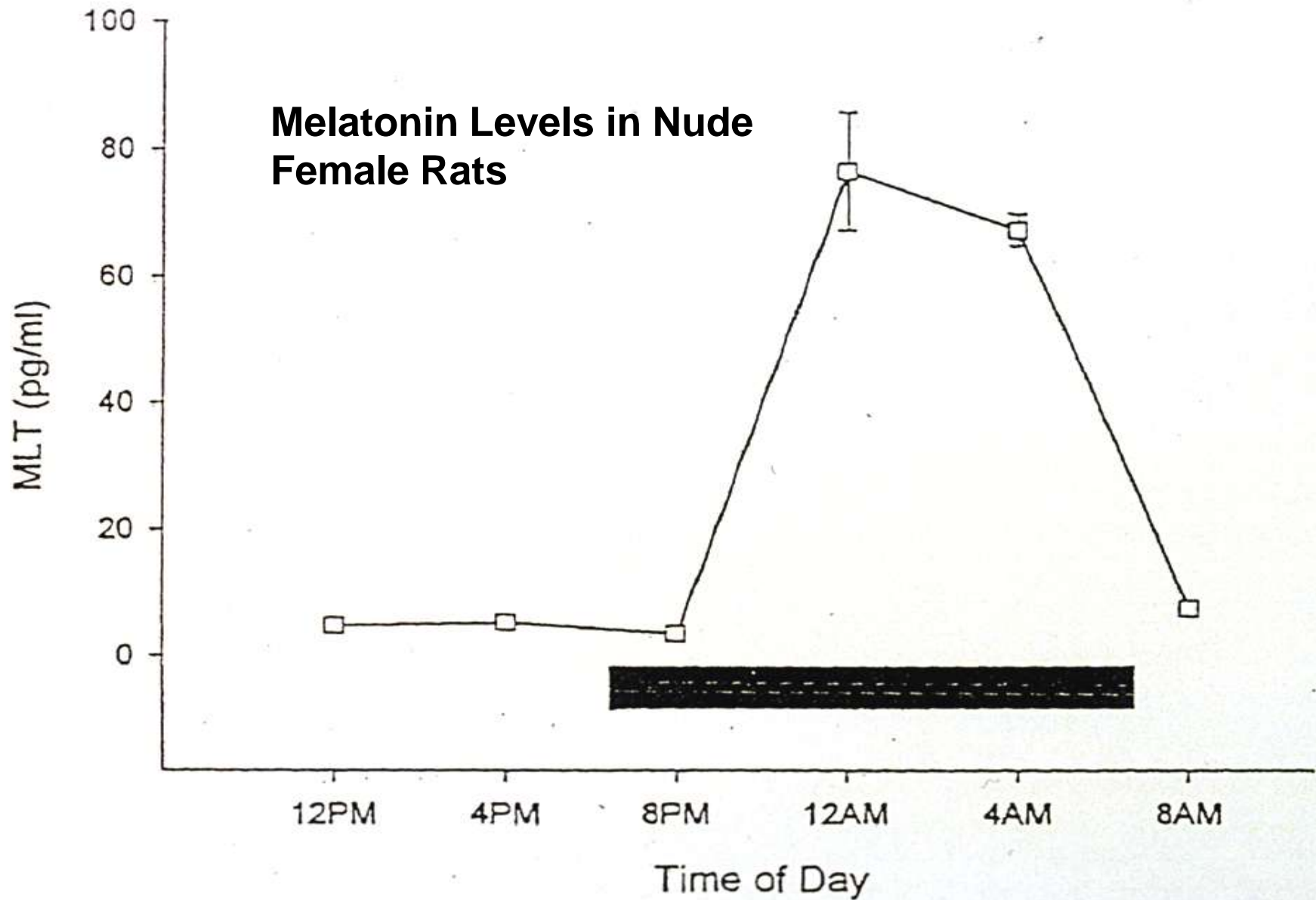


LIGHT DURING THE NIGHT

BAD!!!



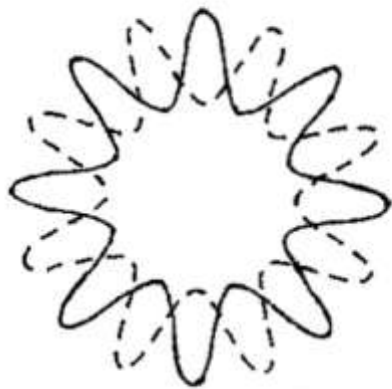
Melatonin Levels in Nude Female Rats



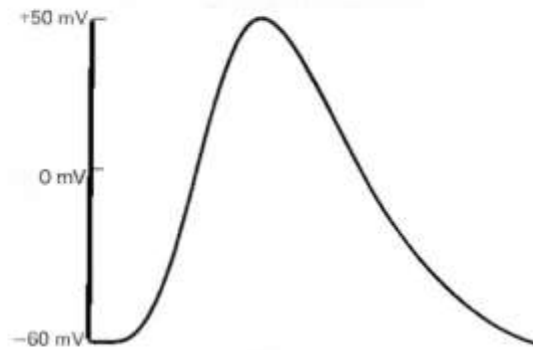
PROSTATE CANCER

- **Epidemic in westernized societies - incidence increasing as mortality decreases**
- **Second leading cause of death in men**
- **Rotating night shift workers have a 3-fold increased risk as compared with day shift workers**
- **Decreased risk in blind men**

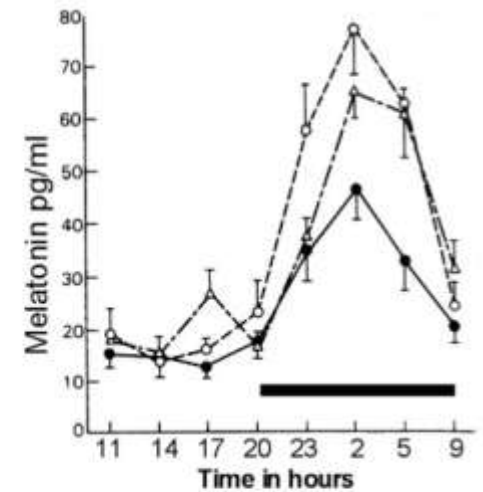
From the Cosmos to the Pineal Gland



Photon



Action Potential



Melatonin Rhythm

LIGHT DURING THE DAY

GOOD!!!



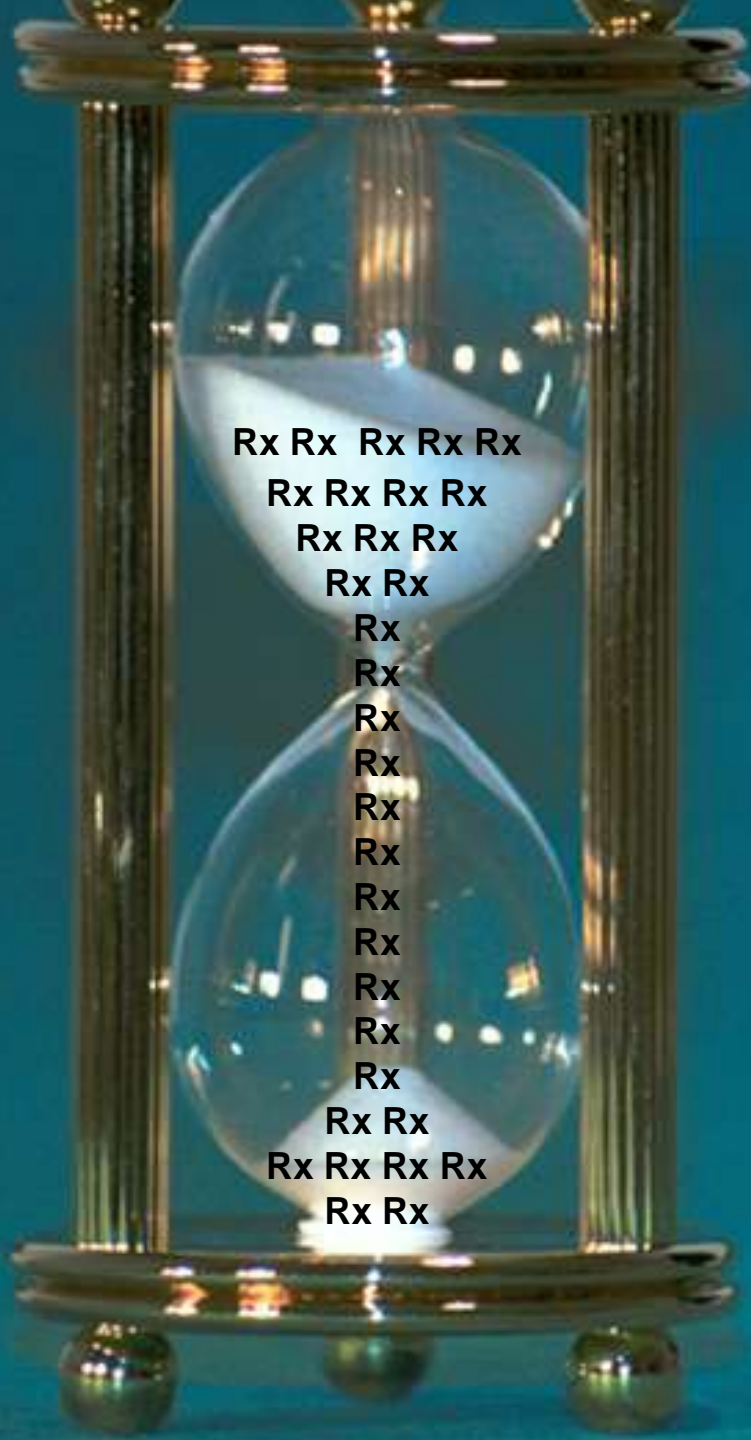
LIGHT DURING THE NIGHT

BAD!!!



CONCLUSIONS

- **Light at night-induced melatonin suppression as new risk factor for breast cancer may account for some of the 50% of breast cancer that is currently unexplained**
- **Light at night-induced melatonin suppression as a new risk for breast cancer may explain some of the increased breast cancer risk in female shift workers**
- **A unique approach to breast cancer prevention may include: 1) prudent of avoidance of light at night, 2) new, intelligent architectural lighting design and/or, 3) supplementation with melatonin or new melatonin agonists**



Rx Rx Rx Rx Rx
Rx Rx Rx Rx
Rx Rx Rx
Rx Rx
Rx
Rx
Rx
Rx
Rx
Rx
Rx
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Rx
Rx
Rx
Rx
Rx Rx
Rx Rx Rx Rx
Rx Rx

CIRCADIAN OPTIMIZED MELATONIN ADMINISTRATION FOR CANCER THERAPY AND PREVENTION

Light at night in U.S. 400 years ago

Ramelteon in Oncology

- Cancer Rx - single agent; combinatorial in cancer patients
- Cancer Prevention - shift workers; general population
- Sleep Promotion - cancer patients
- Correct Circadian Disruption - cancer patients
- Cachexia Rx - cancer patients
- Improve Quality of Life - cancer patients

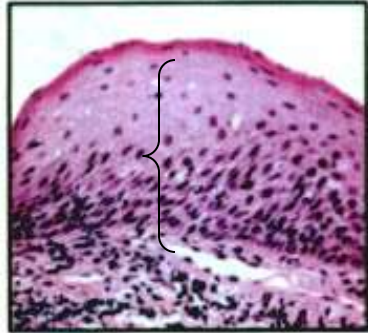
Future Priorities for Pre-Clinical Testing for Rx and 2° Prevention during Promotion/Progression (Blask) - Step 2

- **Oral ingestion of Ramelteon at increasing doses by nude rats bearing tissue-isolated human cancer xenografts following implantation → assess dose-response of long-term tumor growth activity/regression, signaling and LA metabolism**
- **Oral ingestion of Ramelteon at increasing doses by nude rats prior to implantation of tissue-isolated human cancer xenografts → assess dose-response of long-term tumor growth activity, signaling and LA metabolism**

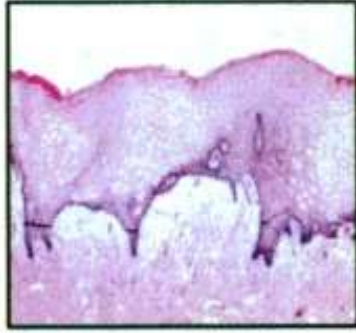
Double-Blind Study of the Effects of Ramelteon (5 ng/ml) on Signaling, LA Metabolism and Proliferative Activity in Tissue-Isolated (ER-) MCF-7 Human Breast Cancer Xenografts Perfused *In Situ* (n = 6; values are mean \pm SD; p < 0.001 vs control)

Treatment	LA Uptake (% of Supply)		13-HODE Production (ng/min/g)		cAMP (nmol/g tumor)	3H-Thymidine Incorporation (dpms/ μ g DNA)
	Pre treatment	Post treatment	Pre treatment	Post treatment	Post treatment	Post treatment
Control Vehicle	19.6 \pm 2.5	19.9 \pm 3.1	1.60 \pm 0.22	1.63 \pm 0.14	1.116 \pm 0.164	45.7 \pm 3.7
Ramelteon (5ng/ml)	20.7 \pm 3.8	0	1.59 \pm 0.15	0	0.092 \pm 0.07	7.1 \pm 1.4

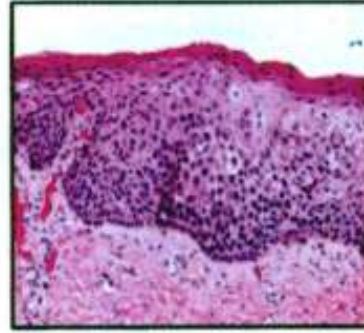
DEVELOPMENT, GROWTH AND SPREAD OF CANCER: POTENTIAL ROLE OF RAMELTEON?



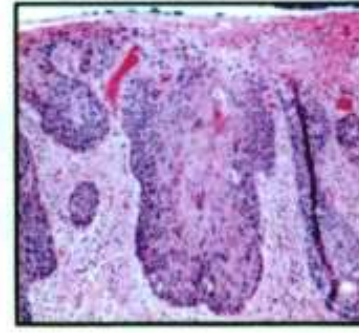
Normal mucosa



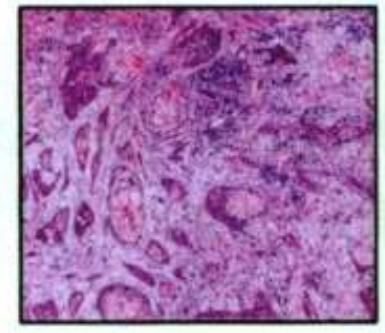
Hyperplasia



Dysplasia



CIS



Carcinoma

Initiation → Promotion/Progression → Invasion/Metastasis

Ramelteon ?

Initiation (DNA Damage/Repair), Differentiation, Proliferation/
Survival (Apoptosis), Signal Transduction, Metabolism,
Invasion/Metastasis

LIGHT DURING THE NIGHT

BAD!!!



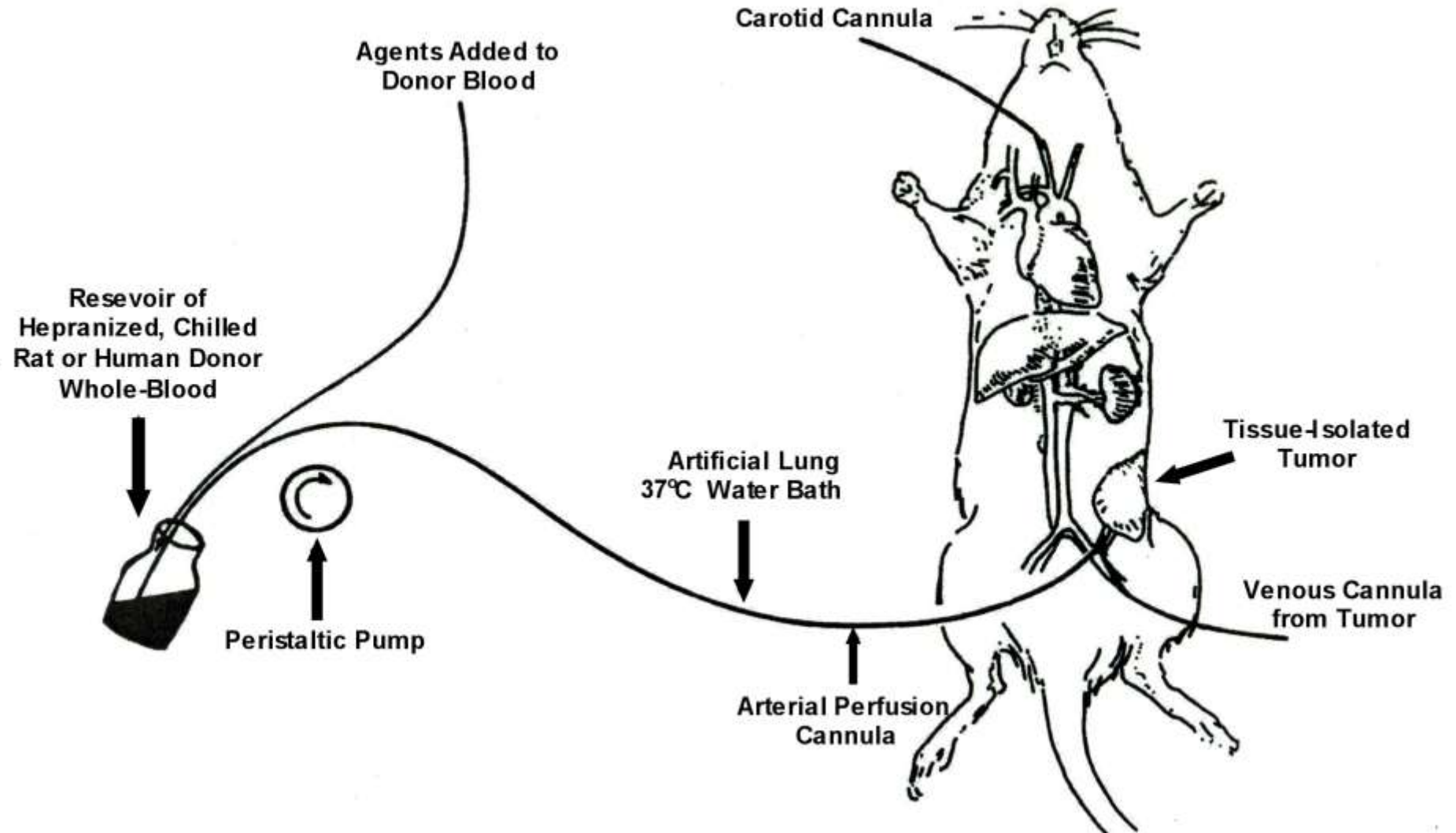
Future Priorities for Pre-Clinical Testing for Rx and 2° Prevention during Promotion/Progression (Blask and Dubocovich) - Step 1

- Oral ingestion of Ramelteon at increasing doses by human subjects → perfusion of tissue-isolated human cancer xenografts with Ramelteon-rich blood → assess dose-response of acute tumor proliferative activity, signaling and LA metabolism
- Acute perfusion of tissue-isolated human cancer xenografts with liver metabolite M-II → assess dose response of acute tumor proliferative activity, signaling and LA metabolism
- MT₁ receptor pharmacology and function in human cancer xenografts and cell lines

ENVIRONMENTAL LIGHT/DIETARY INTERACTIONS AND BREAST CANCER

- **BIOLOGICAL TIME (CIRCADIAN RHYTHMS)**
- **LIGHT/DARK CYCLES (MELATONIN)**
- **LIGHT AT NIGHT (MELATONIN SUPPRESSION; CIRCADIAN DISRUPTION)**
- **DIETARY FAT (LINOLEIC ACID)**
- **BREAST CANCER GROWTH**

PERFUSION OF TISSUE-ISOLATED (ER-) MCF-7 HUMAN BREAST CANCER XENOGRAFTS *IN SITU* WITH RAMELTEON (5 ng/ml)

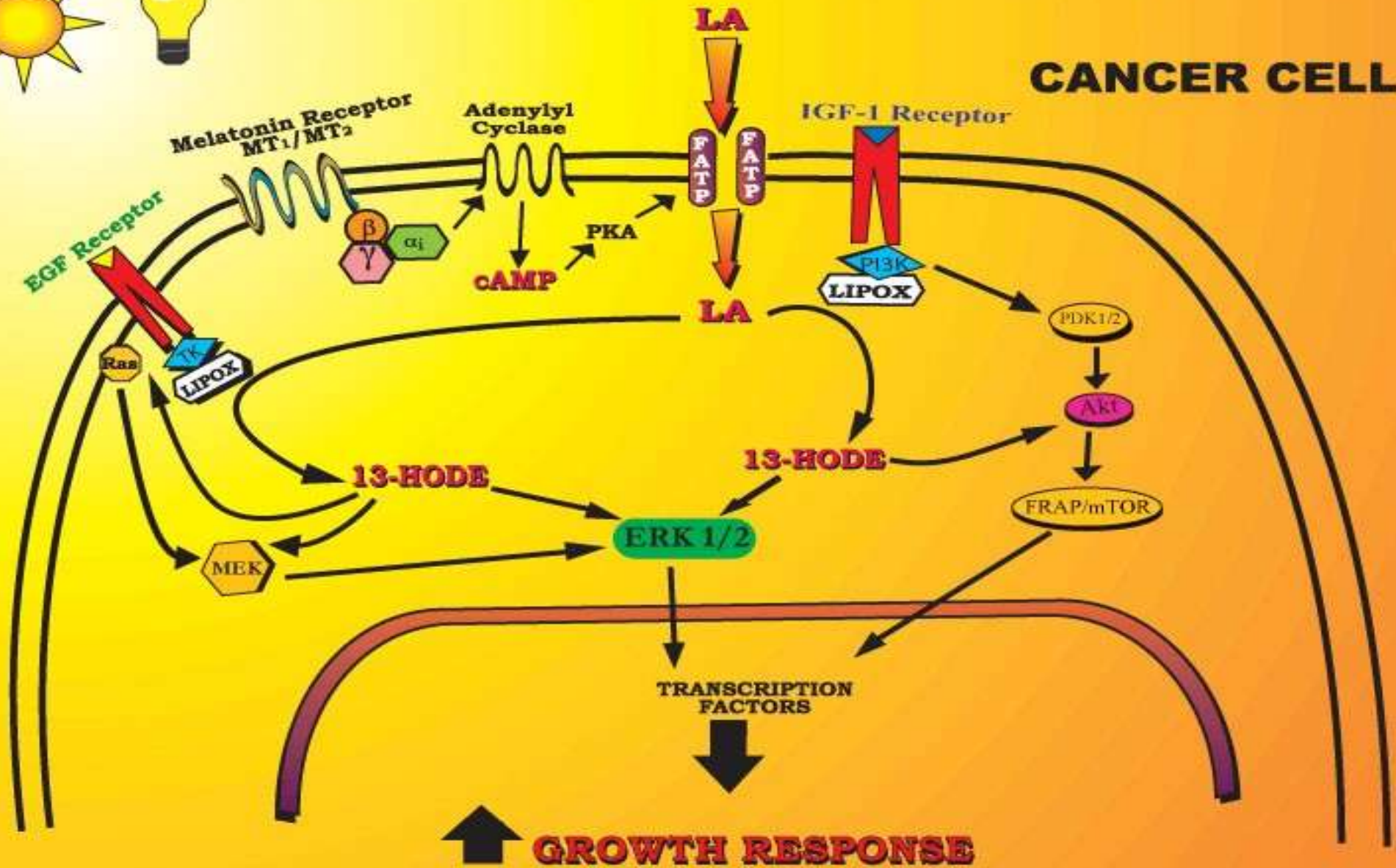


Future Priorities for Pre-Clinical Testing for Rx and 2° Prevention during Promotion/Progression (Hill) - Aim 1 (cont'd)

- **Assess dose-response effects of Ramelteon and metabolite M-II on proliferation and apoptosis in human cancer cell lines *in vitro***
- **Assess dose-response effects of Ramelteon and metabolite M-II on molecular and signal transduction mechanisms involved in cell proliferation and apoptosis in human cancer cell lines *in vitro***



CANCER CELL



Future Priorities for Pre-Clinical Testing for Rx and 2° Prevention during Invasion/Metastasis (Hill) - Aim 2

- **Assess dose-response effects of Ramelteon and metabolite M-II on invasion of human cancer cell lines *in vitro***
- **Assess dose-response effects of Ramelteon and metabolite M-II on metastases of human cancer xenografts *in vivo* in nude and/or SCID mice**

Future Priorities for Pre-Clinical Testing for Combinatorial Rx and 2° Prevention (RA and Omega-3 FAs) during Promotion (Hill and Blask) - Aim 3

- **Oral ingestion of Ramelteon in combination with RA or omega-3 FAs by human subjects → perfusion of tissue-isolated human cancer xenografts with Ramelteon-rich blood → assess dose-response of acute tumor proliferative activity, signaling and LA metabolism**
- **Acute perfusion of tissue-isolated human cancer xenografts with Ramelteon liver metabolite M-II in combination with RA or omega-3 FAs → assess acute tumor proliferative activity, signaling and LA metabolism**

Future Priorities for Pre-Clinical Testing for Combinatorial Rx and 2° Prevention (RA and Omega-3 FAs) during Promotion (Hill and Blask) - Aim 3

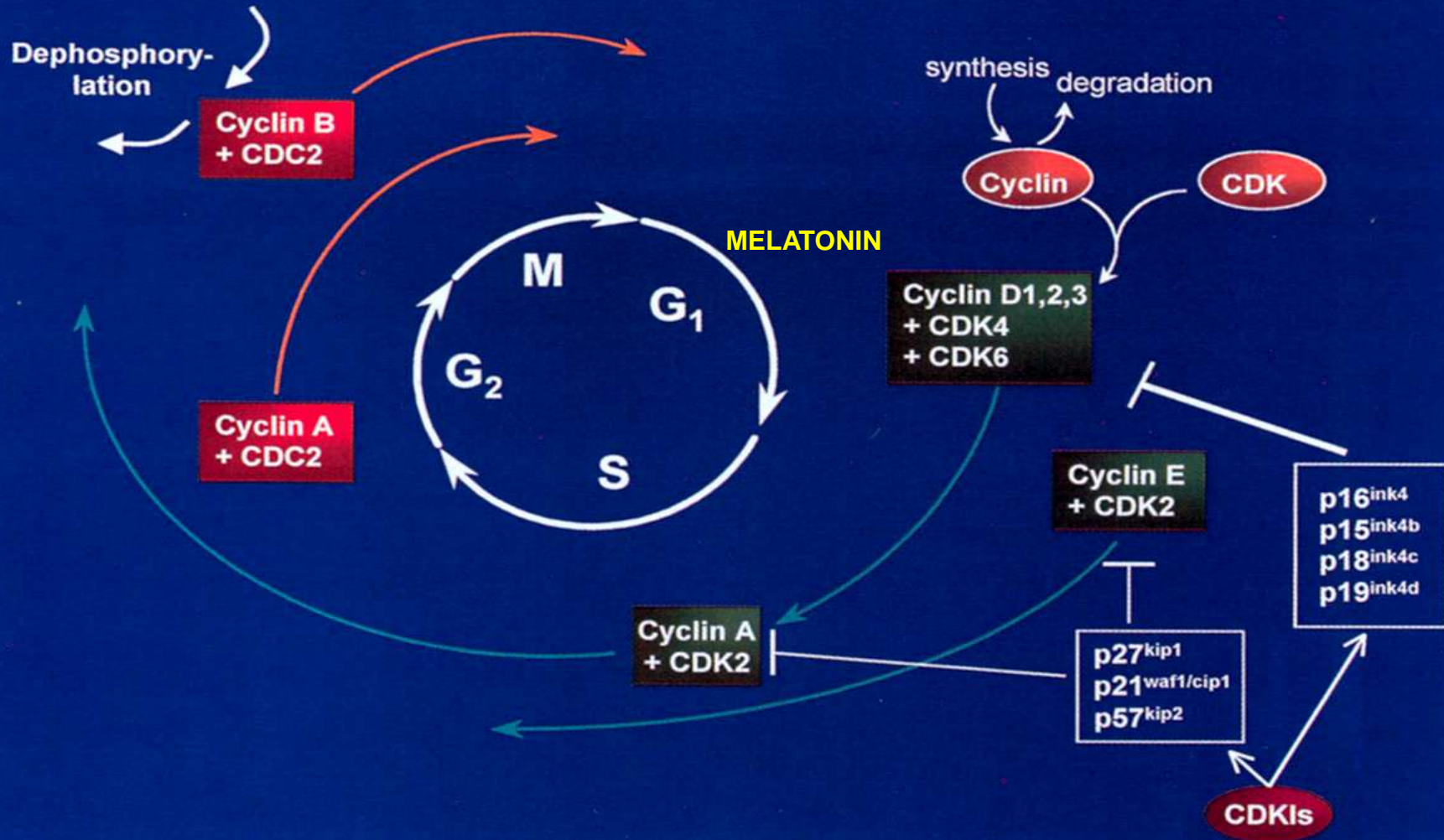
- **Oral ingestion of Ramelteon in combination with RA or omega-3 FAs by nude rats bearing tissue-isolated human cancer xenografts following implantation → assess dose-response of long-term tumor growth activity/regression, signaling and LA metabolism**
- **Oral ingestion of Ramelteon in combination with RA or omega-3 FAs by nude rats prior to implantation of tissue-isolated human cancer xenografts → assess dose-response of long-term tumor growth activity, signaling and LA metabolism**

Future Priorities for Pre-Clinical Testing for Combinatorial (RA and Omega-3 FAs) Rx and 2° Prevention during Invasion/Metastasis (Hill and Blask) - Aim 3

- **Assess dose-response effects of Ramelteon in combination with RA or omega-3 FAs on invasion of human cancer cell lines *in vitro***
- **Assess dose-response effects of Ramelteon in combination with RA or omega-3 FAs on metastases of human cancer xenografts *in vivo* in nude and/or SCID mice**

MELATONIN INHIBITION OF CELL CYCLE

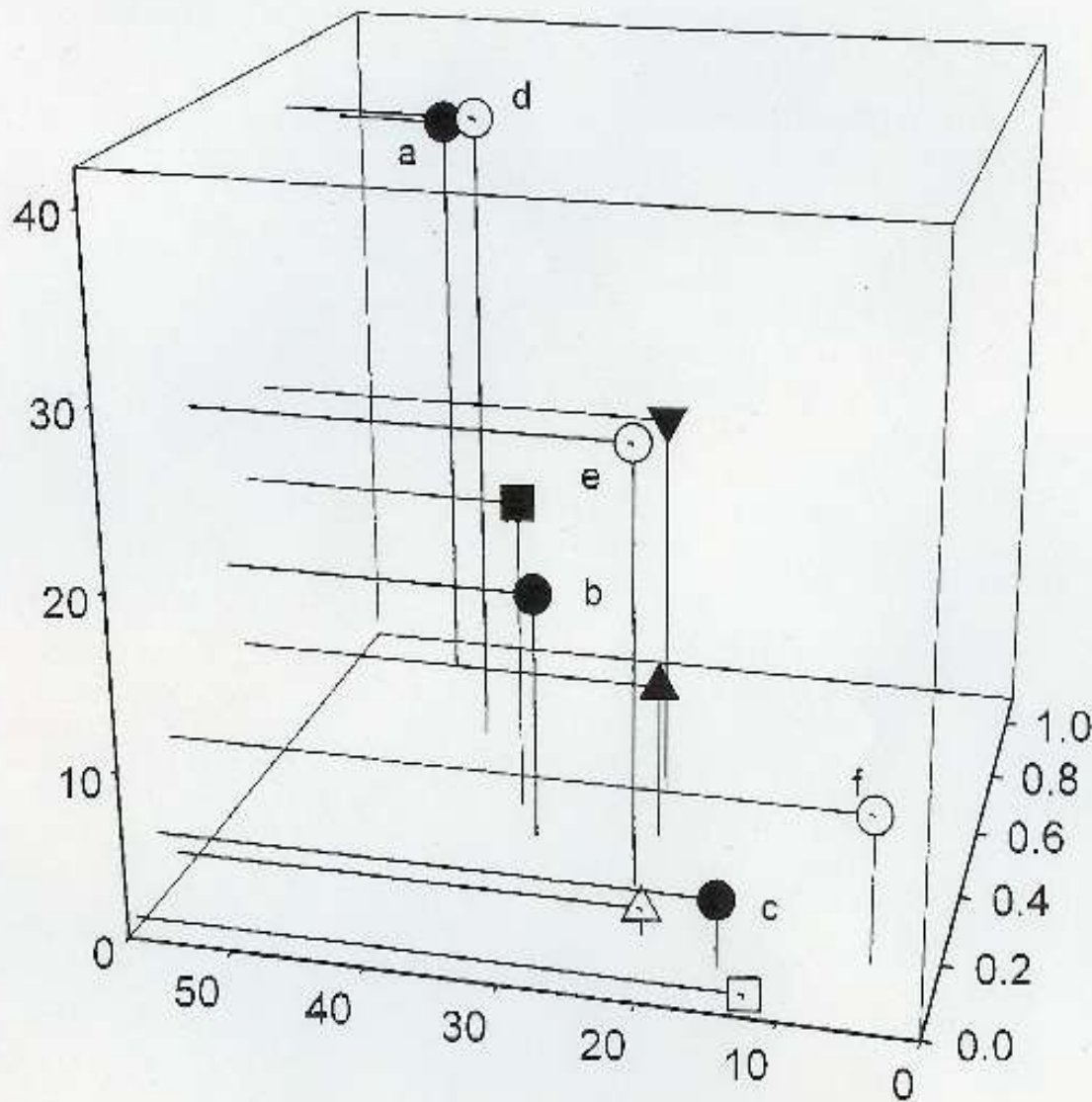
Cell Cycle Regulation



ESSENTIAL QUESTIONS

- **What is the relative contribution of the melatonin suppression component of circadian disruption by light at night to cancer risk?**
- **What is the relative contribution of the circadian phase shifting component of circadian disruption by light at night to cancer risk?**

13-HODE
RELEASE
ng/min/g



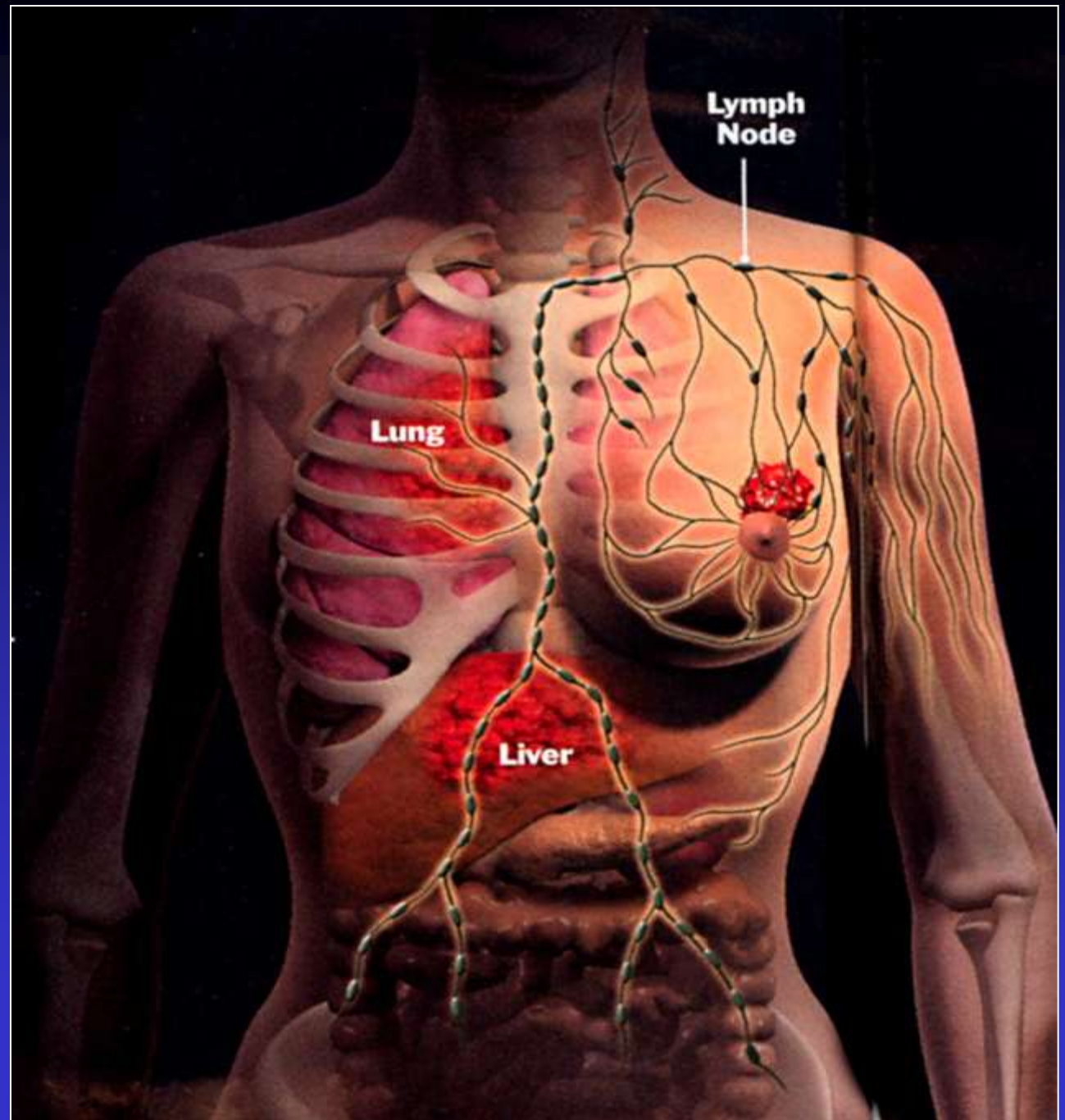
LINOLEIC ACID UPTAKE

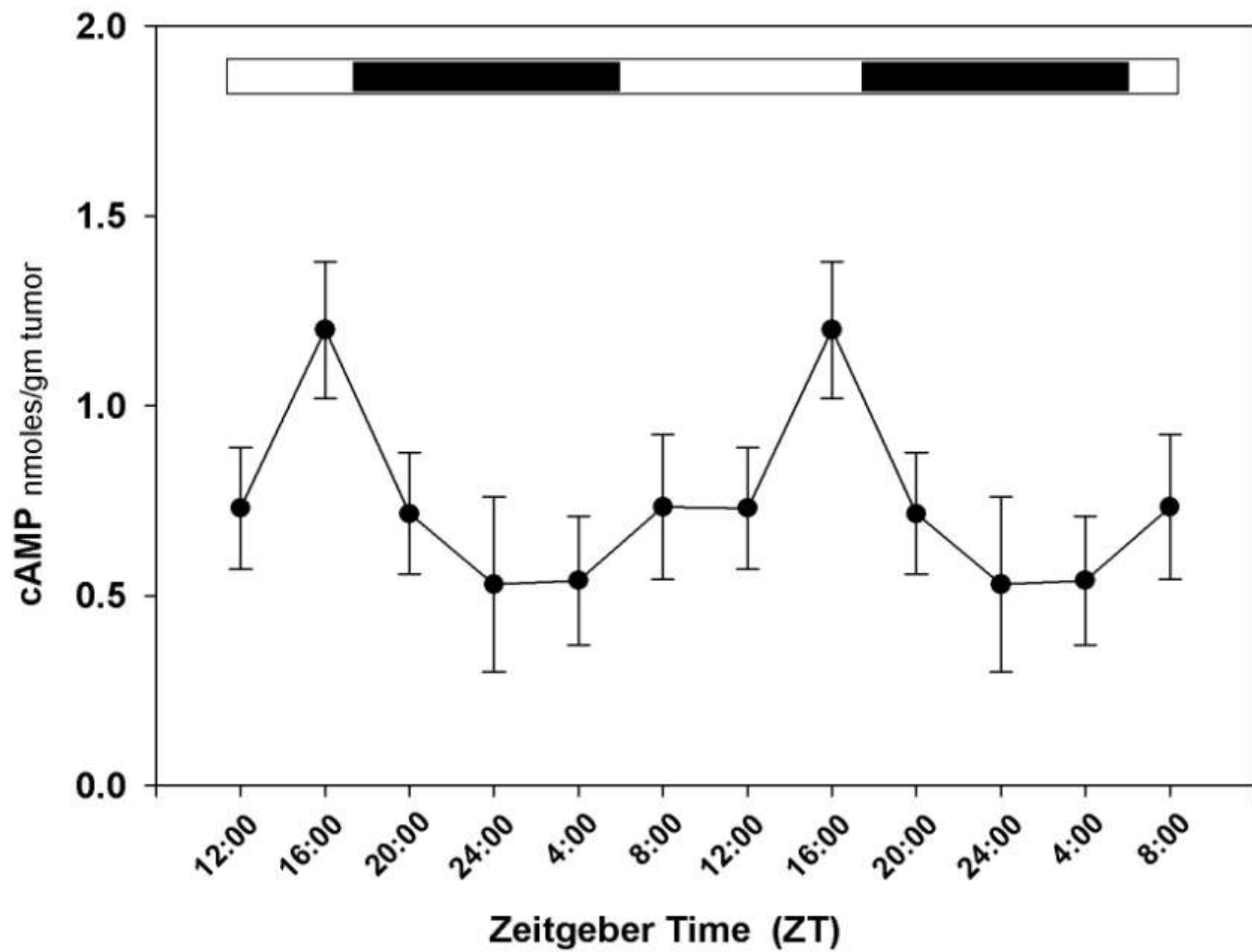
% of Supply Rate

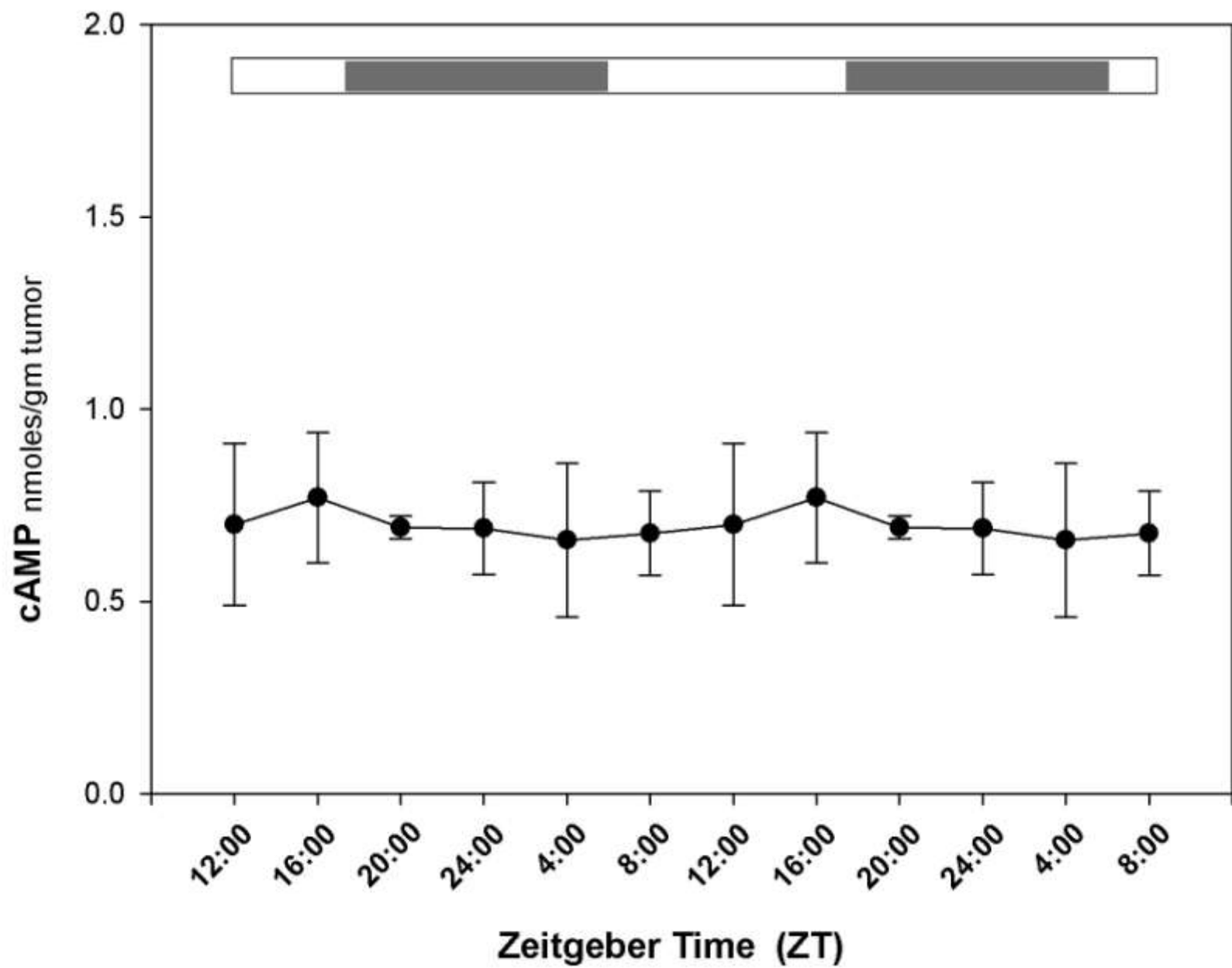
- HEPATOMA 7288CTC + fish oil treatment
- HEPATOMA 7288CTC + melatonin treatment
- △ MCF-7 (ER-)
- MCF-7 (ER+)
- ▼ PC3
- ▲ CFDT1
- FaDu

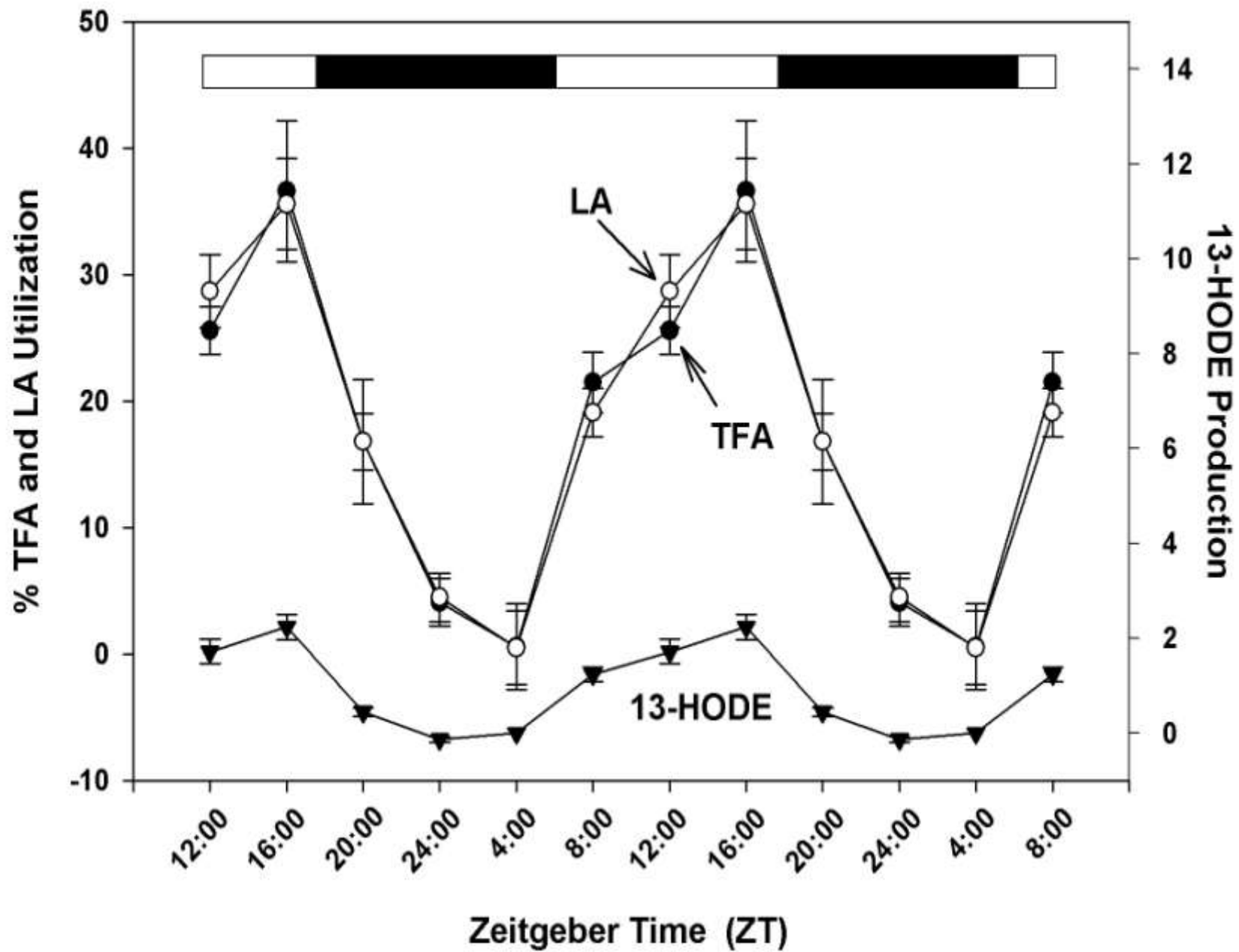
TUMOR GROWTH
RATE
g/day

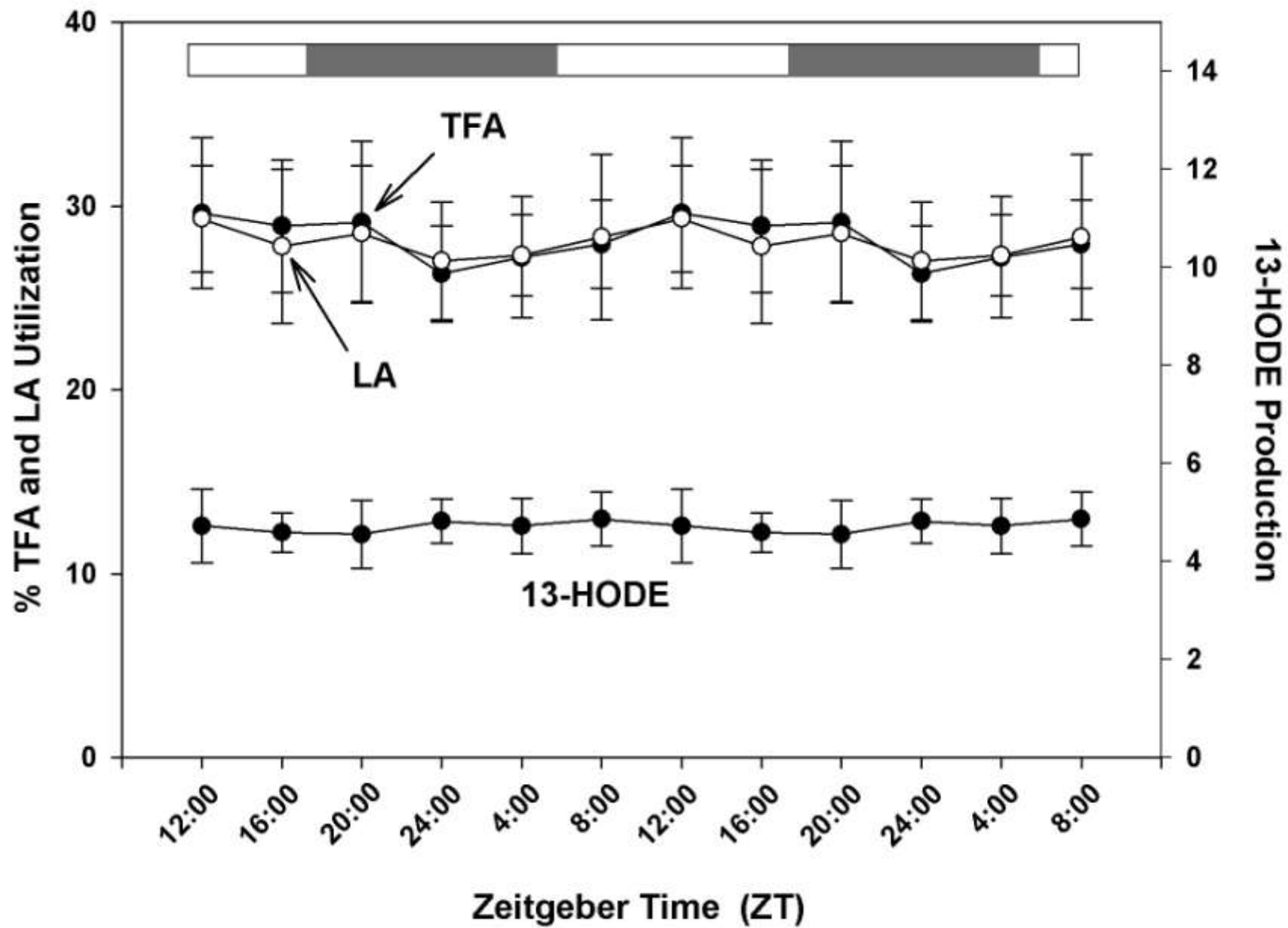
EXPOSURE TO LIGHT AT NIGHT AND BREAST CANCER

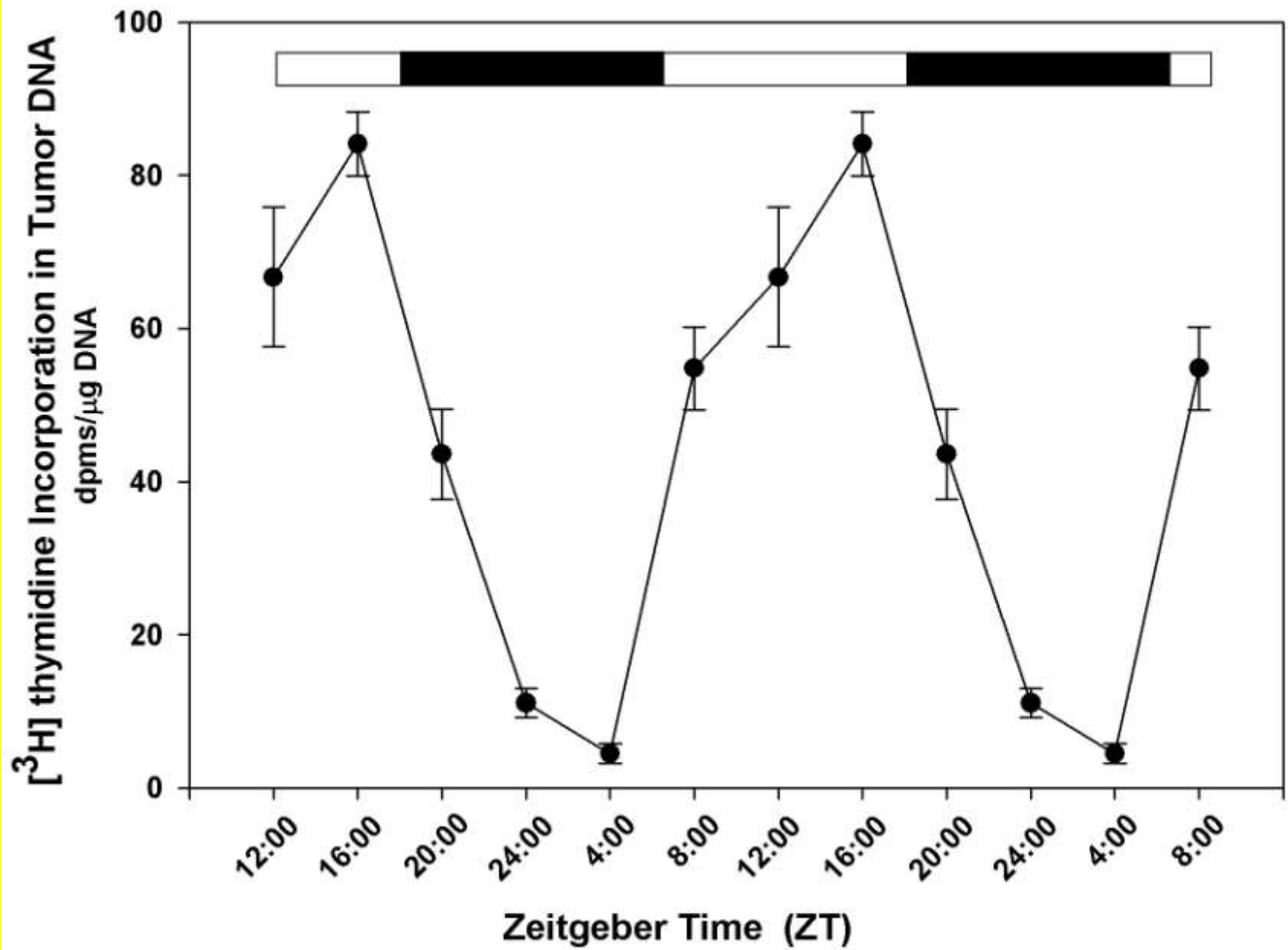




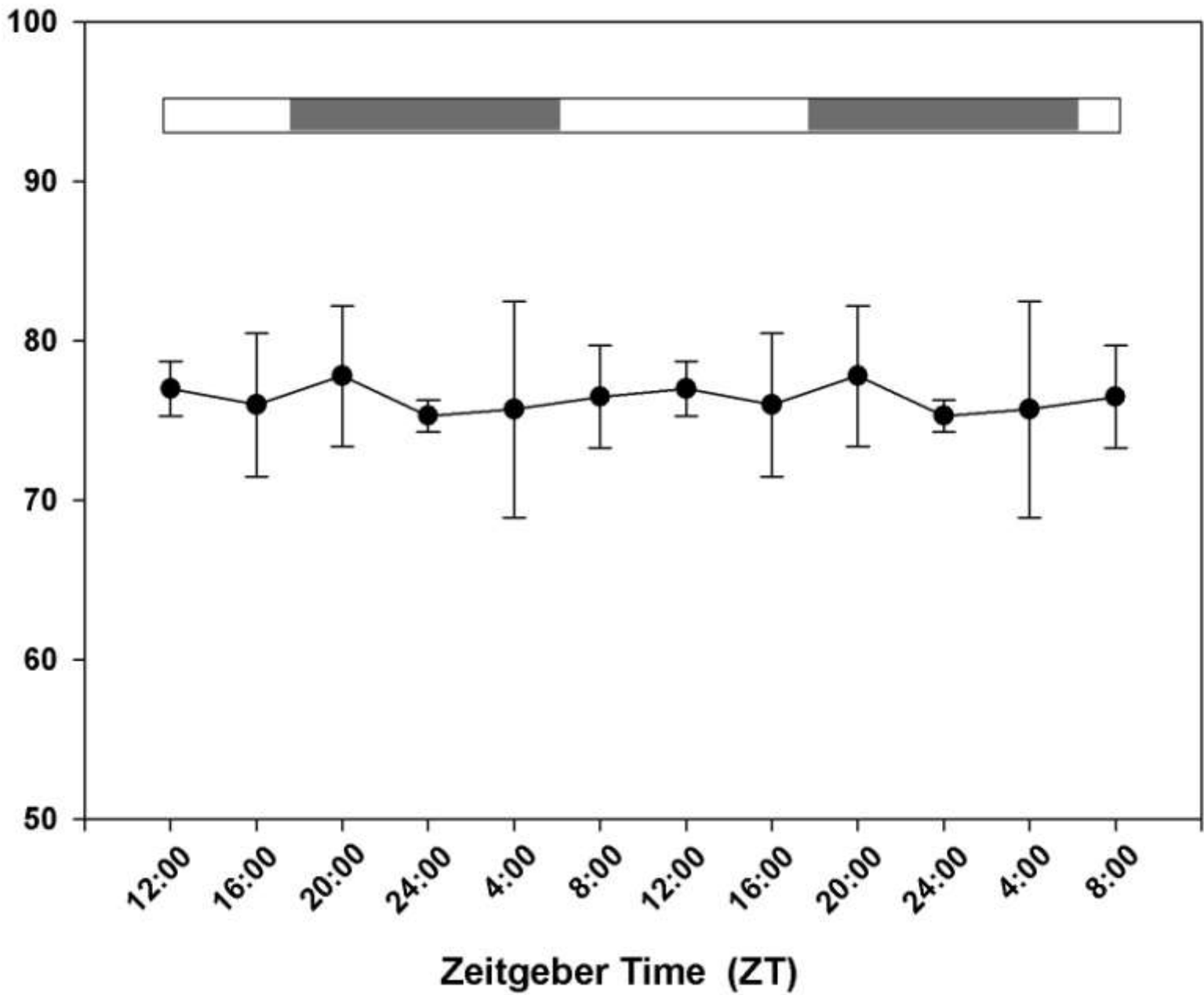


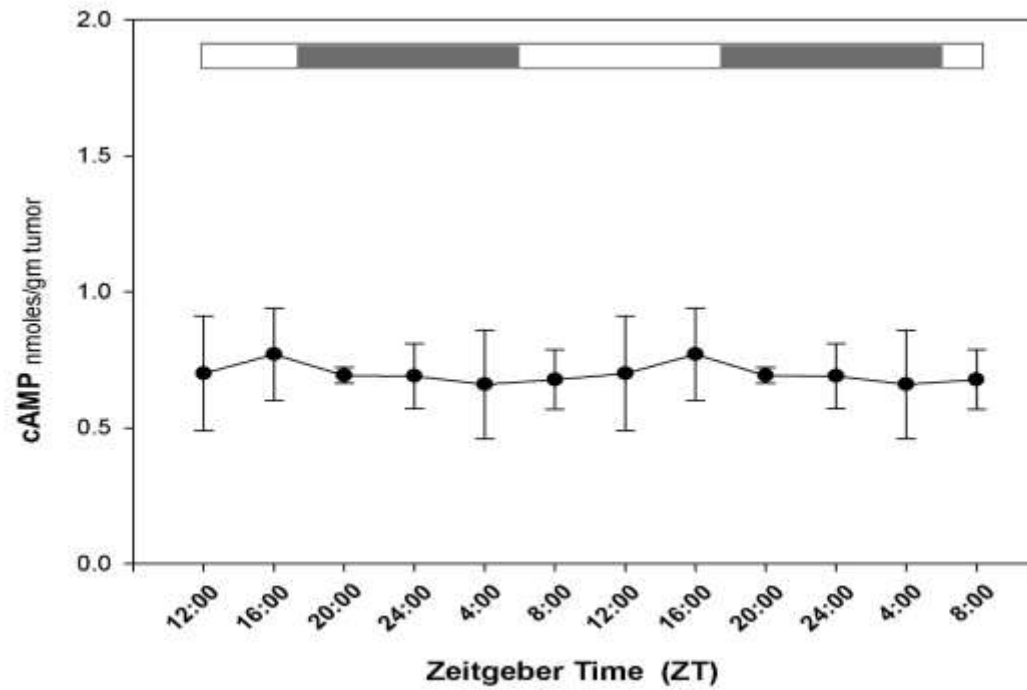
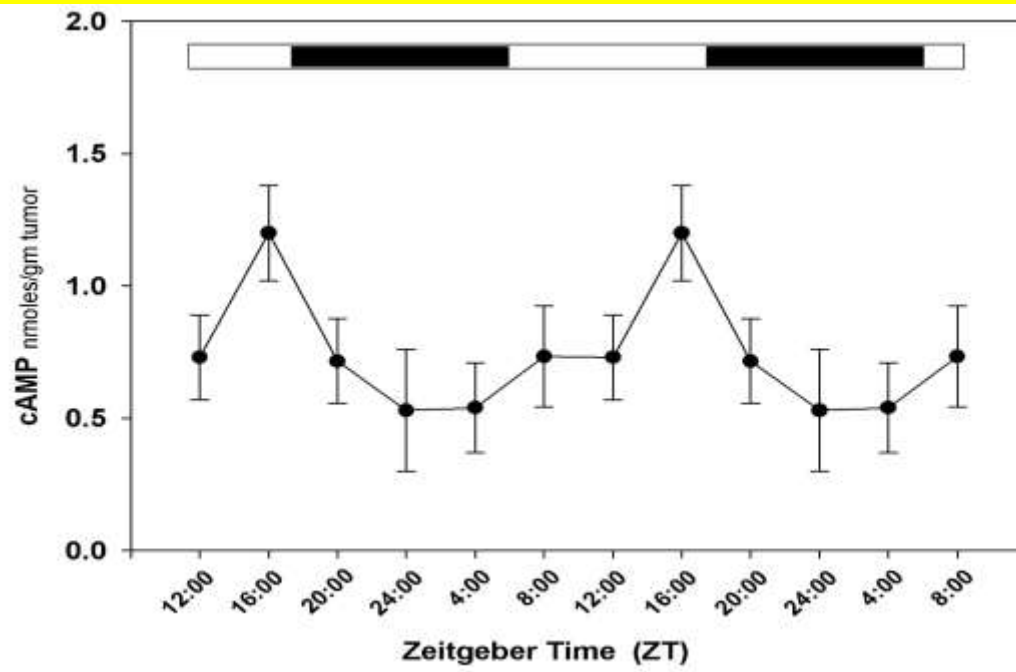






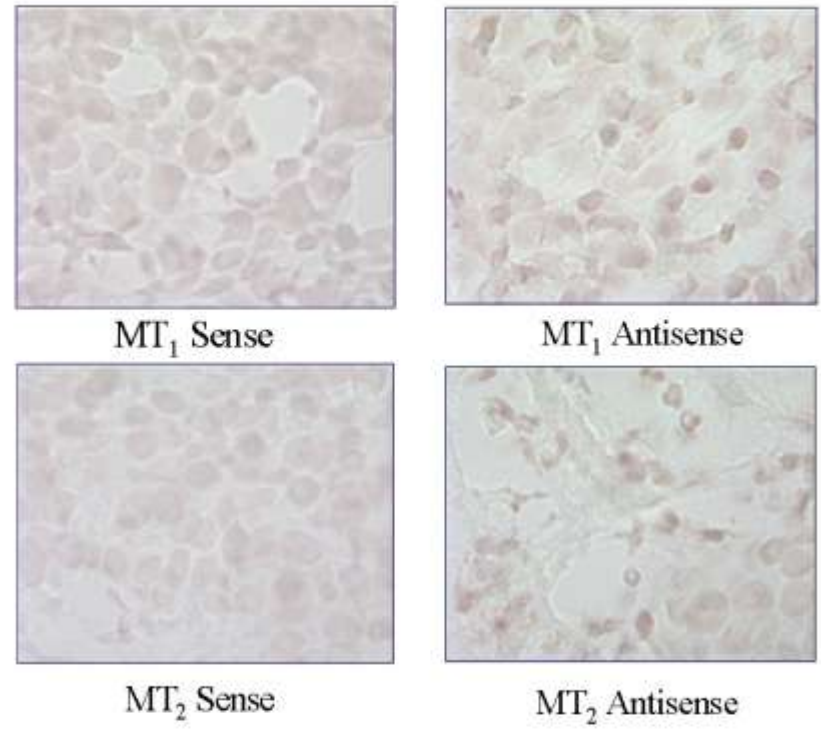
[³H] thymidine Incorporation in Tumor DNA
dpm/μg DNA



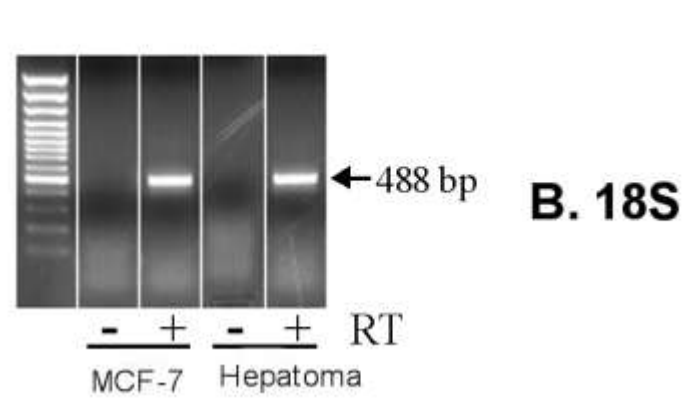
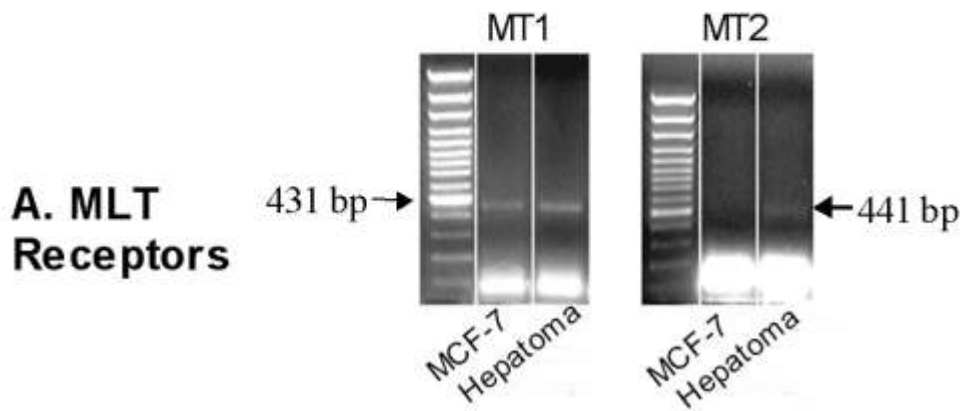
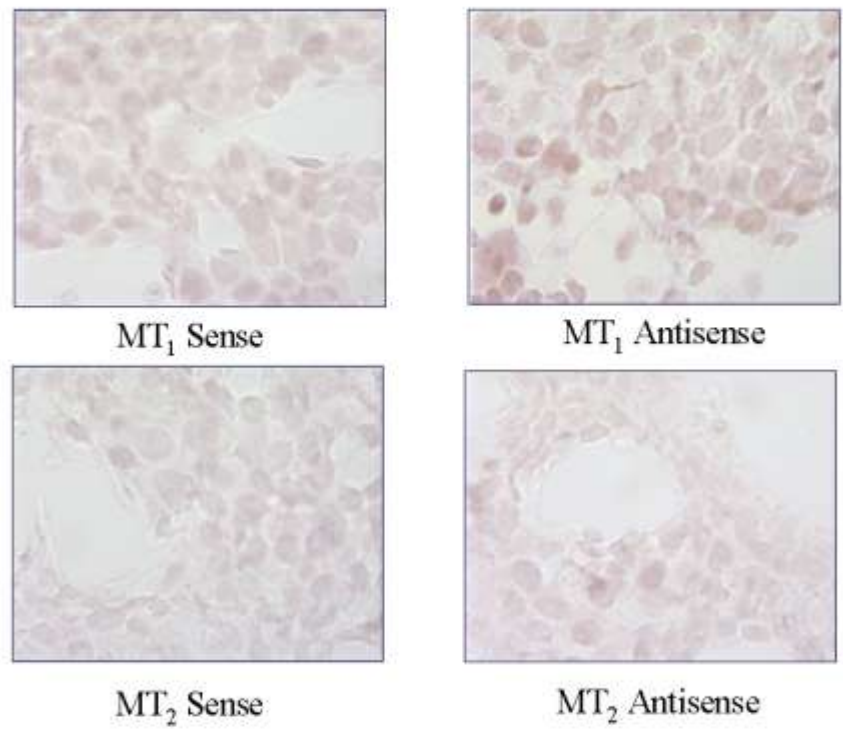


MELATONIN RECEPTOR EXPRESSION IN TISSUE-ISOLATED TUMORS

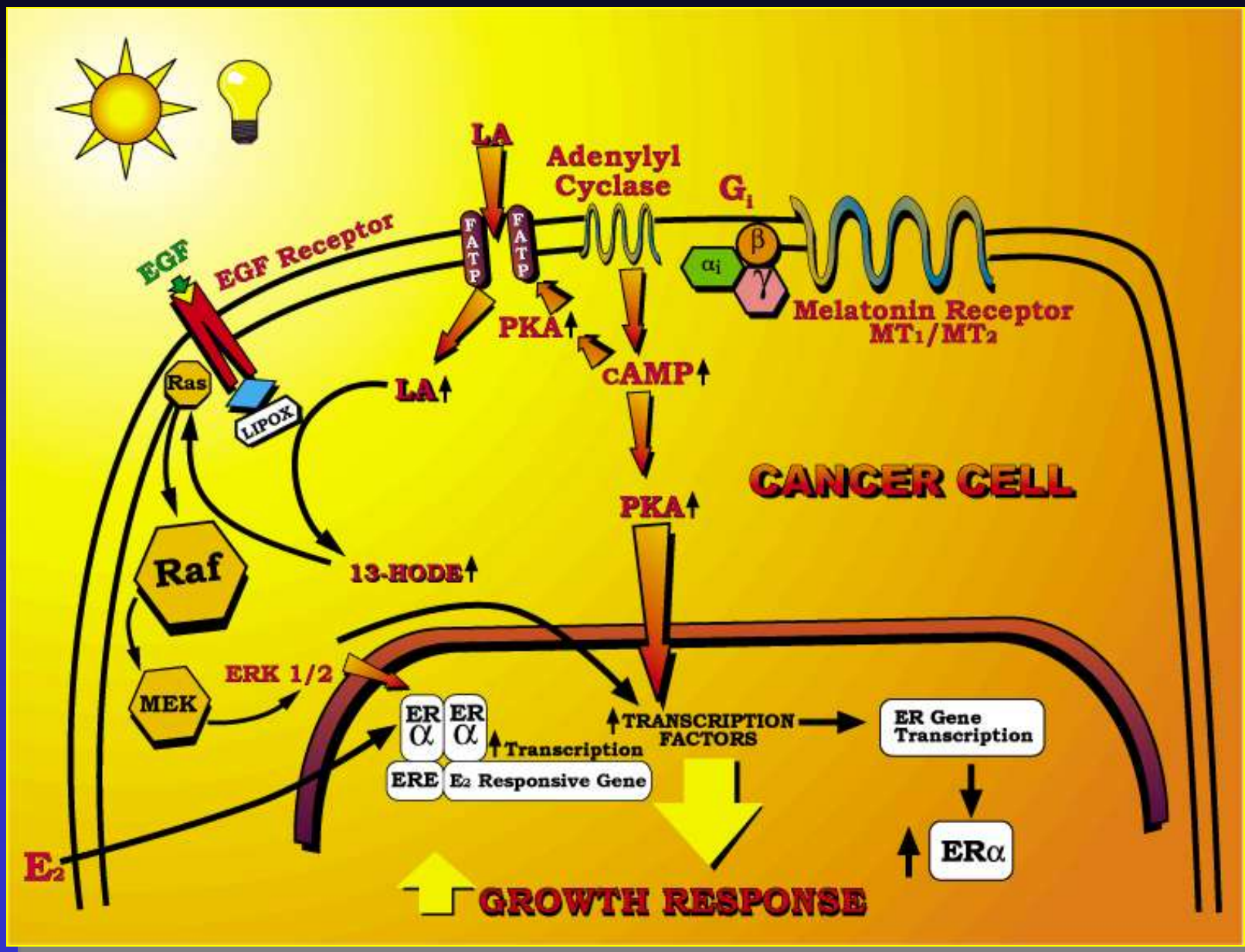
HEPATOMA



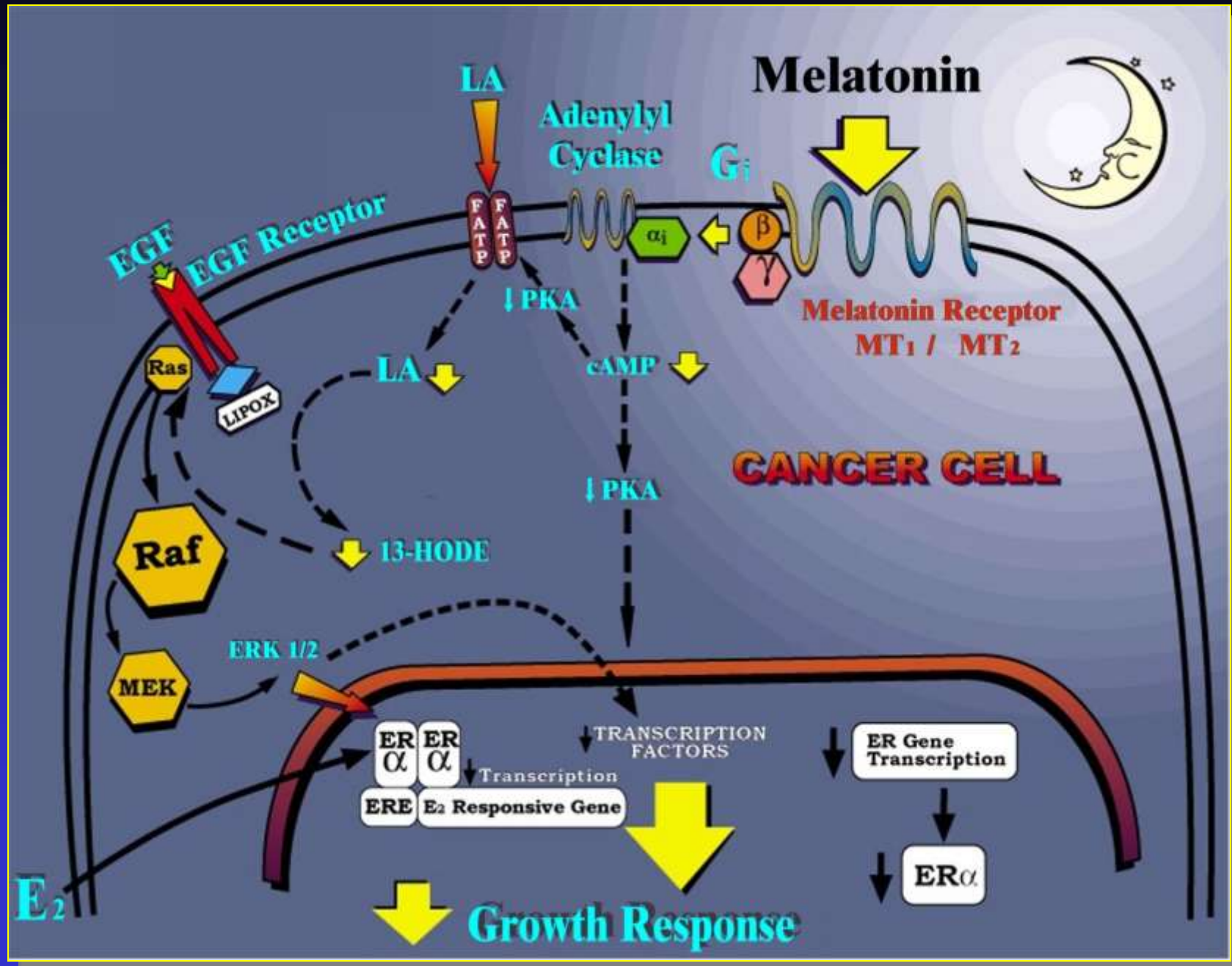
MCF-7 (SR-) Xenograft



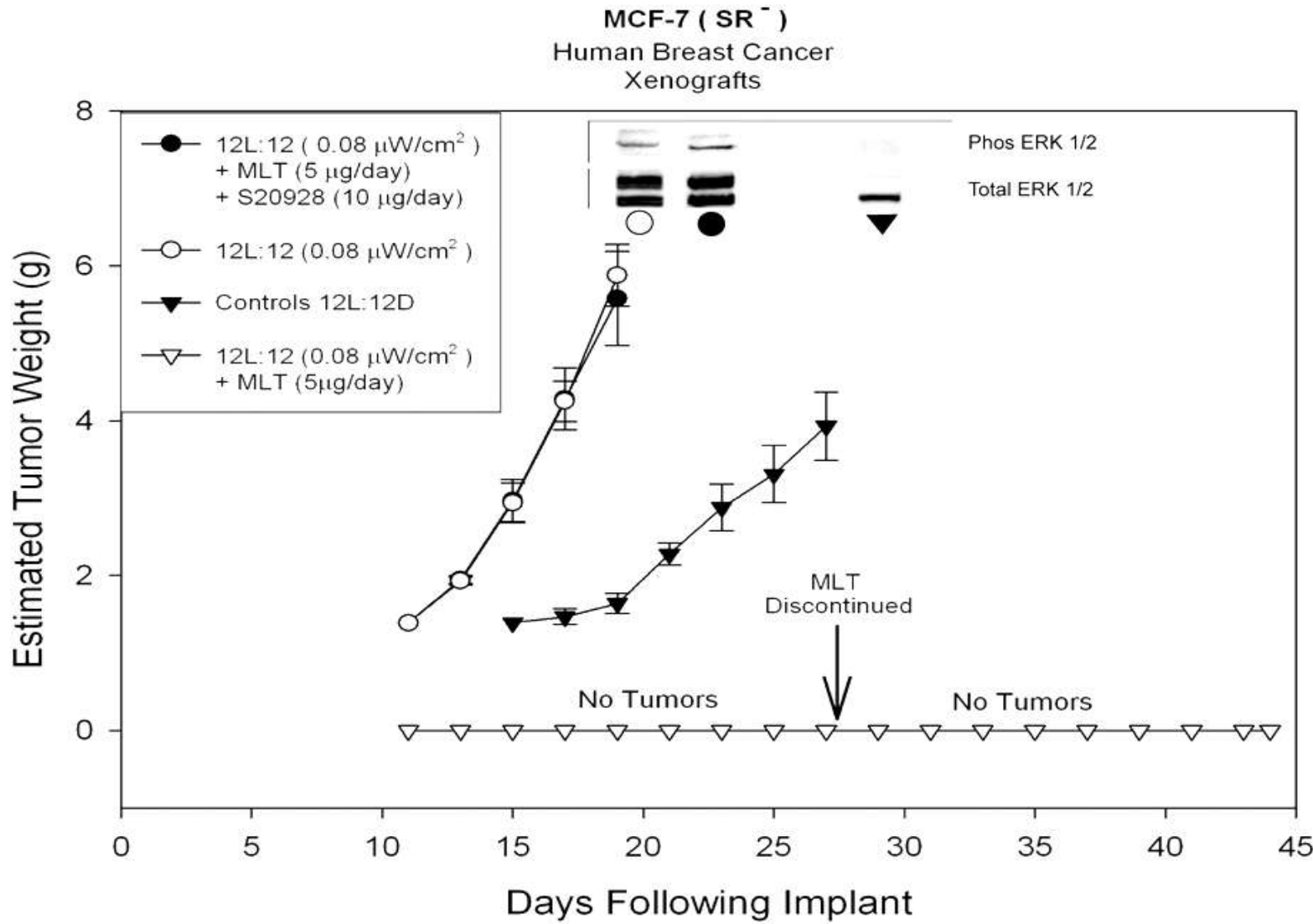
SIGNAL TRANSDUCTION IN TISSUE-ISOLATED TUMORS



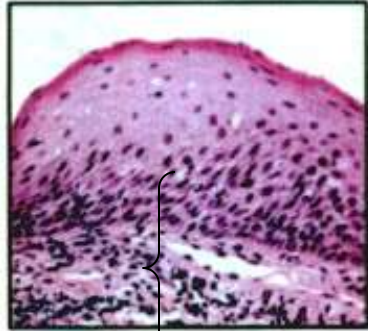
SIGNAL TRANSDUCTION IN TISSUE-ISOLATED TUMORS



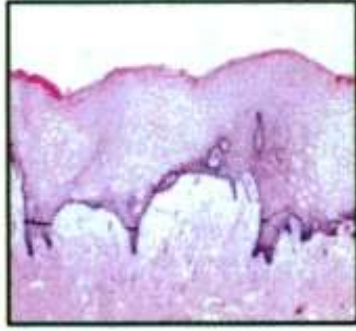
MELATONIN SUPPLEMENTATION IN REDUCING BREAST CANCER RISK DUE TO EXPOSURE TO LIGHT AT NIGHT



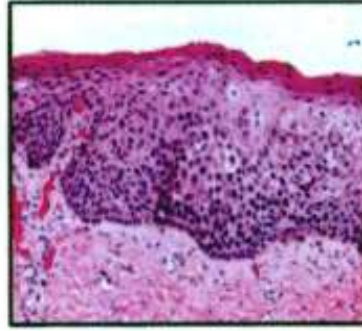
SESSION 9: MELATONIN AND CANCER



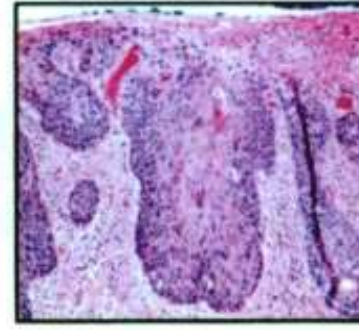
Normal
mucosa



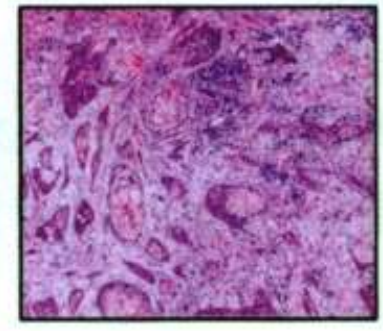
Hyper-
plasia



Dysplasia



CIS



Carcinoma

David Blask

Steven Hill

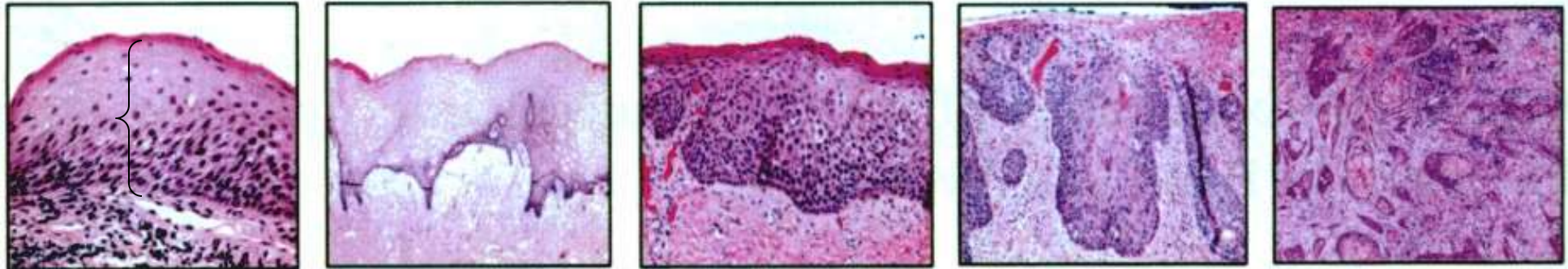
Samuel Cos

Stephen Shiu

William Hrushesky

Lulu Mao

SESSION 9: MELATONIN AND CANCER



Normal
mucosa



Hyper-
plasia



Dysplasia



CIS



Carcinoma

Initiation → Promotion/Progression → Invasion/Metastasis

Melatonin

Initiation (DNA Damage/Repair), Differentiation, Proliferation/
Survival (Apoptosis), Signal Transduction, Metabolism,
Invasion/Metastasis

MELATONIN: A CIRCADIAN ANTICANCER SIGNAL, ITS SUPPRESSION BY LIGHT AT NIGHT AND IMPLICATIONS FOR BREAST CANCER RISK, GROWTH PREVENTION AND THERAPY

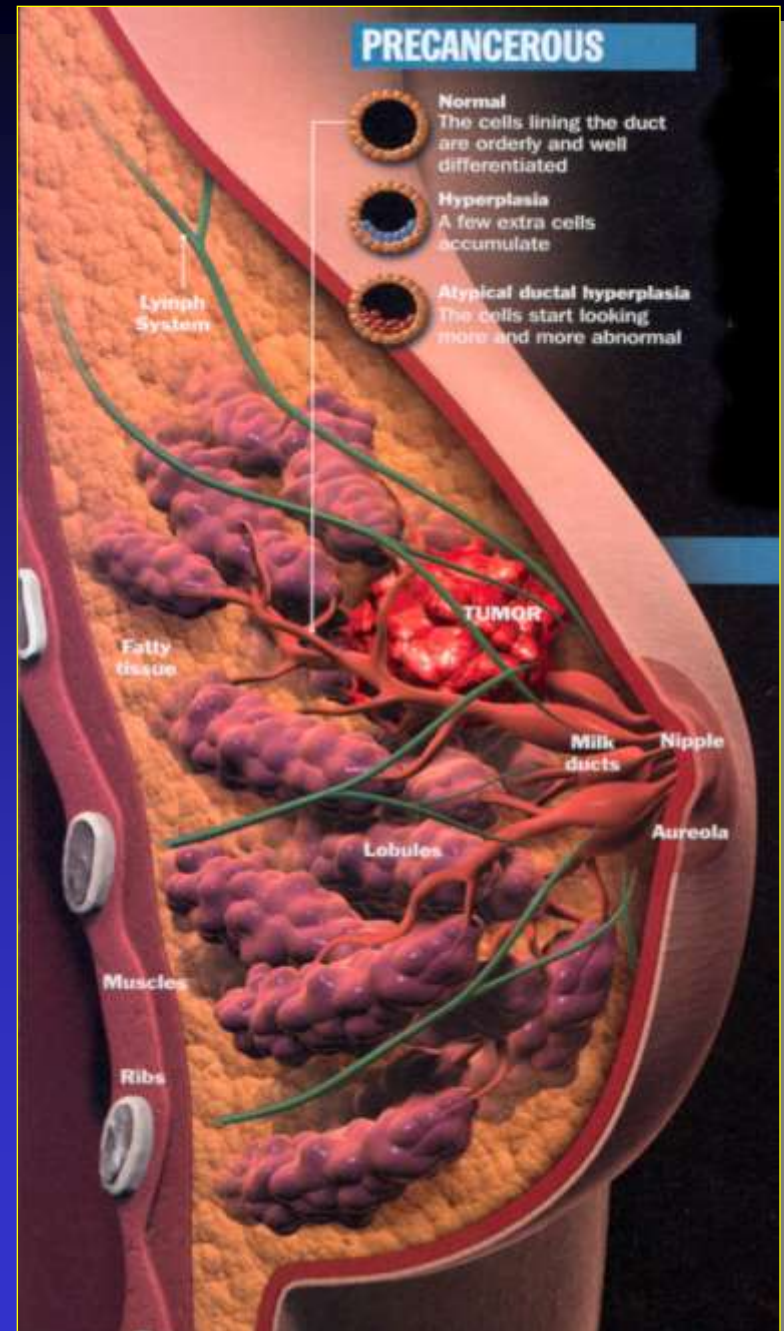
David E. Blask Ph.D., M.D.

Laboratory of Chrono-Neuroendocrine Oncology

Bassett Research Institute

Cooperstown, NY

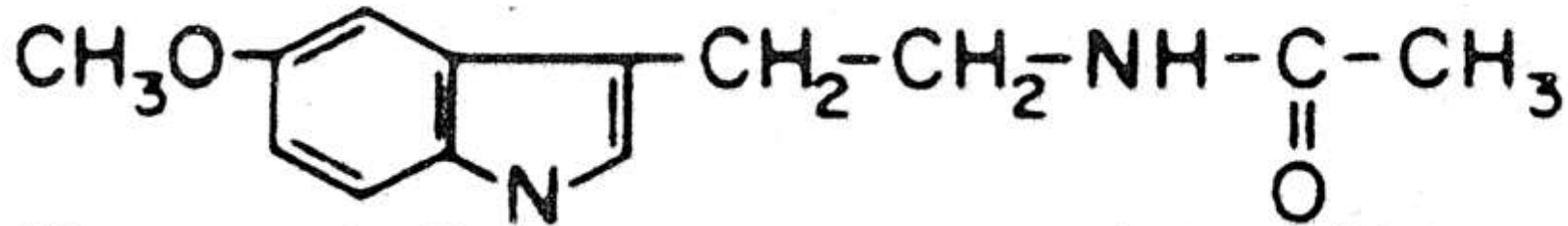
FASEB Summer Research Conference
Melatonin Receptors: Actions and
Therapeutics August 10-15, 2008
Snowmass Village, CO



Light At Night Worldwide



MELATONIN'S CHEMICAL STRUCTURE



N-acetyl-5-methoxytryptamine (Melatonin)