Light Treatment and Biological Rhythms

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Opinion

CLEARED!

After 89 days of being the focus of the FBI's investigation into the bombing in Atlanta's Centennial Park, Richard Jewell was finally, officially cleared as the agency's primary "target". His dismissal as the prime suspect in this tragedy did not come easily. He and his lawyers had protested publicly and often, but to no avail. The news media, some of whom were at the forefront of the rush-to-blame in the first place, raised its collective voice to call for an investigation of the investigators. Still Jewell was surveilled. Finally, a judge with jurisdiction declared that Mr. Jewell should no longer be viewed as target material, and a few days later the FBI grudgingly concurred. In fact, the timing probably had less to do with the judge's decision, than it did with the fact that the O.J. civil trial was starting to gain momentum, stealing headlines and garnering a certain curiosity among the G-men. Time to turn down the heat and turn on Court TV.

Like most everyone who did not spend the late Summer and early Fall on another planet, I was more-or-less forced to follow the daily news dispatches from Jewell's personal hell—the humiliation of indiscriminant search and seizure (they even took his poor mother's Tupperware collection), the oppressiveness of round-the-clock surveillance. I watched. I read. I vacillated. One day I would be inclined to believe the vicious rumors and accusations leaked by the law enforcement authorities: The suspect was a fat-cheeked law-officer wannabe with an interest in explosives, and a desire for the spotlight. The next day I would be swayed by the fervor of the suspect's attorney: "The man is innocent! He's a hero! He's a saint, fercrissake!"

Imagine yourself in Jewell's shoes. I did. The frustration. The depression. Just imagine. One day on CNN I even imagined that I heard Richard Jewell tell Bernard Kalb, or was it Morley Safer on 60 Minutes, that he used bright light to ease his situational depression.

"Oh, the irony!" I shouted.

"Did I forget to switch it off?" my mate replied from the kitchen.

"Not the iron, my dear, the irony!" I was forced to reiterate.

The irony indeed. Here was a man living under the darkest cloud of suspicion and mistrust seeking emotional rescue from a treatment modality caught up in a fog of doubt and innuendo. For years the debate had raged—was light treatment truly effective, or was it, as some suspected, simply an elaborate placebo? Not only had the question echoed through the halls of Academia, it had been the focus of a *Wall Street Journal* expose, it had been considered, by a few of us anyway, in the Flash(light) Poll; it had even been a topic of at least one memorable after-dinner speech.

Like most everyone in the field who did not spend the last few years in another specialty, I felt obliged to follow the periodic reports from the placebo abyss—the mortification of non-discrete effects (even disconnected negative ion generators seemed to work as well), the onerous weight of inconclusive results. I listened. I read. I vacillated. At one meeting I would be captured by the hypotheses explaining the lack of a time-of-day effect. The next meeting I would find myself shaking my head in front of the poster that

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explained exactly how 60 lux from a visor really could look the same to my retina as 10,000 lux from a light box.

Oh the irony. A man suspected of the worst sort of malevolence and deception taking comfort in a treatment threatened by the specter of disrespect and trickery. But the irony does not stop there. Nosir. The irony continues. It has to. The editor demanded 1000 words.

The greater irony, of course, is that both patient and cure have been redeemed. Cleared! That was the headline in *Time* magazine. His attorney had been right. Jewell was a saint after all. A "poster boy for the Bill of Rights" someone observed. The FBI revealed that, from the start, they had never really had much reason to focus on Jewell, and certainly didn't have enough to warrant a search of his home and his mother's Tupperware.

And light treatment? Cleared! as well. If not the headline, that was at least the bottom line of a report presented at the SLTBR Meeting in Bethesda this past Summer. In their abstract entitled, "Light Treatment for Winter Depression is More Than a Placebo", Eastman and colleagues seemed to eliminate our namesake intervention as a target of suspicion. Here was the group whose findings had helped get us into this mess in the first place saying, "wait a minute here . . . "

It turned out that if you followed outcome for long enough, and you analyzed your data with strict enough criteria, morning bright light perhaps could be viewed as more effective than placebo. Not the most convincing result, but then, they were preaching to the choir.

One day not long ago, while watching the aftermath of Jewell's redemption on CNN, it occurred to me that more than a few FBI agents could probably benefit from a good regimen of our newly-acquitted treatment modality. The depression of a stake-out gone to seed must be quite debilitating. I phoned a family friend with ties to the FBI (actually a retired tailor who used to do Hoover's hems) to suggest this course of action, but he cut me off in mid-sentence.

"You think they're finished with this case?" he asked. "Not on your life. The trail may have cooled, but believe you me that Jewell fella remains under suspicion. A year from now, there'll still be G-men in his bushes. Look, it's not like they've come up with a new suspect after all"

"True, but even the Feds admit a lack of any hard evidence against the guy." I countered. "Why pursue it"

My friend's voice took on a knowing tone. "Hey, you can call it paranoia if you want, but it's that kind of healthy skepticism that keeps the Bureau on its toes. You very well may hear the name Richard Jewell again, my friend."

I thought to myself, "there's a lesson here someplace . . . .". But then I did a word count. I was already well over my limit.

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MELATONIN SITES OF ACTION

Introduction

Melatonin is the primary hormone secreted from the pineal gland. The putative functions of this hormone are many and include convincing data concerning the control of several seasonal reproductive, metabolic and thermogenic responses (for review see: Bartness and Wade, 1985; Wade et al., 1989), its action as a retinal neuromodulator (Dubocovich, 1983), as well as its role as an entrainer (Zeitgeber) of circadian rhythms (Cassone et al., 1986). More recently the effects of melatonin on aging processes (for review see: Retier, 1992) and as an anticarcinogenic substance (for review see: Maestroni, 1993) have been hypothesized. The purpose of this review is to describe the putative sites of action for melatonin in the central nervous system along with some of its more recently identified peripheral sites. Because the sites of action of melatonin for the control of seasonal reproductive and metabolic responses have been studied the most frequently and thoroughly, this research will receive the greatest emphasis in this article.

In mammals, photoperiod cues are received by the retina and transmitted through a multisynaptic pathway to the pineal gland (Moore, 1996). The pineal gland transduces the photic cue into an internal endocrine signal. Pineal and plasma melatonin concentrations are at their daily nadir during the light and at their peak during the dark phases of the photocycle (for review see: Bartness et al., 1993; Hastings, 1991) for all species studied, regardless of whether they show nocturnal, diurnal, or crepuscular activity patterns (for review see: Bartness and Albers, in press).This pattern of melatonin synthesis and release results from regulation of the pineal gland by an endogenous circadian oscillator that is entrained to the light:dark cycle (Elliot and Tamarkin, 1994). Thus, the duration of the night is coded by the duration of the secretion of melatonin and serves to trigger seasonal responses—long duration melatonin secretion profiles signal "fall/winter,"whereas short duration profiles signal "spring/summer" (Bartness et al., 1993).

Criteria for a Melatonin "Site of Action"

Hastings et al. (Hastings, et al., 1991) describe three approaches of the search for melatonin sites of action. These have been modified here and are presented as three criteria for a *bona fide* melatonin site of action. First, melatonin neuronal binding must be exhibited at the site. Second, appropriately timed, direct application of melatonin into the site must trigger the seasonal response in question. Third, lesions of this putative site should block the effects of melatonin. These criteria have been difficult to fulfill, partly because of technical difficulties in selectively accessing some of the putative sites of action (second and third criteria), and partly because the application of all three approaches in the same paradigm has been rare.

Concerning the fulfillment of the first criterion, the arrival of an iodinated melatonin analogue (2-[125I]-iodomelatonin (IMEL) with sufficient specific activity to permit autoradiographic localization of melatonin binding sites has proven invaluable (for reviews see: Bittman, 1993; Morgan et al., 1994; Weaver et al., 1991). IMEL central nervous system binding sites have been reported in a wide range of mammals (i.e., sheep; deer; goats; rabbits; laboratory rats; Syrian, Siberian and European hamsters; white-footed mice; golden-mantled ground squirrels; Guinea pigs; nine-banded armadillos; bats; and humans), as well as in other non-mammals (Atlantic hagfish; river lamprey; hedgehog skates; rainbow trout; clawed toads; for review see: Bittman, 1993; Morgan et al., 1994). In mammals, one brain and one peripherally associated brain structure have consistently shown IMEL binding—the suprachiasmatic nucleus of the hypothalamus (SCN) and/or the pars tuberalis (PTu) of the pituitary gland (Bittman et al., 1994; Duncan and Meade, 1992; Reppert et al., 1988; Williams et al., 1989). Many of the mammals examined to date show binding in both the SCN and the PTu (see Table 1); however, some seasonally breeding species only show IMEL binding in the PTu (Duncan and Meade, 1992; Weaver and Reppert, 1990). The latter finding appears to have turned the focus of the search for the site of action of melatonin toward the PTu. The inaccessibility of the PTu for melatonin stimulation or for PTu lesion experiments (criterion 2 and 3) has curtailed some of the enthusiasm for its role in the photoperiodic control of reproduction by researchers studying rodents; however, this has not been a problem in sheep. In addition, the size of the sheep PTu has provided ample tissue for cell culture studies (e.g., McNulty, 1995; Morgan et al., 1989a,b).

Pituitary Pars Tuberalis

Most of the studies examining the molecular mechanisms underlying melatonin stimulation were accomplished using the PTu in tissue culture and provided much needed information at that level of analysis (see above). *In vivo* studies that have been interpreted as supporting the role of the PTu in receiving photoperiod-driven melatonin signals have involved implantation ofmelatonin into the medial basal hypothalamus. For example, implants of melatonin into the medial basal hypothalamus of white-footed mice (Glass and Lynch, 1981), Syrian hamsters (Hastings et al., 1988), Mongolian gerbils (DeVries et al., 1989), and sheep (Lincoln, 1994, 1994; Lincoln and Maeda, 1992; Malpaux et al., 1993; Malpaux et al., 1995) have been re-interpreted to suggest

that melatonin spread from the brain site and reached the PTu (Malpaux, 1995). A possible and reasonable model focusing on the PTu as the primary melatonin site of action was developed (Morgan et al., 1994); however, the results of several recent *in vivo* studies cast doubt on the role of the PTu in melatonin-induced changes in the control of luteinizing hormone (LH) release and, therefore, on the control of reproductive status. Specifically, microimplants of melatonin into the medial basal hypothalamus in reproductively inhibited sheep result in stimulation of the reproductive system, as assessed by increased LH release (Malpaux et al., 1993). However, PTu implants (which are feasible to do in sheep) did not have such an effect, but when an additional subcutaneous implant was given, LH secretion increased (Malpaux et al., 1995). Therefore, it does not appear that melatonin stimulation of the PTu is necessary for alterations in reproductive status in sheep (Malpaux et al., 1995). Similar studies in rams also support the view that the PTu has no role in the control of reproductive status by melatonin (Lincoln, 1992; Lincoln et al., 1992; note that the author of these studies in rams was reluctant to make this conclusion, but instead suggested that the results occurred because of possible technical difficulties with the microimplants).

Suprachiasmatic Nucleus

Possible sites of action for melatonin in the brain also exist. Siberian hamsters show IMEL binding in the SCN, paraventricular nuclei and the reuniens nuclei of the thalamus (Duncan et al., 1993; Weaver et al., 1989). Therefore, as a first attempt at experimentally determining which of these structures is a site for the reproductive and metabolic effects of melatonin, SCN lesions were made in Siberian hamsters. These animals were given daily, long duration, subcutaneous infusions of melatonin that mimic the short day natural secretion of the hormone (using the so-called "timed infusion

Table 1. Comparison of IMEL binding sites in the brain and pituitary of several mammalian species.*

Species	SCN	Reuniens	PVT	PTu	References
Sheep	+		+	+	(1)
Siberian hamsters	+	+	+	+	(2, 3)
Syrian hamsters	+	+	+	+	(3, 4)
Laboratory rats	+	+	+	+	(3)
White-footed mice	+	+	+	+	(5)
Golden-mantled ground squirrels	+	+	+	+	(6)
Guinea pigs	+		+	+	(6)
Ferrets				+	(7)
Western spotted skunk				+	(8)
Musk shrew	+				(9)
European rabbits	+			+	(9, 10)
Little brown bat	+			+	(9)
Humans	+				(11)

*Modified from tables in Bittman, 1993, and Morgan, et al. 1994. SCN=suprachiastmatic nucleus of the hypothalamus, PVT=paraventricular nucleus of the thalamus, Reuniens=Reuniens nucleus of the thalamus, PTu=pars tuberalis of the pituitary gland. References: (1) Bittman and Weaver, 1990; (2) Duncan, Takahashi and Dubocovich, 1989; (3) Bartness and Albers, in press; (4) Bartness, et al. 1993; (5) Carlson, Weaver and Reppert, 1991; (6) Bittman, Thomas and Zucker, 1994; (7) Weaver and Reppert, 1990; (8) Duncan and Mead, 1992; (9) Bittman, 1993); (10) Stankov, et al. 1991; (11) Reppert, et al. 1988.

paradigm," for review see: Bartness et al., 1993). The short day-like gonadal regression, serum follicle-stimulating hormone (FSH) concentrations and body mass responses were blocked by the SCN lesions (Bartness et al., 1993). However, in Syrian hamsters, a species that shows similar melatonin binding patterns in these three structures (but also shows IMEL binding in other areas; Weaver et al., 1989; Williams et al., 1989), a similar timed infusion of melatonin in hamsters bearing SCN lesions failed to block the short day-like gonadal regression (Maywood et al., 1990). Instead, lesions of the anterior hypothalamus, an area that surrounds the SCN, block the effects of short day melatonin signals in this species (Maywood et al., 1990). In an attempt to reconcile these apparent species differences in the brain areas necessary for the reception/transmission of melatonin signals, we recently made lesions of the anterior hypothalamus and explicitly spared the SCN (Song and Bartness, 1996). This was done because we assumed that our original SCN lesions resulted in ancillary destruction of the adjacent anterior hypothalamus. Hamsters with anterior hypothalamic lesions that were given timed infusions of short day-like melatonin signals showed reproductive and metabolic responses that were similar to hamsters bearing sham anterior hypothalamic lesions without short day-like melatonin signals (Song and Bartness, 1996). Therefore, the differences in brain regions necessary to receive/transmit melatonin signals between the two species appear to be real: Siberian hamsters require an intact SCN; Syrian hamsters do not, but they do require an intact anterior hypothalamus.

Further support for the involvement of the SCN as a site of melatonin action in Siberian hamsters comes from direct application of melatonin (criterion #2 above). Melatonin or the vehicle was administered daily through reverse microdialysis to decrease the probability of non-specific (hydrostatic) lesions. The three central nervous system structures (i.e., SCN, paraventricular thalamic or the reuniens nuclei; Carlson et al., 1991; Duncan et al., 1993) that show IMEL binding (criterion #1 above) were each infused daily in separate groups of juvenile hamsters housed in constant light to eliminate endogenous melatonin secretion (instead of pinealectomy). All three areas were responsive to infusion of the short day pattern of melatonin, but the SCN was the area most sensitive to melatonin application. Thus, in *Siberian hamsters, all three criteria for a melatonin site of action seem to have been met by the SCN*; although juvenile, rather than adult animals were used to test criterion #2.

Dorsomedial Hypothalamic Nucleus

Another possible brain site for the effects of melatonin on reproductive status has been found in Syrian hamsters. Syrian hamsters show IMEL binding in several forebrain structures which do not bind IMEL in Siberian hamsters (Maywood et al., 1996; Maywood et al., 1995; Williams et al., 1989), one of which is the dorsomedial hypothalamic nucleus of the hypothalamus. This area is interesting in that both IMEL binding and immunoreactive androgen receptors show distinct regions of overlap (Maywood et al., 1996). Lesions restricted to the dorsomedial hypothalamic nucleus or sham operations were made in pinealectomized hamsters which were then infused with melatonin or the saline vehicle using the timed infusion paradigm. Hamsters with dorso medial hypothalamic nucleus lesions receiving short day melatonin signals did not exhibit gonadal regression or decreased serum LH concentration as did their sham lesion counterparts. Thus, the dorsomedial hypothalamic nucleus of Syrian hamsters, through its response to short day melatonin signals, may affect gonadotropin releasing hormone that, in turn, affects reproductive status. Moreover, the dorsomedial hypothalamic nucleus also may be the central nervous system region responsible for the photoperiod-induced changes in steroid feedback (Maywood et al., 1996).

Conclusions

When taken together, the results of the IMEL binding, and lesion plus stimulation experiments in the two hamster species suggest that the melatonin sites of action for its effects on photoperiod-induced reproductive and metabolic responses have not been highly conserved during evolution. Rather than finding one melatonin site that is uniformly important for the control of seasonal responses, it may be that many species have unique, but functionally equivalent, melatonin sites of action. Such a circumstance will make it more difficult to construct a unified model of the reception/ transmission of melatonin signals. Table 1 shows a comparison of the distribution of IMEL binding sites across several mammalian species.

Finally, although the PTu was mentioned as a peripheral tissue showing IMEL binding, other peripheral tissues also exhibit IMEL binding and therefore, as least for the first criterion presented above, may be important sites for some of the effects of melatonin. For example, IMEL binding has been found in the vasculature (i.e., arteries of the tail and circle of Willis in rats; Viswanathan, 1990, 1993) and is thought to participate in thermoregulatory responses; in the adrenal gland (i.e., sheep, rats, chickens and ducks; Brown et al., 1994; Helliwel et al., 1994) and is thought possibly to be involved in seasonal changes in glucocorticoid secretion and stress responses; in the spleen (i.e., Guinea pigs, ducks, pigeons and mice, but not rats; Yu et al., 1991) and is thought possibly to influence seasonal changes in the immune system; in the liver (i.e., laboratory rats; Acuna-Castroviego et al.,

1993) where it may affect seasonal changes in metabolic fuel utilization; and in the heart and lungs (i.e., chickens, ducks, quail, and frogs; Pang et al., 1993) without any clear physiological relevance.

Collectively, these reports of IMEL binding to brain and peripheral tissues in a wide variety of species should be considered when melatonin is taken for the prevention and/ or treatment of a variety of conditions (e.g., jetlag, cancer, aging, insomnia), applications that have catapulted the hormone to the front page of leading weekly news magazines (e.g., Cowley, 1995) and to the front shelves of health and drug stores. Clearly the location of the sites of action for the known, functionally significant, physiological effects of melatonin is still an unanswered, fundamental question in regulatory biology, despite the great strides that have been made toward identifying and describing melatonin receptors at the molecular level of analysis (i.e., Reppert et al., 1995a, b, c; and for review see: Reppert et al., 1996).

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REFERENCES.

Acuna-Castroviejo, D, M I Pabolos, A Mendendez-Pelaez, and R J Reiter (1993) Melatonin receptors in purified cell nuclei of liver. Res Commun Chem Path Pharmacol *82*:263-256.

Badura, L L and B Goldman (1992) Central sites mediating reproductive responses to melatonin in juvenile male Siberian hamsters. Brain Res*598*: 98-106.

Bartness, T J and H E Albers (In press) Activity patterns and the biological clock in mammals. In: Activity patterns in small mammals. edited by S Halle and N C Stenseth. Heidelberg: Springer-Verlag, 1997.

Bartness, T J, J B Powers, M H Hastings, E L Bittman, and B D Goldman (1993) The timed infusion paradigm for melatonin delivery: What has it taught us about the melatonin s ignal, its reception and the photoperiodic control of seasonal responses? J Pineal Res : 61-190.

Bartness,T J and G N Wade (1985) Seasonal body weight cycles in hamsters. Neurosci Biobehav Rev *9*: 599-612, .

Bittman, E L (1993) The sites and consequences of melatonin binding in mammals A. Zool *33*: 200-211.

Bittman, E L, E M Thomas, and I Zucker (1994) Melatonin binding sites in sciurid and hystricomorph rodents: studies on ground squirrels and guinea pigs. Brain Res *648*:73-79,.

Bittman, E L and D R Weaver (1990) Distribution of melatonin receptors in neuroendocrine tissues of the ewe. Biol Reprod *43*: 986-993, 1990.

Brown, G M, C S Pang, and S F Pang (1994) Binding sites for 2[125I] Iodomelatonin in the adrenal gland. Biol. Signals *3*: 91-98.

Carlson, L L, D R Weaver, and S M Reppert (1991) Melatonin receptors and signal transduction during development in Siberian hamsters (Phodopus sungorus). Dev. Brain Res. *59*: 83-88.

Carter, D S and B D Goldman (1983) Antigonadal effects of timed melatonin infusion in pine alectomized male Djungarian hamsters (Phodopus sungorus sungorus): Duration is the critical parameter. Endocrinology *113*: 1261-1267.

Cassone, V M, J M Chesworth, and S. Armstrong (1986) Dose-dependent entrainment of rat circadian rhythms by daily injection of melatonin. J Biol Rhythms *1*: 219-229.

Cowley, G (1995) The melatonin craze. Newsweek 60-68.

DeVries, M J, S A Ferreira, and J D Glass (1989) Evidence that short photoperiod-induced gonadal regression in the Mongolian gerbil is mediated by action of melatonin in the medial hypothalamus. Brain Res 494: 241-246.

Dubocovich, M L (1983) Melatonin is a potent modulator of dopamine release in the retina. Nature *306*: 782-784.

Duncan, M J (1994) Photoperiodic effects on puberty and specific 2-[125I] iodomelatonin binding sites in Siberian hamsters. Brain Res *640*: 316-321.

Duncan, M J, K S Heller, C C Purvis, B T Massey, and M H Stetso (1993) Investigations of the regulation of specific 2-[125I] iodomelatonin binding sites in Siberian hamsters by endogenous and exogenous melatonin. Brain Re. *631*: 107-113.

Duncan, M J and R A Mead (1992). Autoradiographic localization of binding sites for 2-[125I] iodomelatonin in the pars tuberalis of the western spotted skunk (Spilogale putorius latifrons). Brain Res 569: 152-155.

Duncan, M J, J S Takahashi, and M L Dubocovich (1989) Characteristics and autoradiographic localization of 2-[125I] Iodomelatonin binding sites in Djungarian hamster brain. Endocrinology 125: 1011-1018.

Elliott, JA and Tamarkin, L (1994) Complex circadian regulation of pineal melatonin and wheelrunning in Syrian hamsters. J Comp-Physiol A *174*:469-484.

Glass, J D and G R Lynch (1981) Melatonin: Identification of sites of antigonadal action in mouse brain. Science *214*: 821-823.

Goldman, B D, J M Darrow, and I Yogev (1984) Effects of timed melatonin infusions on reproductive development in the Djungarian hamster (Phodopus sungorus). Endocrinology *114*: 2074-2083.

Hastings, M H F (1991) Neuroendocrine rhythms. Pharmac Ther *50*: 35-71.

Hastings, M H, E S Maywood, F J P Ebling, L M Williams, and L Titchener (1991) Sites and mech anism of action of melatonin in the photoperiodic control of reproduction. In: Advances in Pineal Research, edited by J. Arendt and P. Pevet. London: John Libbey, p. 147-157.

Hastings, M H, A P Walker, A C Roberts, and J Herbert (1988) Intrahypothalamic melatonin blocks photoperiodic responsiveness in the male Syrian hamster. Neuroscience *24*: 987-991.

Helliwell, R J A, H E Howell, W. Lawson, P Barrett, and P J Morgan (1994) Autoradiographic anomalyin I 25I-melatonin binding revealed in ovine adrenal. Mol Cell Endocrinol *104*: 95-102.

Lincoln, G A (1992) Administration of melatonin into the mediobasal hypothalamus as a continuous or intermittent signal affects the secretion of follicle stimulating hormone and prolactin secretion in the ram. J Pineal Res *12*: 135-144.

Lincoln, G A (1994) Effects of placing micro-implants of melatonin in the pars tuberalis, pars distalis and the lateral septum of the forebrain on the secretion of FSH and prolactin, and testicular size in rams. J Endocrinology *1 42*: 267-276, 1994.

Lincoln, G A and K .Maeda (1992) Reproductive effects of placing microimplants of melatonin in the mediobasal hypothalamus and preoptic area in rams. J Endocrinology *132*: 201-215.

Maestroni, G J M (1993) The immunoneuroendocrine role of melatonin. J Pineal Re. *14*: 1-10.

Malpaux, B, A Daveau, F Maurice, Y Gayrard, and J C Thiery (1993) Short-day effects of melatonin on luteinizing-hormone secretion in

the ewe: Evidence for central sites of action in the medial basal hypothalamus. Biol Reprod *48*: 752-760.

Malpaux, B, D C Skinner, and F Maurice (1995) The ovine parstuberalis does not appear to be targeted b y melatonin t o modulate uteinizing hormone secretion, but may be important for prolactin release. J Neuroendocrinology *7*: 199-206.

Maywood, E, E L Bittman, and M H Hastings (1996) Lesions of the melatonin and and rogen-responsive t issue of the dorsomedial nucleus of the hypothalamus block the gonadal response of male Syrian hamsters to programmed infusions of melatonin. Biol Reprod *54*: 470-477.

Maywood, E S, R C Buttery, G H S Vance, J Herbert, and M H Hastings (1990) Gonadal responses of the male Syrian hamster to programmed infusions of melatonin are sensitive to signal duration and frequency but not to signal phase nor to lesions of the suprachiasmatic nuclei. Biol Reprod *43*: 174-182.

Maywood, E S and M H Hastings (1995) Lesions of the iodomelatonin-binding sites of themediobasal hypothalamus spare the lactotropic, but block the gonadotropic response of male Syrian hamsters to short photoperiod and to melatonin. Endocrinology *136*: 144-153.

McNulty, S, I L Schurov, P J Morgan, and M H Hasting (1995) The inhibitory action of melatonin in the ovine pars tuberalis is not dependent on changes in plasma membrane potential. J Endocrinology *145*: 471-478.

Moore, R Y (1996) Neural control of the pineal gland. Behav Brain Res *73*: 125-130.

Morgan, P J, P Barrett, E Howell, and R Helliwell (1994) Melatonin receptors: Localizaton, molecular pharmacology and physiological significance. Neurochem Int *24*: 101-146.

Morgan, P J, W Lawson, G Davidson, and H E Howel (1989a). Guanine nucleotides regulate the affinity of melatonin receptors on the ovine pars tuberalis. Neuroendocrinology *50*: 359-362.

Morgan, P J, W Lawson, G Davidson, and H E A Howell (1989b) Melatonin inhibits cyclic AMP production in cultured ovine pars tuberalis cells. J Molec Endocrinology *3*: R5-R8.

Pang, C S, G M Brown, P L Tang, K M Cheng, and S F Pang (1993)2-125I} Iodomelatonin binding sites in the lung and heart: a link between the photoperiod signal, melatonin and the cardiopulmonary system. Biol Signals *2*: 228-236.

Reiter, R J (1992) The aging pineal gland and its physiological consequences. Bioessays *14*: 169-175.

Reppert, S M, C Godson, C D Mahle, D R Weaver, S A Slaugenhaupt, and J F Gusella (1995) Molecular characterization of a second melatonin receptor expressed in human retina and brain: the Mel1b melatonin receptor. Proc Natl Acad Sci USA *92*: 8734-8738.

Reppert, S M. C Godson, C D Mahle, D R Weaver, S A Slaugenhaupt, and J F Gusella (1995) Molecular characterization of a second melatonin receptor expressed in human retina and brain: the melatonin 1b melatonin receptor. Proc Natl Acad Sci USA *92*: 8734-8738.

Reppert, S M, D R Weaver, V . Cassone, C Godson, and L F Kolakowski (1995) Melatonin Receptors Are for the Birds: Molecular Analysis of Two Receptor Subtypes Differentially Expressed in Chick Brain. Neuron *15*: 1003-1015.

Reppert, S M, D R Weaver, and T Ebisawa (1994) Cloning and characterization of a mammalian melatonin receptor that mediates reproductive and circadian responses. Neuron *13*: 1177-1185.

Reppert, S M, D R Weaver, and C Godson (1996) Melatonin receptors step into the light: cloning and classification of subtypes. TIPS *17*: 100-102.

Reppert, S M, D R Weaver, (1988) S A Rivkees, and E G Stopa. Putative melatonin receptors in a human biological clock. Science *242*: 78-81.

Song, C K and T J Bartness (1996) The effects of anterior hypothalamic lesions on short day responses in Siberian hamsters given timed infusions of melatonin. J Biol Rhythms *11*: 14-26.

Stankov, B, B Cozzi, V Lucini, S Capsoni, J Fauteck, P Fumagalli, and F Fraschini (1991) Localization and characterization of melatonin binding sites in the brain of the rabbit (Oryctolagus cuniculus) by autoradiography and in vitro ligand receptor binding. Neurosci Lett *133*: 68-72.

Viswanathan, M, J T Laitinen, and J M Saavedr (1993). Vascular melatonin receptors. Biol Signals *2*: 221-227.

Viswanathan, M, J T Laitnen, and J M Saavedra (1990) Expression of melatonin receptors in arteries involved in thermoregulation. Proc Natl Acad.Sci USA *87*: 6200-6203.

Wade, G N, T J Bartness, and J M Hamilton (1989) Seasonal changes in body weight and brown adipose tissue in hamsters: Role of photoperiod and diet. In: Hormones, Thermogenesis and Obesity, edited by H Lardy and F Stratman. New York: Elsevier, p. 137-147.

Weaver, D R and S M Reppert (1990) Melatonin receptors are present in the ferret pars tuberalis and pars distalis, but not in brain. Endocrinology *127*: 2607-2609.

Weaver, D R, S A Rivkees, L L Carlson, and S M Reppert (1991) Localization of melatonin receptors in mammalian brain. In: Suprachiasmatic nucleus: The mind's clock. edited by D. C. Klein, R. Y. Moore, and S. M. Reppert. New York: Oxford Univ. Press, p. 289-308.

Weaver, D R, SA Rivkees, and S M Reppert (1989) Localization and characterization of melatonin receptors in rodent brain by in vitro autoradiography. J Neurosci *9*: 2581-2590.

Williams, L M, P J Morgan, M H Hastings, W Lawson, G Davidson, and H E Howell (1989) Melatonin receptor sites in the Syrian hamster brain and pituitary. Localization and characterization using [125I] iodomelatonin. J Neuroendocrin *1*: 315-320.

Yu, Z, H Yuan, Y Lu, and S Pang (1991) [125I]Iodomelatonin binding sites in spleens of birds and mammals. Neurosci Lett *125*: 175-178.

DEXFENFLURAMINE FOR SAD?

In 1989, O'Rourke and colleagues from MIT published positive findings from a treatment study of dexfenfluramine (dexfen) for winter depression (O'Rourke et al. 1989). This past spring dexfen (brand-named Redux) was approved in the U.S. and Canada for use in the treatment of obesity. Given evidence of its antidepressant effect and its new availability, off-label use of dexfen in SAD becomes an option. The following is intended as brief review of considerations relevant to this treatment alternative.

Efficacy

Dexfen acts to enhance serotonergic neurotransmission, both through pre-synaptic release and re-uptake inhibition. Results of the initial treatment study (O'Rourke et al. 1989) must be considered strongly positive, though the number of subjects was low and the finding has not, to my knowledge, been replicated. The design was a crossover comparison of dexfen and placebo, with each treatment condition lasting four weeks interrupted by a two-week washout. Results by patient are included in the Table 1 below (reprinted with permission, Physicians Postgraduate Press). Using standard criteria for categorically defined treatment response, 14 of 18 subjects (78%) on dexfen could be considered full responders compared to 3 of 18 (17%) on placebo. Anecdotal

Table 1. Effect of d-Fenfluramine on Depression Scores in SAD Patients. (Reprinted by permission of Physicians Postgraduate Press; O'Rourke et al., 1989)

	Predrug		Postdrug		Preplacebo		Postplacebo	
Patient	HAM-D	AAD	HAM-D	AAD	HAM-D	AAD	HAM-D	AAD
1	31	14	10	4	19	14	20	16
2	23	16	4	4	25	11	21	15
3	18	14	1	0	24	14	12	13
4	18	10	0	0	19	10	17	7
5	18	16	3	2	18	14	16	14
6	26	13	3	1	24	12	26	10
7	22	17	5	1	22	15	22	17
8	10	11	5	0	15	8	13	11
9	22	16	1	0	19	17	18	15
10	21	15	6	2	29	15	28	16
11	16	13	1	3	15	12	19	19
12	22	17	6	1	34	15	32	16
13	15	4	0	0	24	14	14	11
14	15	12	0	4	15	9	1	2
15	19	11	6	4	21	14	4	7
16	22	10	15	12	20	15	2	2
17	27	13	20	14	18	14	17	14
18	32	18	25	14	24	15	22	12
Mean	21	13	6*	3.6*	21	13	17	12
SE	1.3	0.8	1.7	1.1	1.2	0.6	1.9	1.1

*Differs from predrug scores, $p < .001$

Abbreviations: AAD = Addendum for Atypical Depression to the Hamilton Rating Scale for Depression; HAM-D = Hamilton Rating Scale for Depression

experience with dexfen for SAD in Europe and elsewhere has not been systematically assessed.

Safety

Side effects are typically mild and transient and include dry mouth, headache, diarrhea, and asthenia. Dexfen has no stimulant properties.

Widespread use of fenfluramines for obesity outside North America has revealed an associated risk of developing Primary Pulmonary Hypertension (PPH). PPH is a rare and often lethal condition which typically presents with complaints of shortness of breath. The disorder occurs with a background incidence of about 1 case per 800,000 persons per year (Manson & Faich, 1996) and has a median period of survival after diagnosis of 2.5 years, but patients may survive for longer periods, particularly with the use of newer methods of treatment (Rubin, 1997).

A recent two-year, multi-center case control study in Europe estimated that taking appetite-suppressant drugs for more than three months within the past year increased the risk of developing PPH by a factor of 23.1 (Abenhaim, et al., 1996). The incidence among treatment-exposed persons is thus raised to 28 cases per million or 1 case per 35,000 persons per year. While such risk is not trivial, perspective is gained by noting that such risk is "close to the magnitude of the risk of death from penicillin-induced anaphylaxis or oral-contraceptive-associated venous thromboemboli and myocardial infarction" (Manson & Faich, 1996).

Duration of treatment in winter depression, as opposed to typical chronic obesity, may serve to reduce this risk. The study above (Abenhaim, et al., 1996) observed PPM risk to be increased only among those who had used the drug for more than three months and only as recently as within the past year, both characteristic of use patterns necessary to maintain weight reduction in obesity. (O'Connor, et al., 1995) Winter depression, by contrast, requires only intermittent treatment and provides yearly opportunities for prolonged drug holidays, thereby reducing exposure and, possibly, risk.

Obesity and SAD

Co-occurrence within individuals of obesity and SAD has been observed not uncommonly (Wurtman and Wurtman, 1995), though no formal prevalence estimates are available. Given the risks associated with obesity and the well-demonstrated effect of dexfen in promoting weight loss (Guy-Brand et al., 1989; O'Connor et al., 1995), persons with comorbid obesity and winter depression should be considered carefully for dexfen treatment. This is especially so in cases where there is history of substantial seasonal weight gain and loss. O'Rourke et al., (1989), observed weight lossamong 13 of 18 subjects on dexfen (mean weight loss ±

Table 2

BODY MASS INDEX (BMI), kg/m²

	HEIGHT (feet, in.)					
Weight (pounds)	**5'0"**	**5'3"**	**5'6"**	**5'9"**	**6'0"**	**6'3"**
140	27	25	23	21	19	18
150	29	27	24	22	20	19
160	31	28	26	24	22	20
170	33	30	28	25	23	21
180	35	32	29	27	25	23
190	37	34	31	28	26	24
200	39	36	32	30	27	25
210	41	37	34	31	29	26
220	43	39	36	33	30	28
230	45	41	37	34	31	29
240	47	43	39	36	33	30
250	49	44	40	37	34	31

BMI value of 30 or greater is be considered an indication for dexfen. Values of 27 to 29 are considered candidates if one other cardio-vascular risk factor coexists.

SE=1.2± 0.5 kg; p=.033) compared to 5 of 18 on placebo (0.3±0.7 kg; p=.69).

Severity criteria for justifying dexfen treatment in obesity are operationalized based on *Body Mass Index* (BMI). BMI is calculated by dividing body weight, in kg, by height, in meters, and squaring the result. (BMI=kg/m^2; see table 2 below). Persons with BMIvalues of 30 or greater may be considered candidates for dexfen on this basis alone. Those with values of 27 to 29 are considered candidates if they have one other cardio-vascular risk factor (e.g., hypertension, diabetes, family history of heart disease, cigarette smoking, high cholesterol).

Dosing/Cost

Drug dosing is standardized at 15 mg twice a day, the dose used in both the SAD treatment study above and in recommended use for obesity. The drug retails for about $70 U.S. for a 30-day supply and is not covered by most prescription reimbursement plans in the U.S.

Conclusions

Dexfenfluramine is not recommended as a first-line treatment for winter depression. Reservations are based on limited efficacy data, potential risk of Primary Pulmonary Hypertension (PPM) and the availability of other proven treatments. Nevertheless, its use may be justified in cases where other treatments are ineffective or contraindicated or where there is comorbid obesity and seasonal depression. The cyclical course of SAD should provide predictable opportunity for medication discontinuation thereby, possibly, limiting the risk of treatment-associated PPM.

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References

Abenhaim L, Moride Y, Brenot F, et al. (1996) Appetite-Suppressant Drugs and the Risk of Primary Pulmonary Hypertension. New Engl J Med; *335*:609-616.

Guy-Grand B, Apfelbaum M, Crepaldi G, et al. (1989) International Trial of Long-Term Dexfenfluramine in Obesity. Lancet; Nov 11:1142-1145.

Manson JE, Faich GA (1996) Pharmacotherapy for Obesity - Do the Benefits Outweigh the Risks? New Engl J Med; *335*:659-660.

O'Connor HT et al. (1995) Dexfenfluramine Treatment of Obesity: Adouble-Blind Trial with Post-Trial Follow-Up. Int J Obesity Relat Metab Dis; *19*:181-189.

O'Rourke D, Wurtman JJ, Wurtman RJ, et al. (1989) Treatment of Seasonal Depression with d-fenfluramine. J Clin Psychiatry; *50*:343-347.

Rubin, LJ (1997) Primary Pulmonary Hypertension. New Engl J Med. *336*:111-117.

Wurtman RJ, Wurtman JJ (1995) Brain serotonin, carbohydrate-craving, obesity and depression. Obes Res; 3 Suppl 4:477S-480S.

Commentary

NOCTURNAL MELATONIN EXCRETION AND CHANGES OF IMMUNOLOGICAL STATUS IN ISCHEMIC STROKE PATIENTS

In a recent letter to the journal *Lancet*, Fiorina et al. (1996) reported impaired nocturnal urinary excretion of melatonin (see synopsis below) and altered cell-mediated immune function in patients with ischemic stroke. Urinary melatonin excretion during a 12-hour period during the day (0800-2000) and a second 12-hour period during the night (2000-0800) was measured in a group of 13 patients who were admitted to the hospital with a diagnosis of ischemic stroke. In addition, tests of immunological status, including an anergy battery were performed in all patients. These results were compared to those obtained from an age-matched healthy volunteer group. Their findings indicate a marked decrease in nocturnal melatonin excretion and a lack of response to the anergy battery (indicating impaired cell-mediated immune function) in stroke patients.

Several factors in the experimental design may limit the interpretation of these findings. Details regarding the environmental conditions and some essential characteristics of the experimental subjects were not provided in this report. Thus, it is not clear whether efforts were made to control for several important factors that can modify the 24-hour profile of melatonin secretion in humans. If the healthy volunteers were residing in the community, their level of exposure to environmental zeitgebers and personal schedules could be different from the hospitalized patients. For example, lighting conditions in the hospital, particularly during the night,may have significant effects on melatonin levels. Similarly, various medications, including non-steroidal anti-inflammatory drugs, can modify pineal function and/or the metabolism of melatonin (Arendt, 1995) and may represent a significant confounding variable in both groups of experimental subjects. Furthermore, stress due to illness may alter autonomic nervous system function which in turn could affect melatonin secretion. Finally, since less than 1% of melatonin in the circulation is excreted by the kidneys in its native form, the determination of 6-sulfatoxymelatonin in urine collection samples provides a highly superior and more reliable integrative measure of human pineal function during various periods of the 24-hour cycle.

The finding of impaired cell-mediated immune response in stroke patients is very interesting; however, it is non-specific and can be seen in other types of illnesses. Similarly, decreased melatonin levels and/or disruption of its 24-hour profile have been reported in a variety of states which involve damage to the central nervous system, such as normal pressure hydrocephalus following aneurysm rupture, hemorrhagic stroke, Lennox-Gastaut syndrome and others (Pang et al., 1990; Yamada et al., 1991). Therefore, alterations of the diurnal rhythm of melatonin and immunological dysfunction may not be directly related to cerebrovascular disease. Further clinical investigations are necessary to evaluate the scope, underlying mechanisms and practical implications of these findings. Nevertheless, these results raise the intriguing possibility that alterations in circadian rhythmicity and melatonin profile may be associated with increased morbidity in hospitalized patients with cerebrovascular disease.

Synopsis

Urinary melatonin and immuno-neuroendocrine measures were assessed in 13 patients (5 female, 8 male; mean age = 64) suffering from ischemic stroke with the results compared to those from 5 healthy controls (mean age = 63).Mean (± SD) 12 hr nocturnal melatonin of controls was 30.0 ± 3.0 ng/ml compared to 0.05 ± 0.01 ($p<.001$) for the patients. The groups did not differ with respect to 12 hr daytime melatonin levels. Cell-mediated immunity was also depressed in the patients (0.77 ± 1.24) compared to controls (2.8 ± 0.45; $p<.01$).

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References

Arendt J. (1995) Melatonin and the mammalian pineal gland. Chapman and Hall, London.

Fiorina P, Lattuada G, Ponari O, Silvestrini C, Dall'Aglio P (1996) Impaired nocturnal melatonin excretion and changes of immunological status in ischaemic stroke patients. Lancet *347*:692-693.

Pang SF, Li Y, Jiang DH, Chang B. Xie BL. (1990) Acute cerebral hemohharge changes the nocturnal surge of plasma melatonin in hu-mans. J. Pineal Res. *9*:193-208.

Yamada N, Iwase H, Noris S, Kurokawa N, Fujimoto K, Kawashima K, Masuzawa T, (1991) Melatonin secretion in normal pressure hydrocephalus after aneurysm rupture — Investigation before and after ventriculoperitoneal shunt. Neurologia Medico-Chirurgia *31*:490--497.

BULLETIN BOARD

SLTBR YOUNG INVESTIGATOR AWARD

The Society for Light Treatment and Biological Rhythms offers an annual award of $500 for the purpose of stimulating international research in clinical aspects of biological rhythms and light therapy by young investigators.

GUIDELINES

1. Candidates shall not have passed their 35th birthday on February 15, 1997. When more than one investigator is listedas an author of the submitted work, all must meet this requirement.
2. Although the research is not to be judged in comparison with the work of the more senior investigators in the field, special consideration will be given to the originality of the approach and independence of thought evident in the submission.
3. The candidate(s) must be still actively involved in the area of research described in the submission.
4. The submission should be in the form of a manuscriptthat describes empirical studies recently completed but notpublished or currently under consideration. The studies and data must not have been published elsewhere at the time of submission.
5. The winning authors will receive their awards and will be invited to present a scientific abstract based on their pa - per at the 9th Annual Meeting of the Society in the spring of 1997
6. Submission will be welcomed from young investigators in any country.
7. Candidates need not be current members of the Society for Light Treatment and Biological Rhythms.

INSTRUCTIONS

1. Please submit manuscripts (six copies) of 10-20 pages (double-spaced) in length, excluding references.
2. A brief biographical sketch (six copies) should be sent with the manuscript.
3. The deadline for receipt of submissions is January 31, 1997.
4. Submissions should be sent to Young Investigator Award, SLTBR, 10200 W. 44th Avenue, Suite 304, Wheat Ridge, CO 80033-2840.

1997 ANNUAL MEETING IN VANCOUVER, B.C.

SLTBR's 9th Annual Meeting will be held June 7, 8 and 9, 1997, in Vancouver, B.C., Canada. This meeting will take place immediately preceeding the annual APSS meeting being held in San Francisco, Calif., a short 1.5 hour airplane flight away.

Look for details of the meeting in the next issue of *LTBR*.

MEETING ANNOUNCEMENTS

Journées Internationales d'Endocrinologie Clinique - Symposium on Endocrine Rhythms—Roles of the Sleep-wake Cycle, the Circadian Clock and the Environment: Diagnostic and Therapeutic Implications, Paris, France, May 29-30, 1997.

For information contact Dr. G. Copinschi or Dr. A. Caufriez, Laboratoire de Médecine Expérimentale, Faculté de Médecine - CPI 618, Université Libre de Bruxelles, Route de Lennik 809, B-1070 Bruxelles, Belgium: Tel: +32-2-555-62-38 (8 a.m. to noon) Fax: +32-2-555-62-39.

International Congress on Chronobiology, Sept. 7-11, 1997, Paris, France. The Congress will focus on significant advances in all aspects of biologic rhythms and their applications, especially in clinical fields. Proposed topics will cover: molecular and cellular clock mechanisms; circadian oscillators and their regulation; circadian, ultradian and infradian rhythms; sleep wake cycle; desynchronisation (jet lag and shift work); melatonin and the pineal gland; development and aging; chronopharmacology; chronotoxicology; chronotherapeutics; rhythms in endocrine and neuroendocrine functions; rhythms in metabolic functions; cancer and chronotherapy of cancer; rhythms of systems and their therapeutics (gastrointestinal, cardiovascular, blood pressure and hypertension, renal, respiratory and immunoallergology).

The format will consist of symposia with invited lectures by leading investigators in each session as well as short oral presentations and poster sessions on original research in the field. The proceedings will be published by Elsevier.
For information, contact Pr Yvan Touitou, Conference President, International Congress on Chronobiology, Service de Biochimie Médicale, Faculté de Pitié-Salpêtriére, 91 boulevard de l'Hôpital, 75634 Paris CEDEX 13, France, Tel:(+33-01) 40 77 96 63, Fax: (+33-01) 40 77 96 65, e-mail: touitou@ccr.jussieu.fr.

WELCOME NEW MEMBERS

Welcome to the following new members of the Society who have joined since publication of the September 1996 issue of *LTBR*.

Horst Boger
Dong Chen
Arvinder Grewal
Heather Larkin
Tadeusz Pracki
Craig M. Rudasics
Steven J. Young
Jan-Erik Broman
Andrew JP Francis
Ron B. Kerr
Daria Pracka
Maria Rosa Ramundo
Rae Silver

AUTHORS ASKED TO USE *JBR* STYLE GUIDELINES

Authors contributing articles to *LTBR* are reminded that submissions should be written in the style consistent with the *Journal of Biological Rhythms*. This is especially true of references. Articles not submitted in the correct style may be returned to the authors for revision.

General guidelines for references include: Authors' given names are abbreviated as initials with no periods following surnames; volume numbers of periodicals are italicized with no space after the colon following the volume number, and journal titles are abbreviated as appropriate with no periods.

Examples of proper reference style are as follows:

Campbell SS, Dawson D, and Anderson M (1993) Alleviation of sleep maintenance insomnia with timed exposure to bright light. J Am GeriatriSoc *41*:829-836

Okawa M, Hishikawa Y, Hozumi S, and Hori H (1991) Sleep-wake rhythm disorder and phototherapy in elderly patients with dementia. In *Biological Psychiatry*, G Racagri and T Fukuda, eds, pp 837-840, New York, Elsevier Science Publishers.