BIOLOGICAL RHYTHMS BULLETIN

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NEW JBR EDITOR

The nominating committee is pleased to announce the selection of the next editor of the Journal of Biological Rhythms - Martin Zatz, Ph.D., M.D. The committee was faced with an excellent slate of candidates. The committee appreciates efforts that were made by members of the Society for Research in Biological Rhythms in making nominations, and by the candidates in sharing their thoughts regarding possible future directions of the journal. This information will be forwarded to Dr. Zatz.

Martin Zatz, Ph.D., M.D., who holds the position of Chief, Laboratory of Cellular and Molecular Regulation (LCMR), NIMH, has agreed to serve as editor. He will take over responsibilities for review of manuscripts in October 1999, and will serve a 5 year term. Dr. Zatz can be reached at the "loci" indicated below:

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Nominating committee members: Jay Dunlap, Gene Block, Robert Y. Moore, Rae Silver and Irving Zucker

Charlotte Remé receives Bühlmann award

Dr. Charlotte E. Remé from the University Hospital Zurich received the ALPCO/Bühlmann Award during the 11th Annual Meeting of the Society for Light Treatment and Biological Rhythms. The award is granted by the Society to an internationally-recognized scientist working in the field of light treatment or biological rhythms. The award is sponsored by ALPCO and Bühlmann Laboratories, producers of a melatonin and 6-sulfatoxymelatonin RIA. In the First Annual ALPCO/Bühlmann Distinguished Lecturer Presentation, chaired by Dr. Dan Oren, President of the Society for Light Treatment and Biological Rhythms, Dr. Remé gave a lecture titled, "Retinal Gene Expression, Cell Death, and the Spectral Distribution of Visible Light."

From the Editors:

This is the third issue of the Biological Rhythms Bulletin. By now, everyone in both the SLTBR and the SRBR should be generally aware of the breadth of content acceptable to the Bulletin. Let this editorial be further encouragement for you to contribute something.

To date, most of the material published has been solicited. We would much prefer a better balance between the solicited and unsolicited. Also, because nothing has yet been rejected, we clearly have not reached our limits of acceptability, at least with respect to subject matter. Try us! Publication in the Bulletin is about as painless as the process can be. For example, did you have a grant funded recently? Is that wonderfully convincing "Background and Significance" section you wrote for that grant just gathering dust? Is it something others in the field would benefit from seeing? Send it to the Bulletin and try it out! Would you like to become a regular columnist or cartoonist or doggerel specialist? Contact us! Do you know any anecdotes about Aschoff or Pittendrigh or any rhythm-related history that you would like to share? Write it up and send it in. And have fun doing so.

Somehow, the above announcement from the SRBR that Marty Zatz will "serve a 5 yr term" as new JBR editor does not sound quite right. Well, congratulations anyway, Marty.

The following questions have been raised by SRBR members: How many chronobiologists does it take to screw in a light bulb? Wind a watch? Any help with the answers would be greatly appreciated. Heard on the grapevine: A reviewer of a manuscript submitted to Pineal Research recommended that the authors publish it in a "more specialized" journal.

In This Issue

Commentary on a paper by Calderone and Jacobs (1999): UV Sensitivity in the Syrian Hamster

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Most rodent species have two spectrally distinct cone photoreceptor classes in their retina. For example, the mouse has a middle wavelength-sensitive cone class (M-cones, λ_{max} 511 nm) and a short wavelength-sensitive cone class (S-cones, λ_{max} 359 nm).[1] Retinally degenerate mice have been used to assess the role of rod and cone photoreceptors in the photic regulation of circadian rhythms. Most of these naturally-occurring or transgenic animal models suffer from genetic lesions that specifically target the rods but result in a protracted secondary loss of cones.[2, 3] A major criticism has been that perhaps a few surviving cones are sufficient to maintain the full sensitivity of the circadian system to light. To put this criticism in perspective, one must understand that cones comprise only 3 to 5% of the total complement of murine photoreceptors.[4] By nine weeks of age, the *rd* (*retinal degeneration*) mouse has lost all of its rods and greater than 95% of its cones.[5] Although these few remaining cones (approximately 4000) contain opsin, the protein moiety of retinaldehyde-based photopigments, they lack outer segments, the light-capturing apparatus of photoreceptor cells.[3] Nevertheless, critics have contended that this sickly contingent of cells may be sufficient to maintain the observed, unattenuated circadian photosensitivity. To address this lingering criticism, Freedman *et al.*[6], crossed a transgenic rodless strain of mouse (*rdta*)[7] with a transgenic coneless strain of mouse (*cl*).[8] Progeny homozygous for both of these trans-genes (*rdta cl/rdta cl*) continued to exhibit robust, unattenuated phase shifts of circadian locomotor activity rhythms in response to 15 minute monochromatic light pulses (λ_{max} 509 nm). In fact, at the highest irradiance tested (5.7 μW/cm^2), *rdta* mice show greater phase shifts than wild-type controls.[6]

One caveat to these studies is that *rdta cl/rdta cl* mice are probably not completely coneless. S-cone opsin mRNA remains detectable by RT-PCR.[6] Mice homozygous for the coneless transgene (*cl/cl*) retain about 5% of their short wavelength-sensitive cones (S-cones).[8] Again, a critic may contend that this small cohort of S-cones is capable of carrying the entire burden of photoentrainment. The phase shifting stimulus used in these studies is centered around 509 nm which is spectrally distant from the 359 nm λ_{max} of the murine S-cone. If this reduced set of S-cones were indeed mediating phase shifting, then one would expect a greatly attenuated ability to capture photons relative to wild-type controls because of the dramatic attrition of S-cones. This most likely would be reflected as an attenuated sensitivity of the circadian system to light. To address the role of S-cones in photoentrainment conclusively, a mouse model missing all S-cones would prove extremely useful.

No murine model exists that completely lacks S-cones, but in 1997, von Shantz and colleagues showed that another rodent, the Syrian hamster (*Mesocricetus auratus*), has no S-cones.[9] (See Table 1) This conclusion was based upon a failure to detect hamster S-cone opsin mRNA by probing a Northern blot with a mouse S-cone opsin probe. S-cone opsin-specific antibodies also failed to label cones in fixed retinal sections.

This year, in a comprehensive study comparing the cone classes of Syrian and Siberian hamsters, Calderone and Jacobs exhaustively confirmed the absence of S-cones in the Syrian hamster.[10] Using electroretinographic techniques, they showed that the Siberian hamster, like the mouse, has two spectrally discrete photopic sensitivities (360 nm and 500 nm). Furthermore, they demonstrated that the photopic short wavelength sensitivity could be attributed to a distinct photopigment. This was accomplished by eliminating the contribution of the long wavelength photopigment to the electro-retinogram by presenting an intense long wavelength adaptation background upon which the short wavelength test stimulus was given. This chromatic isolation paradigm, however, failed to isolate a short wavelength sensitivity in the Syrian hamster. The lack of a short wavelength sensitivity in the Syrian hamster was corroborated by immunohistochemical studies using S-cone opsin-specific antibodies distinct from those used by von Schantz and coworkers. Calderone and Jacobs also failed to find cone labeling of retinal whole mounts with their anti-S-cone antibodies. The Siberian hamster provided a convenient, closely related, positive control.

Kacher-Cobb and colleagues presented a poster at this year's meeting of the Association of Research in Vision and Ophthal-mology (ARVO) showing that the absence of S-cone opsin in the Syrian hamster is a result of a mutation in the S-cone opsin gene.[11] Specifically, a single nucleotide has been deleted in codon 90 of exon 1. This deletion causes a frameshift and results in a premature termination codon in the beginning of exon 2.

Paradoxically, the absence of S-cones has not prevented the Syrian hamster from detecting ultraviolet (UV) stimuli. Thirteen years ago, Brainard *et al.* showed that monochromatic UV radiation (λ_{max} 360 nm) is capable of suppressing elevated nocturnal levels of pineal melatonin in intact, but not bilaterally enucleated, Syrian hamsters.[12] In a follow-up study, this group determined that 10 times the number of 360 nm photons compared to 500 nm photons, were required to elicit a 50% suppression of pineal melatonin.[13] Although this is consistent with rhodopsin-mediated pineal melatonin suppression, the authors caution that other known or yet unidentified photopigments within the retina may mediate the melatonin response.

A "light at night" protocol by Bertoni and coworkers demon-strated that the stimulated gonadal status of male Syrian hamsters could be maintained by 1 hour pulses of UV in the dark portion of a nonstimulatory (10:14 h) light:dark cycle.[14] Pulses were given at equal irradiances (0.2 μW/cm^2) and not corrected for photon number. Because the photons of shorter wavelengths are more energetic than those of longer wavelengths, stimuli of equal irradiances resulted in a greater number photons being given at the longer wavelengths. Even this bias toward the longer wavelengths could not exceed the effectiveness of UV photons in the maintenance of the *reproductive axis.*

Phase shifting of circadian locomotor rhythms in response to UV pulses has also been observed in the Syrian hamster. von Shantz *et al.* have shown that administration of a subsaturating pulse of 515 nm light (3.4 X 10^{15} quanta s^{-1}m^{-2}) at circadian time (CT) 14 re-sults in phase shifts of 48.5 ± 13.2 min (n=2).[9] A similar pulse of UV radiation (360 nm, 2.3 x 10^{15} quanta s^{-1}m^{-2}) at CT 14 results in virtually the same magnitude of phase shift (40.6 ± 7 min, n=5).

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TABLE 1

Rodent Model	Photoreceptor Status	Circadian Responses	Ref.
wild-type mouse	full complement of rods full complement of M-cones full complement of S-cones	normal wild-type response	3
rd/rd mouse	no rods very few (approx. < 5%) M-cones very few (approx. < 5%) S-cones	unattenuated relative to wild-type	3
rdta/rdta mouse	no rods very few (approx. < 5%) M-cones very few (approx. < 5%) S-cones	unattenuated relative to wild-type	2, 6
cl/cl mouse	full complement of rods no M-cones very few (approx. < 5%) S-cones	unattenuated relative to wild-type	6
rdta cl/rdta cl mouse	no rods no M-cones very few (approx. < 5%) S-cones	unattenuated relative to wild-type	6
Syrian hamster	full complement of rods full complement of M-cones no S-cones	normal wild-type response	9

What is the mechanism by which a rodent species with no known short wavelength-sensitive photopigment in the retina can detect UV radiation? This remains unknown. Mechanisms invoking extraocular photoreception, intraocular fluorescence, or even fluorescence of the Harderian gland have been shown to be unlikely mediators of UV-induced responses.[9,15] Perhaps the most parsimonious explanation is that an unidentified photopigment sensitive in the UV range regulates these responses to short wavelengths. RGR[16], peropsin[17], and melanopsin[18] are all novel opsins identified in non-rod, non-cone cells of the human and murine retinas. A homolog of one of these or an as yet unidentified photopigment may be the elusive UV-sensitive pigment of the Syrian hamster. The impending completion of the human and mouse genome projects may help to narrow the candidate of homologs.

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Society for Light Treatment and Biological Rhythms 12th Annual Meeting and CME Course

Sunday (noon), May 7, through Tuesday (noon), May 9, 2000

Holiday Inn Evanston, 1501 Sherman Avenue, Evanston IL 60201; (847)491-6400 or (800)382-6786; Fax (847)328-3090; www.holiday-inn.com chies@worldnet.att.net. Evanston Location and Transportation Information: Easy access to Interstate 94 and 294; 20 minutes from Downtown Chicago; 4 blocks from Northwestern University; 15 minutes from O'Hare Airport; 40 miles from Midway Airport; Public Transportation 1 block from hotel. Area Attractions: Walking distance to boutiques and restaurants; Lake Michigan Beaches 4 blocks; Chicago Museums 25 minutes away; Local Downtown Shopping

Course and Program Details, Registration Information, Call for Abstracts and travel information will be listed at later dates.

BETTER THAN AND/OR DIFFERENT FROM AMELIA? Will there ever be another meeting site?

Every two years, the SRBR meets. One of the topics discussed at the business meeting is where to hold the next meeting. Ambivalence reigns. Prepare now for that discussion. Think about alternatives sites and make suggestions during the next few months and, most importantly, provide some support for them. Send suggestions to the next SRBR President, Dr. Rae Silver (Email: qr@columbia.edu) or to Larry Morin (Email: lmorin@epo.som.sunysb.edu). Santa Fe, NM, has been suggested already. Is this a good or a bad choice? Has anybody been to a meeting there?

Integrating Psychological and Physiological Mechanisms of SAD: The Dual-Vulnerability Model

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Theory and research on the etiology of seasonal affective disorder (SAD) have had a primarily biological focus. Among the reasons for this are the parallel with seasonal physiological changes in animals, the role of light in circadian rhythms, the effectiveness of light treatment for SAD and the theoretical orientation of early workers in the SAD field. For affective disorders in general, there is a great deal of evidence that both biological and psychological factors play a role in terms of vulnerability, onset, course, severity, and treatment response. This statement applies to both unipolar and bipolar disorders, although the specific influences of biological factors and psychological factors are not necessarily equivalent. The purpose of this paper is to connect the literatures of SAD and the psychology of depression and to comment on issues related to integrating physiological and psychological perspectives on SAD in the context of the dual vulnerability model.

Understanding the interaction of physiological and psychological variables[1] is important because it will lead to a better understanding of each domain separately as well as to an understanding of how together they can be more than the sum of their parts. This issue arises more often in human research because cognitive processes are more sophisticated in higher organisms. Also, psychological processes often are manifested as individual differences and animal research often reduces individual differences through breeding and homogeneous environments. However, researchers have recognized that animal responses may differ based on the individual's history of interaction with the environment; Crabbe and colleagues (1999) recently stressed the impact of this phenomenon on research in behavioral genetics.

This paper focuses on the Dual-Vulnerability Hypothesis as an interactional model to understand the etiology of SAD. In 1991 we proposed (Young, et al., 1991) that individuals who meet criteria for SAD—DSM criteria for major depressive episodes and a consistent seasonal pattern of winter episodes and summer remissions—have two vulnerabilities. The first is a tendency for seasonality in vegetative functions, specifically sleep, appetite and energy/motivation. This vulnerability is a normal phenomenon and varies in magnitude across individuals from negligible to severe and debilitating. The second vulnerability is a tendency to develop affective and cognitive symptoms in response to particular stressful conditions, in this case the stress presented by changes in vegetative functioning. (We use "stress" here in a broad sense to potentially include physiological and psychological aspects.)

The dual vulnerability model was developed to explain our data concerning when during an episode the various SAD symptoms begin (Young, et al., 1991). The onsets of hypersomnia, hyperphagia and fatigue, usually considered the vegetative core of SAD, were found to be closely tied to the beginning of the episode. In contrast, other DSM depression symptoms, such as sad mood, decreased self-esteem, guilt, and difficulty concentrating, were equally likely to begin throughout the episode. These different time patterns suggested to us that there are different underlying mechanisms responsible for these different groups of symptoms. This grouping of symptoms is supported by cross-cultural research on depressive disorders in general (Marsella, 1980) which finds that vegetative symptoms are consistent manifestations of depression across cultures, whereas the cognitive and affective symptoms vary more from culture to culture. The latter symptoms presumably are more influenced by cultural/psychological differences. Also consistent with this view is a factor analysis of the Seasonal Pattern Assessment Questionnaire (SPAQ) which found separate factors for vegetative symptoms and mood/social withdrawal (Madden, et al., 1996).

If one has a strongly biological perspective, one might argue that the vegetative symptoms are the "real" depression and that everything else is ancillary. However, this is not how depressive disorder is defined. DSM-IV criteria require the presence of either depressed mood or anhedonia; after that, all symptoms are given equal weight. Because a total of 5 of 9 symptoms is required, it is difficult to meet criteria for major depression without a combination of vegetative, cognitive and affective symptoms. Thus, a theory of depression must explain a wide range of symptomatology.

Research has indicated that there is a heritable component to SAD (Madden, et al., 1996). However, what is inherited is not necessarily the disorder of SAD as defined diagnostically. It may be that one component, such as the vulnerability to vegetative seasonality, is heritable, while other components are not heritable or are heritable through different genes. In a review of genetics and SAD, Sher, et al. (1999) state the issue concisely: "Is SAD inherited as a distinct entity or are seasonality and depression separate heritable traits that happen to coincide in certain individuals?" (P. 204). In addition, a genetic component per se does not indicate whether its effect on SAD is due to a direct physiological mechanism or is mediated by a psychological mechanism. For example, personality traits are heritable. The effect of personality-related genes on SAD could be direct, by their control of the person's physiological functioning, or indirect through psychological processes involving personality. Although Jang, et al. (1998) found that the relationship between personality and SAD was due to genetic rather than environmental factors, it is still not clear whether the relevant genes directly affect both SAD-related physiology and personality expression or whether they directly affect personality which, in turn, affects SAD.

Returning to the dual vulnerability model, what is the empirical evidence for the existence of two vulnerabilities in SAD? The evidence for a normal vulnerability to seasonal variations in vegetative functions comes primarily from general population surveys. Terman.(1988) and Kasper, et al. (1989) conducted such surveys in New York and Maryland, respectively. Their results were remarkably similar in finding a positively skewed distribution of general seasonality scores (GSS) from the SPAQ. Spoont, et al. (1991) obtained similar results in college students assessed with the Inventory of Seasonal Variation (ISV). Hardin et al. (1991) and Thompson et al. (1988) also reported seasonality in normal samples with the SPAQ. Both the GSS from the SPAQ and the ISV emphasize vegetative symptoms and so these results represent normal seasonal variation in these functions. It is important that results have been replicated with different instruments because each instrument has its own idiosyncrasies. In the future studies should assess a wider range of depressive symptoms, including all those in DSM-IV criteria. Also, it is worthwhile to note that self-reported mood typically blends affective, motivational and cognitive aspects so that it is difficult to classify mood items in terms of these distinctions.

What is the evidence for a psychological vulnerability related to SAD? Although there has been an enormous amount of work on

[1]The distinction we draw between physiological and psychological factors refers specifically to the underlying mechanisms involved.In one sense, everything related to an organism has a biological component–your reading this article changes your brain chemistry. However, we would not say that the impact of the ideas presented here is through physiological mechanisms. Rather, it is on a higher level of organization we call cognitive and belongs to the psychological domain.

psychological factors in depression, little work has attempted to apply these theories to SAD.[2] Regarding personality, neuroticism is a dimension of personality that is related to many kinds of psychopathology, including SAD (Murray, et al., 1995; Bouhuys, et al., 1998). Murray et al. (1995) also found that SAD sufferers had a more external locus of control, which might be consistent with the strong seasonal component of their experience.

Studies employing broad measures of personality have yielded diverse results. Using the Five-Factor Model of personality, Bagby et al. (1998) found that SAD patients scored higher than those with non-seasonal depression on Openness, a personality dimension associated with increased sensitivity to internal and external environments. Meesters (1992) reported that 64% of remitted SAD patient had MMPI scores comparable to psychiatric patients, whereas Lillie, et al. (1990) reported that only 38% of remitted SAD patients had scores outside normal limits. In small samples, Schultz et al. (1988) found that SAD patients generally scored between normals and non-seasonal depressives on several MMPI scales and more similarly to non-seasonal depressives on SCID-II Cluster A and Cluster B Personality Disorder scores. These findings are strictly empirically based and the absence of an associated theory makes it difficult to know what they mean.

In the past 30 years several theories have related particular types of information processing or cognitive style to the development of depressive symptoms. These theories have generated a great deal of research and substantial, although not unanimous, empirical support. Most of these models follow a diathesis-stress paradigm in which symptoms are the result of an underlying vulnerability (diathesis) in combination with environmental events (stress). Learned Helplessness Theory began as an animal model of depression and was reformulated (Abramson, et al. 1978) to better describe human phenomenon. According to this theory, individuals with a negative causal attributional style respond to negative events by identifying as their causes factors that are internal, stable and global. As a result they are likely to develop feelings of helplessness and a persistent and pervasive depressive syndrome. Levitan, et al. (1998) found that the level of negative attributional style in SAD patients was similar to that in non-seasonal depressed patients; however, since there was no control group it is not possible to say whether these levels were "high".

Most recently, Young and Azam (1999) applied Response Style Theory (Nolen-Hoeksema, 1991) to SAD. This theory proposes that individuals vary in their response to initial symptoms of depression (including those of normal experience). One style of responding is rumination, in which thoughts and behaviors focus attention on one's symptoms and their possible causes and consequences. Rumination is hypothesized to contribute to increased intensity, duration, and range of symptoms through three mechanism that are well established in normal human functioning: (a) the biasing effect of mood state on information processing, (b) the reduction of positive reinforcement and perceived control due to interference with instrumental behavior, and (c) interference with effective problem solving. Young and Azam found that the degree of ruminative response style (assessed in September) predicted the severity of SAD in February, suggesting that rumination may play a role in the development of SAD.

Other studies also draw attention to the role of response style in SAD. Bouhuys, et al. (1998) found that, compared to nondepressed controls, SAD subjects in remission reported reacting to stressful situations with greater expressed emotion and depression. In addition, for SAD subjects, the greater their emotional response to stress the earlier in the winter their Beck Depression Inventory scores reached 14. Jang et al. (1998) and Bagby, et al. (1996) also reported relationships between SAD and personality factors related to sensitivity, reactivity, rumination and amplification of moods.

Contrary to expectations, Young and Azam (1999) found that ruminative response style was equally related to vegetative and cognitive symptoms. A possible explanation of this is that rumination also affects the experience and self-report of vegetative symptoms. This would parallel the theory of anxiety sensitivity (Reiss, 1987). Individuals with high anxiety sensitivity are more likely both to monitor their physiological state and to believe that their symptoms will have negative outcomes; consequently they report higher levels of subjective distress to the same physiological arousal and experience additional anxiety-related symptoms.

The results on response style are consistent with the dual vulnerability model. Because of their physiological vulnerability, individuals with SAD experience vegetative symptoms of depression early in their episodes. Because of their psychological vulnerability, they then ruminate in response to these symptoms. This contributes to their developing cognitive and affective symptoms later in the episode, as well as possibly intensifying their vegetative symptoms.

What about individuals with only one vulnerability? Those with only the psychological vulnerability may be similar to other depressives.[3] However, it also is possible that the psychological vulnerability in SAD is a diathesis specifically related to seasonal vegetative changes and so is not identical to that of those depressives who are sensitive to other types of stresses. Persons with only the vegetative vulnerability would be those with only seasonal sleep, appetite and energy/motivation symptoms. As such their total scores on depression scales would be lower than SAD subjects and they would probably be less impaired. Thus, these individuals could be similar to subjects currently called sub-SAD (Kasper, et al., 1988). However, sub-SAD has been defined in terms of less symptom severity and less impairment rather than a specific cluster of symptoms. It would be useful to study the validity of a vegetative-only "SAD" syndrome.[4]

This discussion has been speculative and is hardly comprehensive. The amount of empirical research in this area is small. However, the empirical literature on the psychology of depression is large and one purpose of this paper is to connect SAD research to that literature. The dual vulnerability hypothesis needs further conceptual development and empirical testing. However, it can be the basis for designing research and interpreting results. It also is one of many potential theories which can integrate physiological and psychological mechanisms to increase our understanding of SAD. Others, one hopes, will propose complementary and competing formulations. Well elaborated theories, which focus on mechanisms, and not simply correlates, will benefit the field. If this article stimulates people's thinking about SAD and stimulates research that integrates the biological and psychological aspects, as has begun to happen for other affective disorders, it will have succeeded in its purpose.

[2] The results that would be expected in comparing SAD patients to other groups on psychological variables will depend on one's perspective. For example, if the psychological processes are similar regardless of the type of depression, SAD and non-seasonal depressives should be similar to each other and different from non-depressives. On the other hand, if one considers SAD a strictly biological phenomenon, SAD subjects and normals should be similar to each other and different from unipolar depressives (in whom many positive findings already exist.) Finally, SAD could be a unique subtype and produce results different from both normals and non-seasonal depressives.

[3] Cognitive models of depression have been applied almost exclusively to unipolar depression. The role that these factors may or may not play in bipolar disorder is unknown.

[4] In a similar kind of study, we examined the validity of the Reserach Diagnostic Criteria subtype of endogenous depression. We found that this classification was a composite of two subtypings, one of which consisted of the presence or absence of vegetative symptoms (Young, et al., 1986).

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Circadian Rhythms and Genetics in Zebrafish

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The zebrafish (*Danio rerio*) is widely used in genetic analysis of vertebrate embryonic development. This has driven rapid development of the genetic and genomic techniques, information and resources required for mutagenesis, mapping and cloning of zebrafish genes. Recently, research on the zebrafish circadian system has produced efficient measures of rhythmicity than can be used to screen for clock mutations, as well as some information on the molecular, cellular and physiological organization of this system that can be used to interpret mutant circadian phenotypes. These developments now make it possible to use zebrafish in the type of forward genetic approach that has been so successful in defining molecular circadian clock mechanisms in other species.

Zebrafish Genetics and Genomics. The zebrafish was selected by developmental biologists as a genetic model system in part because its embryogenesis is so easy to study, but also because of its small size and vigor under laboratory conditions (up to 2,000 adults can be kept in 4 sq. ft. of laboratory space), high fecundity (each female can produce hundreds of eggs per week), rapid development (from fertilization to free-swimming larvae in 4 days) and relatively short generation time (2-3 months), all of which are important practical considerations for any genetic analysis. Streisinger et al. (1981), who first recognized the potential of this species, developed methods for manipulation of the ploidy of zebrafish that can also be very useful in genetic studies. They found that haploid embryos, produced by fertilization of eggs with UV-treated sperm, would survive and develop for a few days so that haploid genetic techniques could be used in studies of genes that are expressed early in development. Haploid embryos can be made into gynogenetic diploids by hydrostatic "early pressure" treatments that interrupt the second meiotic division, or by heat shock treatments that interrupt the first mitotic division in the embryo. Early pressure embryos are homozygous for all loci close to the centromere, while heat shock embryos are homozygous at all loci. These "instant" homozygotes can be used to accelerate recovery of recessive mutations, and have been very useful in genetic mapping (Postlethwait and Talbot, 1997). Zebrafish can be mutagenized most efficiently by immersion of adult males in N-ethyl-N-nitrosourea (ENU), which results in high rates of fixed point mutations in spermatogonial stem cells (Mullins et al. 1994, Solnica-Krezel et al. 1994). Two large-scale screens for ENU-induced mutations that affect embryonic development recovered over 2,000 mutants that identify over 600 essential genes (Haffter et al. 1996, Driever et al. 1996).

The need for new tools and information to clone all of these mutant genes led to the Zebrafish Genome Initiative (http://zfish.uoregon.edu/zf_info/catch/resources.html), which is designed to develop several critical components of the infrastructure required for genetic mapping and cloning of mutated zebrafish genes. The genetic and genomic information produced under this initiative will eventually be centralized within the developing Zebrafish Information Network (http://zfish.uoregon.edu/ ZFIN). A major component of this initiative is a meiotic map based on simple sequence length polymorphisms (SSLPs). This map now has over 2,000 markers, providing an average resolution of 1.2 cM (Knapik et al. 1998, Shimoda et al. 1999), and addition of 5,000 more markers to increase the resolution to 0.3-0.5 cM (about 200-350 kb) within the next three years is planned (http://zebrafish.mgh.harvard.edu/). This will enable the high-resolution genetic mapping of mutations required for positional cloning. A comprehensive chromosomal deletion panel is also being developed, which will aid in mapping and interpretation of mutants (http://www.ciwemb.edu/zdp.html). An expressed sequence tag (EST) project is in progress to provide partial sequence information for 100,000 zebrafish genes. Over 28,000 zebrafish ESTs are now in the dbEST database, with new ESTs being added at a rate of ~3,000/month (http://zfish.wustl.edu/). Framework maps have been developed for two zebrafish-mammalian radiation hybrid (RH) panels, which will enable rapid physical mapping of cloned zebrafish genes and ESTs (Kwok et al 1998, 1999, Chevrette et al 1998, http://zfish.wustl.edu/rh.html, http://www.eb.tuebingen.mpg.de/abt.3/). Coordinated efforts to map thousands of cloned genes and ESTs on both meiotic mapping panels and RH panels are beginning to provide candidate genes for *mapped* mutations (http://cmgm.stanford.edu/~tallab/Frontpage.html).

Comparison of the first-generation maps of zebrafish genes with the maps of mouse and human genes indicates that many genes that are linked to one another (i.e. syntenic) in mammals are also linked to one another in zebrafish, so the more extensive mammalian gene maps can be used to identify candidate genes for mutations in zebrafish (Postlethwait et al. 1998). These initial gene mapping efforts also showed that, for many single-copy mammalian genes, there are two

homologous genes (or paralogs) in zebrafish, possibly as the result of an ancient whole-genome duplication event in the teleost lineage. The larger number of genes may complicate genetic analysis in some cases, but it may be useful in others. For example, some paralogs have evolved to support complementary subsets of the ancestral gene's functions (*eg*, each may be expressed in a different subset of tissues), which may be useful in defining molecular mechanisms. The presence of paralogous genes with partially overlapping functions might also make it possible to recover viable mutations that would be lethal in the absence of a functional paralog.

Large-insert libraries of the zebrafish genome have recently become available for chromosome walking, and have been used successfully in the purely positional cloning of two mutated zebrafish genes (Zhang et al. 1998, Brownlie et al. 1998). Positional cloning of zebrafish genes is still a difficult undertaking, but it will become easier with the expansion of the resources described above. There also have been major advances recently in the technology for production of transgenic zebrafish, with the achievement of high rates of germ line transmission and tissue-specific transgene expression (Higashijima et al. 1997, Long et al. 1997, Jessen et al. 1998, Yan et al. 1998). These techniques will be important for providing conclusive evidence that a candidate gene is in fact the cause of a mutant phenotype.

The Zebrafish Circadian System. Exploitation of zebrafish genetics in studies of circadian clock mechanisms requires efficient measures of circadian rhythmicity that can be used in screening for mutants, as well as information on the organization of the circadian system for interpretation of mutant phenotypes. During the past three years, studies on molecular, physiological and behavioral circadian rhythmicity in zebrafish have begun to fulfill these requirements.

The first studies of the zebrafish circadian system demonstrated that cultured pineal gland contains a self-sustaining circadian oscillator that controls melatonin synthesis, and that a damped oscillator is present in cultured retina (Cahill, 1996). Pineals cultured in a flow-through superfusion system in constant darkness produce exceptionally robust circadian rhythms of melatonin release for at least seven days. The zebrafish pineal, like the pineals of many other non-mammalian vertebrates, is directly photosensitive; exposure to light in vitro suppresses pineal melatonin production, and resets the phase of the circadian oscillator. However, zebrafish pineal melatonin rhythms are not affected by catecholamines, which regulate melatonin rhythms in the pineals and retinas of many vertebrates (Cahill 1997). The rhythm in pineal melatonin synthesis results at least in part from rhythmic regulation of mRNA for arylalkylamine N-acetyltransferase (AANAT, penultimate enzyme in melatonin synthesis), while the acute suppression of melatonin synthesis by light appears to result from post-transcriptional mechanisms (Bégay et al. 1998). The melatonin rhythm of cultured zebrafish pineals provides an easy and reproducible physiological assay for cell- and tissue-level rhythmicity. However, this tiny organ provides relatively little tissue for biochemical or molecular studies, so the recent identification of circadian oscillators in other, more abundant tissues is important.

Zebrafish retina also contains a circadian oscillator that regulates melatonin synthesis. In our initial studies of cultured retinas, we found that rhythmicity in melatonin release damped within two cycles in vitro (Cahill 1996). In more recent studies, we have found that retinal melatonin rhythms can be made to persist for four or more cycles under some culture conditions, indicating that the retina does contain mechanisms sufficient for sustained rhythmicity (DeBruyne and Cahill, unpublished). Research from other laboratories has also demonstrated retinal circadian rhythmicity in vivo, both in retinal photoreceptor gene expression (Rajendran et al. 1996) and in visual sensitivity (Li and Dowling, 1998). A recent study of *Clock* gene expression in zebrafish demonstrated rhythmicity in many adult tissues in vivo, including the pineal, retina and brain (Whitmore et al. 1998). These studies also showed that circadian oscillators, capable of sustaining at least two cycles in vitro, are functional in cultured heart and kidney. This study was the first to demonstrate directly that differentiated peripheral tissues in a vertebrate are capable of independent circadian rhythm generation. From a practical standpoint, it also showed that these more abundant tissues may be useful for molecular and biochemical studies of circadian rhythmicity.

Screens for clock mutants are most easily performed by automated measurement of rhythmicity in vivo, so we developed methods for measurement of circadian locomotor activity (swimming) rhythms from larval (9-18 day old) zebrafish (Cahill et al. 1998). We measure these rhythms with an automated video image analysis system that tracks the movements of fish housed in individual 0.7 ml wells. With this system, a single video camera can monitor the activity of up to 150 individuals simultaneously for up to a week. Over 95% of larval zebrafish express robust circadian rhythms under these conditions, with highest activity during the subjective day. For our purposes, the phase of the activity peak at the end of a week in constant conditions is the most precise measure of the circadian timing of individual fish. The standard deviation in phases of wild-type zebrafish at the end of week in constant conditions ranges from 0.8 h to 1.2 h, which corresponds to standard deviations in freerunning period of ~0.15 h to 0.20 h, assuming synchronous initial phases. We therefore should be able to detect mutants with period-lengths >0.5 h different from wild-types. We have also examined locomotor activity rhythms of adult zebrafish, using a recording system based on infrared beam detectors (Hurd et al. 1998). We observe more variability in the activity patterns of adults than those of larval zebrafish. Patterns range from a single, robust circadian rhythm, to splitting of the activity rhythm into two rhythms with different periods, to complete arrhythmicity. Under optimal conditions, ~70% of adult zebrafish exhibit significant circadian rhythmicity in activity with this system. With this kind of variability, the adult behavioral rhythm would not be useful for screening purposes, but we have found it to be useful for some physiological experiments.

Screens for zebrafish clock mutants. We have begun to screen for dominant, ENU-induced mutations that alter the freerunning period or phase of the larval swimming rhythm. Because recording of these activity rhythms is largely automated, it requires relatively little human effort to screen a few hundred mutagenized animals every week. Barrett et al (1998) have also begun a screen for dominant circadian clock mutations, exploiting the exceptional precision of zebrafish melatonin rhythms. They have shown that excretion of melatonin by living zebrafish is rhythmic, and developed automated sampling methods that enable measurement of circadian timing from of large numbers of animals in short periods of time. With the rapid emergence of the infrastructure needed for cloning of mutant zebrafish genes, it is now becoming possible to determine the molecular causes of the mutant phenotypes recovered in these and future screens. We are therefore optimistic that studies of zebrafish will increasingly contribute to our understanding of vertebrate circadian clock mechanisms.

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POST DOCTORAL POSITIONS:

A postdoctoral research position will be available starting October, 1999, for a period of at least two years. The individual should be capable of conducting neurophysiological research independently and have excellent writing skills. Experience with *in vitro* and/or *in vivo* single-unit neurophysiological recording methods, and neuropharmacology is required. Primarily neurophysiological studies related to the mechanisms underlying biological rhythmicity in mammals; opportunity also exists for involvement in behavioral, anatomical and molecular biological studies related to this topic. Available immediately for applicants of any nationality within their first two postdoctoral years or for any Canadian applicants. Others may apply and will be considered if no suitable candidates in these categories are identified. Submit curriculum vitae [email: rusak@is.dal.ca; fax: (902) 494-6585] and have at least two letters of reference sent to Dr. Benjamin Rusak, Department of Psychology, Life Sciences Centre, Dalhousie University, Halifax, Nova Scotia, Canada B3H4J1.

Two NIH funded positions are available for postdoctoral research using Drosophila as a model system to study the molecular bases of circadian rhythms. Our lab is interested in identifying and characterizing all the clock components that comprise a circadian oscillator in Drosophila and determining how it responds to external stimuli, such as light and temperature. We use a wide range of experimental approaches including protein biochemistry, immunotechniques, molecular biology (DNA and RNA), tissue culture, behavioral studies and standard Drosophila techniques. Current interests focus on the PER-TIM-dCLOCK-CYC transcriptional feedback loop, light and temperature signal transduction pathways, PAS-mediated interactions, identification of cycling transcripts and proteins, and the role of posttranscriptional regulation (e.g., phosphorylation, splicing and translational control) in the clock mechanism. Experience in protein biochemistry and molecular biology highly desirable but not necessary. Seeking individuals with a strong commitment to innovative research. For example, individuals knowledgeable in circadian rhythm concepts that feel Drosophila is a good model system in which to try out ideas or cross-fertilize with other model systems. Salary commensurate with experience. For further information please contact Dr. Isaac Edery. Rutgers University is an equal opportunity employer and the Center for Advanced Biotechnology and Medicine is a recent state-of-the-art research center (see web page at lion.cabm.rutgers.edu) that houses a wide variety of research interests ranging from structural biology to behavioral genetics. Dr. Isaac Edery, Dept. of Mol. Biol. and Biochem., Rutgers University, Centr. for Adv. Biotech. and Med., 679 Hoes Lane, Piscataway, NJ 08854. 732-235-5550 office/5559 lab; 732-235-5318 (FAX); edery@cabm.rutgers.edu

Circadian Clocks and Photoreceptor Cell Biology. A postdoctoral position is available to study cellular/molecular aspects of the photoreceptor circadian clock. The work is directed at identification "clock genes" and "clock regulated genes" and analysis of their role in circadian function, including retinal physiology. Application of biochemical, molecular and cell biological approaches using retinas from Xenopus laevis and zebrafish. The laboratory is currently employing newly introduced transgenic techniques in Xenopus, application of transgenic technology in zebrafish and mouse, and molecular analysis of clock regulated gene expression. Send/FAX resume and references to: Dr. Joseph C. Besharse, Department of Cell Biology, Neurobiology and Anatomy, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee WI 53226-0509 Phone:414-456-8261 Fax 414-456-6517 E-mail:jbeshars@mcw.edu

The Biological Rhythms Research Lab at Rush Medical Center in Chicago needs coordinator for new 3-yr NIH sponsored study. Bright light and melatonin will be tested for facilitating adaptation to simulated night work. Salivary melatonin will serve as a phase marker. Wrist actigraphy and performance testing. Overseeing the daily functioning of the study, training undergraduate research assistants, supervising the recruitment of subjects, supervising data collection, data management, statistical analysis and manuscript preparation. Interaction with many others including research subjects and undergraduate research assistants, will require excellent communication skills and knowledge of the English language. The study takes place during the 4 summer months, leaving ample opportunities during the rest of the year for data analysis, manuscript preparation, initiating other studies, developing an independent line of research, participating in other ongoing studies in the lab, and the writing of grant proposals. The position pays up to $30,000 (depending on experience) plus fringe benefits. Women and minorities are encouraged to apply. Ideal start date April 2000. Send letter, CV and 3 references (with e-mail addresses) to: Charmane Eastman, Ph.D., Director, Biological Rhythms Research Lab, Psychology Department, Rush-Presbyterian-St. Luke's Medical Center, 1653 W. Congress Pkwy., Chicago IL 60612 E-mail: ceastman@rush.edu fax (773) 955-3958