*Behavior Genetics, Vol. 34, No. 1, January 2004 (© 2004)*

# Number of Arginine-Vasopressin Neurons in the Suprachiasmatic Nuclei Is Not Related to Level or Circadian Characteristics of Wheel-Running Activity in House Mice

**Kelly J. Hochstetler,[1] Theodore Garland, Jr.,[2] John G. Swallow,[3] Patrick A. Carter,[4] and Abel Bult-Ito[1,5]**

*Received 13 Sept. 2002—Final 26 May 2003*

House mouse lines bidirectionally selected for nest-building behavior show a correlation between number of AVP cells in the suprachiasmatic nuclei (SCN), the master circadian clock in mammals, and level of nest-building behavior as well as a correlation between wheel-running activity and SCN AVP content. Similar genetic correlations between wheel-running activity and nest-building behavior have been reported in house mouse lines selected for increased voluntary wheel-running behavior. These similarities in genetic correlation structure in independently selected mouse lines allowed us to test whether AVP in the SCN and wheel running activity are truly correlated traits under identical testing procedures. In the mouse lines selected for voluntary wheel-running, no difference was found between the lines selected for high levels of voluntary wheel-running and randomly-bred control lines in the number of AVP immunoreactive neurons in the SCN ($F_{1,6} = 0.09$, NS; replicate line effect: $F_{1,22} = 0.05$, NS). This finding was confirmed at the level of individual variation, which revealed no relationship between number of AVP neurons in the SCN and total daily activity ($R = -0.086$, NS, $n = 24$), or circadian organization (i.e., the chi-squared periodogram waveform amplitude; $R = -0.071$, NS). Therefore our data do not support the hypothesis that differences in activity level and the circadian expression of activity in young adult mice are related to differences in the number of AVP-immunoreactive cells in the SCN.

**KEY WORDS:** SCN; artificial selection; circadian; rhythm stability.

[1] Alaskan Basic Neuroscience Program, Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, Alaska.

[2] Department of Biology, University of California, Riverside, California.

[3] Department of Biology, University of South Dakota, Vermillion, South Dakota.

[4] School of Biological Sciences, Washington State University, Pullman, Washington.

[5] To whom correspondence should be addressed at Behavioral and Evolutionary Neuroscience Laboratory, Alaskan Basic Neuroscience Program, Institute of Arctic Biology, University of Alaska Fairbanks, P.O. Box 757000, Fairbanks, Alaska 99775. Tel: 907-474-7158. Fax: 907-474-6050. e-mail: ffab@uaf.edu.

## INTRODUCTION

The suprachiasmatic nucleus (SCN) of the anterior hypothalamus has long been known as the site of the circadian clock in mammals (Weaver, 1998). This clock allows the body to time biological functions at a precise phase relationship to the light-dark cycle, but whether it regulates other circadian rhythm features (e.g., rhythm amplitude) is currently unknown. Arginine-vasopressin (AVP) neurons are prevalent in the SCN and are extensively interconnected (Castel *et al.,* 1990; Van den Pol and Gorcs, 1986), suggesting the capacity for coordinated

output. However, efforts to link AVP to specific clock functions have largely been inconclusive. For example, work done by Shimizu *et al.* (1996) revealed that selective immunotoxin-induced reduction of the number of AVP neurons in the SCN of rats eliminated the circadian rhythm of water intake. Likewise, AVP mRNA and protein expression is reduced and arrhythmic in the *Clock* mutant mouse, paralleling changes in circadian function (Jin *et al.,* 1999; Silver *et al.,* 1999; Vitaterna *et al.,* 1994). In contrast, studies of circadian rhythms in AVP-deficient Brattleboro rats have shown that AVP is not necessary for the maintenance of coherent circadian rhythmicity (Groblewski *et al.,* 1981; Murphy *et al.,* 1996, 1998; Stoinev and Ikonomov, 1990).

In aging, decreased AVP content in the SCN (Hofman and Swaab, 1994, 1995; Lucassen *et al.,* 1995) is correlated with decreased amplitude of the activity rhythm, increased rhythm fragmentation, and disruption of the normal sleep/wake cycle (reviewed in Myers and Badia, 1995). These correlations parallel relationships found in young adult male house mice (*Mus domesticus*) bidirectionally selected for thermoregulatory nest-building behavior (Bult and Lynch, 2000). In these mice, increased number of AVP neurons in the SCN of the small nest-builders is correlated with increased wheel-running activity and rhythm amplitude, reduced rhythm fragmentation, and delayed time of daily activity maximum compared to the big nest-builders (Bult *et al.,* 1993, 2001). If AVP neurons in the SCN are functionally involved in regulating the level or circadian characteristics of wheel-running activity, then we predict that mice selected for high levels of voluntary wheel-running activity and consequently high rhythm amplitudes (Garland, 2003; Swallow *et al.,* 1998) will have larger numbers of AVP neurons in the SCN compared to the randomly bred control lines. Rhythm amplitude refers to the daily maximum activity level. Using a paradigm similar to the one used to assess the nest-building mice (Bult *et al.,* 1992, 1993, 2001), we compared the number of AVP-positive SCN neurons to circadian rhythm parameters in these activity-selected lines.

## METHODS

In the Bult-Ito laboratory, 24 adult male house mice (*Mus domesticus*) from generation 19 of selection for voluntary wheel-running ($n = 13$) and nonselected control lines ($n = 11$) (see Swallow *et al.* (1998) for details on selective breeding) were individually housed in polypropylene cages ($24 \times 35 \times 21$ cm) equipped with a 27-cm-diameter running wheel. Animals were randomly assigned to running wheels, and all animals were tested at the same time in the same room. Eight additional animals (four high selected and four control) were housed in the same room in cages without wheels. Purina rodent chow and water were available *ad libitum,* and the animals were kept on a 16:8 light-dark cycle (lights on at 0400) for the duration of the experiment. Animals were allowed free access to a running wheel for 17–18 days, after which they were perfused 3.8–6.3 h after lights on and their brains collected for immunohistochemistry as previously described (Van der Zee and Bult, 1995). The final 7–10 days of the activity record were used for circadian rhythm analysis. Running-wheel activity was recorded as previously described (Amy *et al.,* 2000), and parameters of the wheel-running activity rhythm were quantified using ActiView (Minimitter Co., Inc., Bend, OR) and ClockLab (Actimetrics, Evanston, IL) following previously described criteria (Bult *et al.,* 1993, 2001). Measured circadian rhythm parameters included: total daily activity (number of wheel revolutions per day), time of daily activity maximum, amplitude of the wheel-running rhythm, the chi-squared periodogram waveform amplitude (a measure comparable to the tau peak height used by Bult *et al.,* 2001), and rhythm fragmentation (mean number of activity bouts per day). The chi-squared periodogram is a mathematical time-series analysis to determine the period (Sokolove and Bushell, 1978) and robustness (Bult *et al.,* 2001; Levine *et al.,* 2002) of a rhythm. For comparison, mice from the same generation were tested for wheel-running activity in the Garland laboratory as previously described (Garland, 2003; Rhodes *et al.,* 2001; Swallow *et al.,* 1998). Cell counts for AVP-positive neurons were performed in the six most medial SCN sections from each animal, according to Bult *et al.* (1992). Cell counts represent the total number of AVP-positive neurons in the left and right sides of the SCN for all six evenly spaced 20-$\mu$m coronal sections from each animal (section numbers 4, 7, 10, 13, 16, 19). Neurons were also counted in replacement animals (those without running wheels). Cell counts were done blind to line of origin to avoid bias.

Data were analyzed using SAS software (SAS Institute INC., Cary, NC). All values are reported as means $\pm$ standard error. Results from behavioral screening for selective breeding of generation 19 in the Garland laboratory were analyzed by ANOVA (proc mixed), with family and replicate line (four selected lines, four control lines) entered as nested random effects, for the following fixed effects: selected line (high and control), batch (testing group), age, and wheel freeness. For data collected in the Bult-Ito laboratory, the effects of having a running wheel or not, selected line,

and replicate line on the number of AVP neurons in the SCN were tested by ANOVA (proc glm). Differences in total daily activity between selected and control lines were tested by ANOVA (proc glm) for effects of selected line and replicate line. At the level of individual variation ($n = 24$), linear regression analysis (proc reg) was used to test for relationships between the total number of AVP cells in the SCN and total daily activity or the chi-squared periodogram waveform amplitude. In addition, linear regression analysis was also used to test for relationships between the chi-squared periodogram waveform amplitude and total daily activity, time of daily maximum activity, amplitude of the activity rhythm, or rhythm fragmentation.

## RESULTS

In the Bult-Ito lab, we found a nonsignificant trend for increased total daily running in the high-selected mice (10,582 $\pm$ 794 wheel revolutions per day [revs/day], $n = 13$) compared to randomly bred controls (7851 $\pm$ 1153 revs/day, $n = 11$) (selected vs. control $F_{1,6} = 3.06$, NS; replicate line effect $F_{6,16} = 1.80$, NS). However, the replicate control lines tended to have different mean levels of daily wheel running (replicate line #1: 10,132 $\pm$ 1744 revs/day; #2: 5087 $\pm$ 2136 revs/day; #4: 4639 $\pm$ 1744 revs/day; #5: 10,625 $\pm$ 1744 revs/day) compared to the replicate high selected lines (replicate line #3: 10,824 $\pm$ 1173 revs/day; #6: 12,161 $\pm$ 2573 revs/day; #7: 9310 $\pm$ 1893 revs/day; #8: 9956 $\pm$ 1038 revs/day). Male mice ($n = 266$) from the same generation tested in the Garland laboratory showed a 2.67-fold difference in wheel-running activity (control lines: 3232 $\pm$ 540 rev/day; high lines; 8628 $\pm$ 505 rev/day), similar to results from comparable generations (Garland, 2003; Rhodes *et al.,* 2001; Swallow *et al.,* 1998). The replicate line ($F_{1,6} = 53.31$, $p < 0.0003$) and batch ($F_{2,183} = 15.73$, $p < 0.0001$) effects were significant, whereas the age ($F_{1,183} = 1.34$, NS) and wheel freeness ($F_{1,183} = 1.14$, NS) effects were not.

Results of ANOVA showed no difference in the number of AVP neurons in animals with and without running wheels ($F_{1,6} = 0.02$, NS), and therefore the two groups were pooled. No difference was found between the selected (1134 $\pm$ 38, $n = 17$; Fig. 1a) and control (1139 $\pm$ 44, $n = 15$; Fig. 1b) lines in the number of AVP immunoreactive neurons in the SCN ($F_{1,6} = 0.09$, NS; replicate line effect: $F_{1,22} = 0.05$, NS). To confirm that the number of AVP neurons in the SCN was not associated with total daily activity or circadian parameters of wheel-running activity, linear regressions were performed on all animals combined. No relationship was found between number of AVP neurons in the SCN and total daily activity ($R = -0.086$, NS, $n = 24$; Fig. 1c), or the chi-squared periodogram waveform amplitude ($R = -0.071$, NS, $n = 24$). The chi-squared periodogram waveform amplitude is the primary characteristic of the wheel-running rhythm used in this study. This measure incorporates other characteristics of circadian organization (Bult *et al.,* 2001) as exemplified by the following: chi-squared periodogram waveform amplitude was significantly ($p \leq 0.0125$ with Bonferroni correction for four statistical tests) correlated with total daily activity ($R = +0.712$, $p < 0.0001$, $n = 24$; Fig. 1d), rhythm fragmentation ($R = -0.858$, $p < 0.0001$, $n = 24$), amplitude of the activity rhythm ($R = +0.547$, $p < 0.0057$, $n = 24$), and time of maximum activity ($R = +0.652$, $p < 0.0006$, $n = 24$).

## DISCUSSION

Previous studies have shown that the number of AVP cells in the SCN is correlated with activity level and several circadian characteristics of the activity rhythm (see Introduction). We were able to address the relationship between AVP neurons in the SCN and the circadian rhythm of voluntary wheel running independently from other studies by using mouse lines specifically selected for increased wheel-running behavior. Our data do not support the hypothesis that differences in activity level and the circadian expression of activity in young adult mice are related to differences in the number of AVP-immunoreactive cells in the SCN.

In this study we found apparent differences among the replicate control lines with two of the four lines that tended to show lower activity levels, whereas the two remaining control lines tended to be similar to the four high selected replicate lines. Other studies with these lines, including the generation that produced these mice, have shown significant differences in wheel-running activity between all control and all high selected lines with no significant replicate effect (e.g., Koteja *et al.,* 1999; Rhodes *et al.,* 2001; Swallow *et al.,* 1998). A possible explanation for the difference in wheel-running behavior found among the control replicate lines is the difference in the experimental setups for measuring wheel-running activity. Very large diameter running wheels (1.1 m circumference) located in a separate chamber (connected to the home cage by a short tunnel) are used for selection (Koteja *et al.,* 1999; Rhodes *et al.,* 2001; Swallow *et al.,* 1998) as opposed to the relatively small running wheels (located

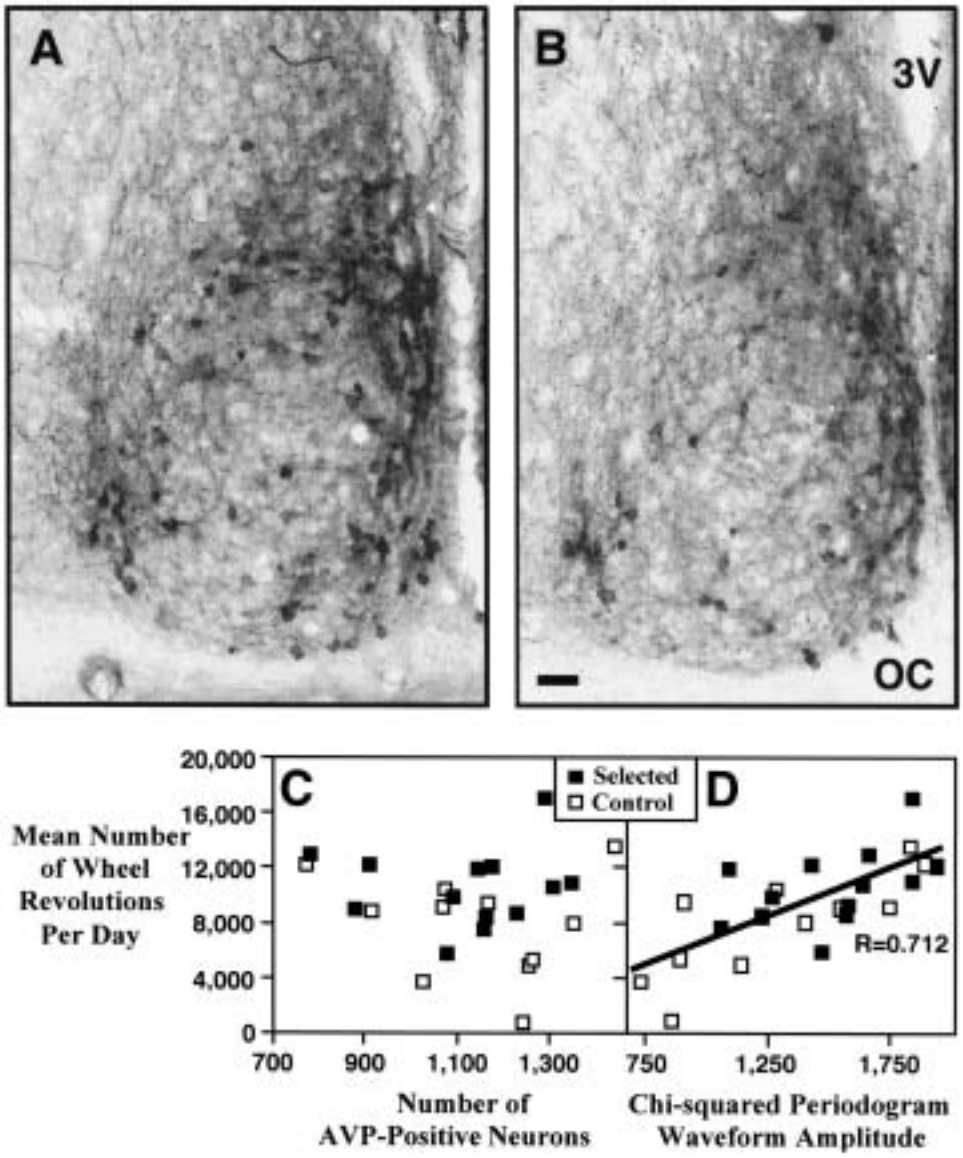


**Fig. 1.** Arginine-vasopressin (AVP) in the suprachiasmatic nucleus (SCN) and its relationship to circadian activity rhythms. A, Representative coronal sections stained for AVP from the medial region of the SCN of a randomly bred control mouse and (B), of a mouse selected for increased wheel-running behavior. C, Mean number of wheel revolutions per day for individual mice vs. number of AVP-positive neurons in six medial SCN sections ($R = 0.0860$, NS, $n = 24$). D, Mean number of wheel revolutions per day for individual mice vs. the chi-squared periodogram waveform amplitude ($p < 0.0001$, $n = 24$). OC, optic chiasm; 3V, third ventricle. Scale bar = 50 μm.

in the home cage) used in this study. Alternatively, the relatively small sample sizes in each replicate line may also have played a role. Because of these differences among the control replicate lines, we used regression analysis to test across selected lines for a relationship between AVP in the SCN and wheel running activity based on total daily activity as opposed to selected line. Again, however, we found no evidence for a relationship between the number of AVP cells in the SCN and total daily activity or characteristics of the circadian activity rhythm. This lack of a relationship is consistent with a recent study that found no difference in the number of Fos-positive cells in the SCN of mice from the selected and control lines (Rhodes, 2002).

Work on house mouse lines bidirectionally selected for nest-building behavior found that small nest-building mice are more active than mice that built large nests (Bult *et al.,* 1993). Similarly, the lines selected

for increased voluntary wheel running used in this study made smaller nests than did less active control lines (Carter *et al.,* 2000). In the lines selected for nest-building (Bult *et al.,* 1993, 2001) and the lines selected for wheel running (this study), significant correlations were measured between the chi-squared periodogram waveform amplitude and total daily activity, rhythm fragmentation, amplitude of the activity rhythm, or time of maximum activity, revealing very similar circadian rhythm organization. The significant correlations also indicate that our data set of the wheel-running selected lines had considerable statistical power. These similarities in genetic correlation structure for nest-building behavior, activity level, and circadian organization in independently selected mouse lines strengthen our conclusion that AVP in the SCN appears to be unrelated to the level or circadian organization of wheel-running activity.

## ACKNOWLEDGMENTS

We would like to thank Ron Tavernier and Dr. Daní K. Raap for helpful comments on an earlier version of the manuscript. This work was funded by NIH Grants 1R15MH58453 (NIMH) and 1U54NS41069 (SNRP: NINDS, NIMH, NCRR, NCMHD) to A. Bult-Ito and by NSF Grants IBN-9728434 and IBN-0212567 to T. Garland.

## REFERENCES

Amy, S. P., Chari, R., and Bult, A. (2000). Fos in the suprachiasmatic nucleus of house mouse lines that reveal a different phase delay response to the same light pulse. *J. Biol. Rhythms* **15**:95–102.

Bult, A., Hiestand, L., Van der Zee, E. A., and Lynch, C. B. (1993). Circadian rhythms differ between selected mouse lines: A model to study the role of vasopressin neurons in the suprachiasmatic nuclei. *Brain Res. Bull.* **32**:623–627.

Bult, A., Kobylk, M. E., and Van der Zee, E. A. (2001). Differential expression of protein kinase C βI (PKCβI) but not PKCα and PKCβII in the suprachiasmatic nucleus of selected house mouse lines, and the relationship to arginine-vasopressin. *Brain Res.* **914**:123–133.

Bult, A., and Lynch, C. B. (2000). Breaking through artificial selection limits of an adaptive behavior in mice and the consequences for correlated responses. *Behav. Genet.* **30**:193–206.

Bult, A., Van der Zee, E. A., Compaan, J. C., and Lynch, C. B. (1992). Differences in the number of arginine vasopressin-immunoreactive neurons exist in the suprachiasmatic nuclei of house mice selected for differences in nest-building behavior. *Brain Res.* **578**:335–338.

Carter, P. A., Swallow, J. G., Davis, S. J., and Garland, T., Jr. (2000). Nesting behavior of house mice (*Mus domesticus*) selected for increased wheel-running activity. *Behav. Genet.* **30**:85–94.

Castel, M., Feinstein, N., Cohen, S., and Harari, N. (1990). Vasopressinergic innervation of the mouse suprachiasmatic nucleus: An immuno-electron microscopic analysis. *J. Comp. Neurol.* **298**:172–187.

Garland, T., Jr. (2003). Selection experiments: An underutilized tool in biomechanics and organismal biology. In Gasc, J.-P., Casinos, A., and Bels, V. L. (eds.), *Vertebrate Biomechanics and Evolution* (pp. 23–56). Oxford, U.K.: BIOS Scientific Publishers.

Groblewski, T. A., Nunez, A. A., and Gold, R. M. (1981). Circadian rhythms in vasopressin deficient rats. *Brain Res. Bull.* **6**: 125–130.

Hofman, M. A., and Swaab, D. F. (1994). Alterations in circadian rhythmicity of the vasopressin-producing neurons of the human suprachiasmatic nucleus (SCN) with aging. *Brain Res.* **651**: 134–142.

Hofman, M. A., and Swaab, D. F. (1995). Influence of aging on the seasonal rhythm of the vasopressin-expressing neurons of the human suprachiasmatic nucleus. *Neurobiol. Aging* **16**: 965–971.

Jin, X., Shearman, L. P., Weaver, D. R., Zylka, M. J., DeVries, G. J., and Reppert, S. M. (1999). A molecular mechanism regulating rhythmic output from the suprachiasmatic circadian clock. *Cell* **96**:57–68.

Koteja, P., Garland, T., Jr., Sax, J. K., Swallow, J. G., and Carter, P. A. (1999). Behaviour of house mice artificially selected for high levels of voluntary wheel running. *Anim. Behav.* **58**: 1307–1318.

Levine, J. D., Funes, P., Dowse, H. B., and Hall, J. C. (2002). Signal analysis of behavioral and molecular cycles. *BioMed Central* **3**:1–25.

Lucassen, P. J., Hofman, M. A., and Swaab, D. F. (1995). Increased light intensity prevents the age related loss of vasopressin-expressing neurons in the rat suprachiasmatic nucleus. *Brain Res.* **693**:261–266.

Meyers, B. L., and Badia, P. (1995). Changes in circadian rhythms and sleep quality with aging: Mechanisms and interventions. *Neurosci. Biobehav. Rev.* **19**:553–571.

Murphy, H. M., Wideman, C. H., and Nadzam, G. R. (1996). Interactions of vasopressin and the photic oscillator in circadian rhythms. *Peptides* **17**:467–475.

Murphy, H. M., Wideman, C. H., and Nadzam, G. R. (1998). The role of vasopressin in modulating circadian rhythm responses to phase shifts. *Peptides* **19**:1191–1208.

Rhodes, J. S. 2002. The neural basis of hyperactive wheel running in mice. Unpublished Ph.D. dissertation. Madison, WI: University of Wisconsin.

Rhodes, J. S., Hosak, G. R., Girard, I., Kelley, A. E., Mitchell, G. S., and Garland, T., Jr. (2001). Differential sensitivity to acute administration of cocaine, GBR 12909, and fluoxetine in mice selectively bred for hyperactive wheel-running behavior. *Psychopharmacology* **158**:120–131.

Shimizu, K., Nagai, K., and Nakagawa, H. (1996). An immunotoxin, anti-VIP antibody-ricin A chain conjugate eliminates neurons in the hypothalamic suprachiasmatic nucleus selectively and abolishes the circadian rhythm of water intake. *Brain Res. Bull.* **41**:369–378.

Silver, R., Sookhoo, A. I., LeSauter, J., Stevens, P., Jansen, H. T., and Lehman, M. N. (1999). Multiple regulatory elements result in regional specificity in circadian rhythms of neuropeptide expression in mouse SCN. *Neuroreport* **10**:3165–3174.

Sokolove, P. G., and Bushell, W. N. (1978). The chi square periodogram: Its utility for analysis of circadian rhythms. *J. Theor. Biol.* **72**:131–160.

Stoinev, A. G., and Ikonomov, O. K. (1990). Effect of continuous vasopressin infusion on circadian rhythms of food and water intake, diuresis, and electrolyte excretion in Brattleboro rats. *Bull. Exp. Biol. Med.* **109**:122–124.

Swallow, J. G., Carter, P. A., and Garland, T., Jr. (1998). Artificial selection for increased wheel running behavior in house mice. *Behav. Genet.* **28**:227–237.

Van den Pol, A. N., and Gorcs, T. (1986). Synaptic relationships between neurons containing vasopressin, gastrin releasing peptide, vasoactive intestinal peptide, and glutamate decarboxylase immunoreactivity in the suprachiasmatic nucleus: Dual ultrastructural immunocytochemistry with gold-substituted silver peroxidase. *J. Comp. Neurol.* **252**:507–521.

Van der Zee, E. A., and Bult, A. (1995). Distribution of AVP and $Ca^{2+}$-dependent PKC-isozymes in the suprachiasmatic nucleus of the mouse and rabbit. *Brain Res.* **701**:99–107.

Vitaterna, M. H., King, D. P., Chang, A. M., Kornhauser, J. M., Lowrey, P. L., McDonald, J. D., Dove, W. F., Pinto, L. H., Turek, F. W., and Takahashi, J. S. (1994). Mutagenesis and mapping of the mouse gene, *Clock,* essential for circadian behavior. *Science* **264**:719–725.

Weaver, D. R. (1998). The suprachiasmatic nucleus: A 25-year retrospective. *J. Biol. Rhythms* **13**:100–112.

Edited by Stephen Maxson