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RESETTING THE SUPRACHIASMATIC NUCLEUS CLOCK

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1. ABSTRACT

Recent research on the cellular basis of circadian rhythmicity has stressed the importance of clock genes for the maintenance of normal rhythmicity. There have been tremendous advances in our understanding of the inter-relationships of the various genes known to generate the rhythms. We know relatively little, however, about the way animals maintain their rhythmicity under normal seasonally changing photoperiods, from the level of neurotransmitters involved in the transfer of photic information to the way the clock genes respond. In this short review, some aspects of entrainment are discussed concentrating on species differences in transmitters used, particularly the role of serotonin. Also the effects of different experimental paradigms on outcomes, for example phase response curves and skeleton photoperiods, are discussed.

2. INTRODUCTION

Life on earth has evolved in an extremely predictable photic environment. The earth's daily rotation and transit around the sun have remained remarkably constant for millennia. On the other hand there have been massive changes in the physical environment reflected in climate change. Given the predictable nature of day and night, it is perhaps not surprising that organisms have evolved the means to measure time. They then use these mechanisms to set up physiological processes for the immediate future. An example might be an animal whose food source becomes available for a short time after dawn.

The animal would be advantaged if it were aroused from rest prior to dawn rather than relying on the perception of sunrise. This could be achieved if the animal possessed the equivalent of an hourglass; when the "sand" ran out, the animal would be aroused and start searching for food. During the day the "hour glass" would be re-charged. But the length of the night changes considerably across the seasons particularly in high latitudes, so there must be some way that duration of the night can be accommodated. The concept of an hour glass has long been discarded and time keeping is now known to involve complex relationships between the environment and genes. In this paper I will address the question of how animals achieve synchronisation of rhythmicity with the external world. It is not intended as a comprehensive review of the subject as there are other recent excellent reviews (1; 2); instead the intention is to highlight some areas that are controversial.

3. CELLULAR RHYTHMICITY

3.1. Rhythmic gene expression

It is now clear that internal time is generated within cells of a wide range of organisms from prokaryotes, to plants, insects and vertebrates. Remarkably the way that cells record time is essentially conserved across organisms,

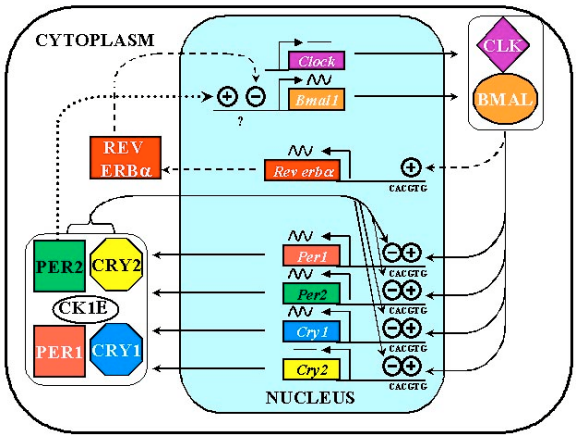

Figure 1. Schematic diagram of the genes underlying cellular rhythmicity. Central to rhythmicity is the CLOCK/BMAL1 heterodimer that binds to the CACGTG E-box and induces period (*per*) and cryptochrome (*cry*) and *rev erbα* genes The protein products of these genes feedback on the promoters to inhibit or induced translation.

although as might be expected higher organisms have the most elaborate systems.

Cellular timing is based upon gene induction, protein synthesis and the feedback of proteins to either induce or repress their own production. In other wordsnegative and positive feedback. Elsewhere in this issue, Urs Albrecht discusses in detail the molecular machinery driving rhythmicity in mammals.

In mammals there are 6 primary genes involved in cellular rhythmicity; *Clock*, *Bmal*1(*MOP*3), *per*1, *per*2, *cry*1 and *cry*2 together with a phosphorylating kinase *CK1E* (3). Figure 1 shows the contemporary understanding of how cellular rhythmicity is generated by negative and positive feedback of bHLH-PAS transcription factors. The central components are the genes *Clock* and *Bmal*1 and their protein products. CLOCK/BMAL1 heterodimers bind to an enhancer region (CACGTG; E-box) in the promoters of *per*1, *per*2, *cry*1 and *cry*2, leading to their induction (4). These 4 proteins form a complex, together with Casein kinase 1*E* and re-enter the nucleus to displace CLOCK/BMAL1 from the E-boxes and so inhibit their own transcription (3). This negative feedback cycle takes approximately 24 hours. The positive drive to the cycle is generated by PER2 inducing *Bmal*1; as a consequence the expression of *Bmal*1 is in antiphase to the other genes. An additional component of the cycle involves the induction of the transcription factor *Rev-erb* α by CLOCK/BMAL1, again via an E-box, leading to inhibition of *Bmal*1 expression by the REV-ERB α protein (5). Recently another component in the control of rhythmicity was reported involving the basic helix-loop-helix transcription factors *Dec1* and *Dec2* which were shown to repress CLOCK/BMAL1 -induced transactivation of the mouse *per1* promoter through direct protein-protein interactions with *Bmal1* and/or competition for E-box elements (6).

3.2. Phase shifting

A fundamental feature of cellular rhythmicity is that in the absence of light, rhythms persist with a period different from 24 hours. In some animals, for example mice, this free running period is <24 hours, whereas in rats and humans it is >24 hours. To remain entrained to the prevailing photoperiod it is clear that the molecular machinery must be re-settable and that this should ideally occur on a daily basis.

Observations in animals and humans indicate that the biological clock can be reset. Importantly, however, the clock system may not reset immediately. An example of this in humans is our physiological response to travel across multiple time zones. A trip from Sydney, Australia (sunrise 05:57, sunset 18:22, local time) to Los Angeles, U.S.A (sunrise 00:08 sunset 11.58, l Australian time) on 12th March results in an apparent advance of the sunset of 6 hours and 24 minutes. A trip from Sydney to London on the other hand results in sunset being delayed by 9 hours 37 minutes. If there were no biological clocks, re-entrainment might be expected to occur spontaneously and there would be no jet lag. As is well appreciated, travelling over these distances by aircraft results in severe disturbances in sleep timing and other physiological disturbances, including hormonal rhythms. The rate of change of objective rhythm markers such as melatonin production indicate that the re-entrainment occurs at about 1 to 2 hours per day.

4. SUPRACHIASMATIC NUCLEUS

Two small clusters of cells located in the anterior hypothalamus just above the optic chiasm have been shown to be the master biological clock in animals. The paired suprachiasmatic nuclei (SCN) are innervated by neurons from the optic nerve which originate in specialised ganglion cells in the retina. The SCN neurons are inherently rhythmic as shown by *in vivo* recording of neuronal firing in SCN islands created by appropriately placed knife cuts (7) and by *in vitro* recording of SCN implants in culture (8). The SCN also provides output signals in the form of secreted factors such as vasopressin (9), TNFα (10) and prokineticin 2 (11) and neural connections to other parts of the brain, pineal gland, pancreas, heart, liver, adrenal etc (12).

5. LIGHT INPUT TO THE SCN

Light is the most potent rhythm-entraining stimulus. In some species (eg, hamsters) behavioural activation e.g. induced wheel running can also reset rhythm phase. The impact of light on SCN function must be neuronal and therefore neurotransmitters are expected to be involved. Excitatory amino acid transmitters particularly glutamate are strongly implicated in the light control of rhythmicity, based upon a wide range of electro physiological and pharmacological experiments *in vivo* and *in vitro*.

5.1. Phase response curves

To maintain entrainment to the prevailing light/dark cycle, mechanisms must exist to bring about re-setting of the molecular rhythms in the SCN. In the case

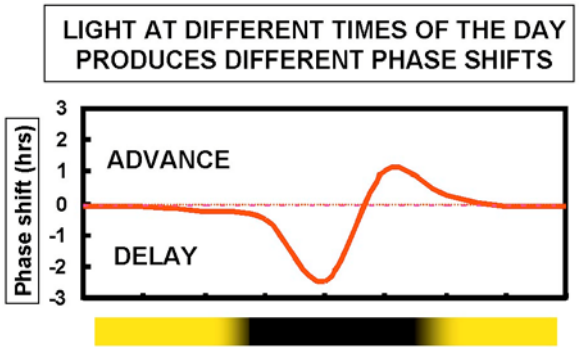

Figure 2. An hypothetical example of a phase response curve (PRC). Light presented at different time of the day or night can either advance or delay the phase of a rhythm including clock gene expression.

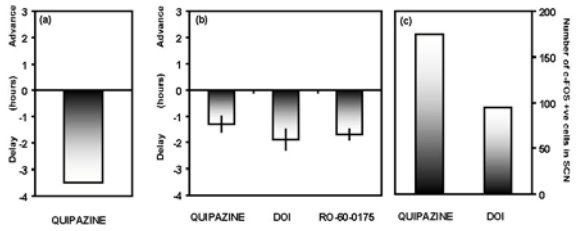

Figure 3. The effects of serotonergic agonist drugs that bind to 5-HT_{2C} receptors on *in vitro* firing rate rhythmicity, melatonin rhythmicity or c-*fos* induction in the rat suprachiasmatic nucleus. (a) data redrawn from (18-20) showing that quipazine administered during early night delays the peak of single unit neuronal firing rate of the rat SCN on the next cycle *in vitro*. (b) The change in onset of the excretion of the melatonin metabolite, 6-sulphatoxymelatonin in rats on the first night following administration of the serotonin agonists quipazine, DOI or RO 60-0175, 4 hours after dark (redrawn from (15; 16) and unpublished results (Kennaway and Varcoe 2003)). (c) The number of cells in rat SCN with detectable c-FOS protein after treatment with quipazine or DOI, 4 hours after lights off (redrawn from (16; 17)).

of animals free running with periods less than 24 hours e.g. mice, the resetting event must occur at sunset, whereas in rats and humans it must occur at dawn. When animals are light pulsed at different times of the light/dark cycle or more commonly at different times of the circadian cycle of rest/activity in constant darkness, the pulses shift the rhythms.

The direction of the shift is dependent upon the time of light presentation; light exposure during early darkness (i.e. early active period of nocturnal rodents) causes a delay in the timing of the rhythms over the subsequent cycles (figure 2). Light in late darkness results in phase advances of the rhythm. As indicated earlier with respect to the jet lag example, the shifts are not complete within one cycle, rather it can take from 5 - 10 days to re-establish the new stable phase. Plots of the phase shift versus the time of the stimulus are termed phase response curves (PRCs). Interestingly the general shape of these curves, including the "dead zone" during subjective day where no shift occurs, is similar across a wide range of organisms. Thus a single exogenous stimulus, light, can have no effect or quite opposite effects (delays or advances of rhythms) depending upon the time of stimulus presentation.

5.2. Retino-hypothalamic tract

Light induced glutamate release at the SCN either has no effect, causes phase delays or phase advances depending upon the time of day. How can this be achieved? If there was a profound rhythm in glutamate receptor number, it could perhaps explain the "dead zone" and either a phase delay or advance but not the opposite responses. There is little evidence for such rhythmicity; in fact in one study NMDA receptors were shown to be high during the day. Perhaps there is gating somewhere in the second messenger cascade. This could be influenced by the clock gene products known to cycle regularly.

5.3. Serotonin

5.3.1. Light mimicking effects

While there is unequivocal evidence for the retino hypothalamic projection, the SCN also receives extensive innervation from the raphe nuclei. These serotonergic neurons terminate in the ventrolateral region of the SCN, the retino recipient region. The role of serotonin in SCN function is not clear, possibly due to marked species differences between the various laboratory animals used in circadian research.

We and others have provided strong evidence that activation of serotonin receptors in the SCN of rats mimics the effects of light on activity rhythms (13), (14) temperature rhythms (15), c-*fos* induction (14-17) and melatonin rhythmicity (15-17) (figure 3). This may provide an additional input signal to the SCN. Extensive pharmacological characterisation of the responses has indicated that the effects are mediated through $5HT_{2C}$ receptors (15). Our studies have always used *in vivo* models and so we cannot be definite that the drugs are acting on the SCN and not at a peripheral site. *In vitro* experiments, however, showed clearly that serotonin and the non-specific agonist quipazine caused phase delays of the neuronal firing rate rhythm of SCN explants *in vitro* (18-20). Furthermore the rat SCN is a rich site of $5HT_{2C}$ receptor mRNA and receptor protein (21). These actions of serotonin are quite distinct from those mediated by pre-synaptic $5HT_7$ receptors in the SCN which appear to be related to behavioural effects on rhythmicity particularly late in the light period.

The idea that a serotonergic pathway may participate in entrainment by light is strengthened by the observation that there is a retinal projection to the raphe nuclei in rats, cats, gerbils and the tree shrew. No such projection is apparent in hamsters and no information is available for mice.

5.3.2. Non-photic effects

The role of serotonergic neurons in mediating even some aspects of the photic environment is controversial. In fact the light mimicking effects of serotonin and the impact of certain agonists have often

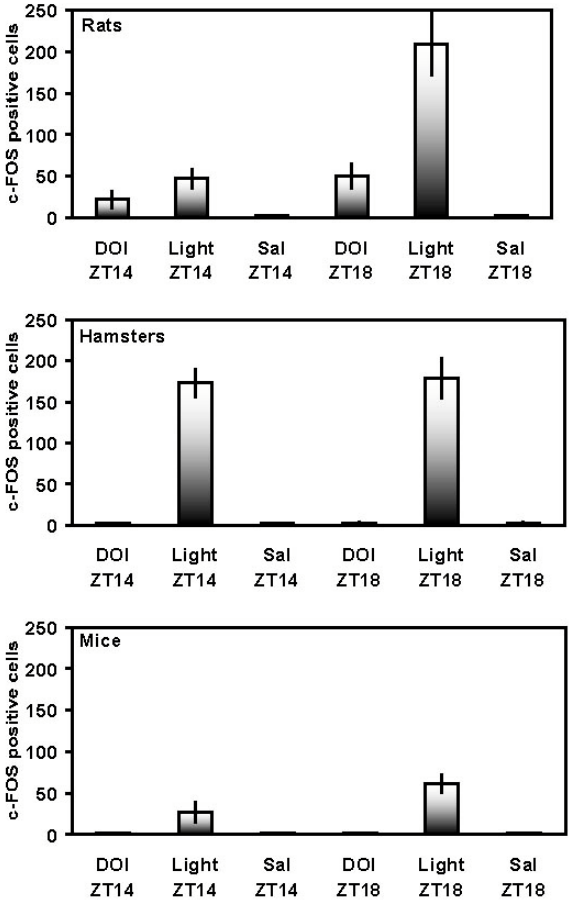

Figure 4. The effects of administration of the 5-HT_{2C} receptor agonist, DOI (2 mg/kg), saline or light exposure (200 lux/15 minutes) on the appearance of c-FOS protein immunoreactivity in the SCN of rats, hamsters and mice. ZT14 is 2 hours after lights off and ZT18 is 6 hours after lights off. Note that DOI only induced c-*fos* in rats (Moyer, Pevet and Kennaway, unpublished results).

been ignored in favour of serotonin playing a predominant role in so called "non-photic" phase shifting. Administration of agonists with an affinity for 5-HT_{1A} and 5-HT_7 receptors has been shown to cause phase advances in the wheel running activity of hamsters. The response is similar to that observed following forced exercise or introduction to a novel environment and is most obvious during the late subjective day. The phenomenon has alsobeen shown to occur *in vitro* in rat SCN slices when single unit neuronal firing rate was investigated, but not with multiple unit recording of the SCN (22). The pharmacological specificity and intracellular mechanisms have not been clearly defined. In many cases there are serious differences between *in vivo* and *in vitro* results. For example in the rat we were unable to show that systemic administration of the 5-$HT_{1A/7}$ agonist, 8-OH-DPAT or Quipazine during late subjective day advanced the melatonin rhythm. In the mouse there is no evidence that similar systemic administration of 8-OH-DPAT shifts wheel running activity (23) whereas the same drug was reported to advance neuronal firing rate (24).

5.3.3. Species differences

There are also differences between species with respect to responses to serotonergic drugs which may have a basis in the anatomical connections between the retina and raphe alluded to earlier. We recently showed that administration of the $5HT_{2A/2C}$ receptor agonist DOI caused the induction of c-*fos* in the SCN of rats, but not hamsters or mice (figure 4, Moyer, Pevet and Kennaway, unpublished results). This supports other results on the effects of this particular agonist on activity rhythms in hamsters and mice. By contrast, when the 5-$HT_{1A/7}$ agonist 8-OH-DPAT was administered to rats, hamsters or mice prior to exposure to a light pulse, the drug potently inhibited the effects of light on c-*fos* induction in all 3 species (figure 5). Thus it appears that appropriate stimulation of serotonin receptors in the raphe or activation of presynaptic receptors in the SCN can also modulate the response to light during the night.

6. CLOCK GENES AND ENTRAINMENT

6.1. Induction of genes by light

There are many studies indicating that the period genes *per*1 and *per*2 are induced by light (25-27). A mouse *per*1 mutant failed to phase advance following a subjective morning light pulse and a *per*2 mutant could not phase delay (28). On the basis of these and other results it was hypothesised that the 2 genes might be responsible for tracking dawn and dusk respectively (29). It is perhaps worth stating here that in considering entrainment of rhythms in animals in the natural world, they are only endeavouring to predict sunrise and sunset as it varies during the year. No wild animal ever has had to prepare for, or respond to trans-meridian travel and the huge adaptations this involves. Similarly animals are never exposed to unexpected bright light exposure at night even though it is often argued that lightning or perhaps moonlight may affect the circadian timing system. To respond to these stimuli, especially lightning, with a 2-3 hour advance or delay in the return to or emergence from a burrow would not, on face value, seem to be a good survival strategy. Thus while phase response curves to light are very impressive with respect to showing large changes in behaviour, hormone secretion and gene expression, they may be misleading. We would argue that it might be more useful to concentrate on the light/dark and dark/light transitions.

As indicated previously mice have an endogenous period of <24 in constant conditions. Thus it must be the light/dark transition that provides the entraining signal, i.e. the cessation of retinal input to the SCN. This is nicely demonstrated by the very precise onset of wheel running activity within minutes of lights off and the poorly defined offset of activity in mice entrained to a 12L:12D photoperiod. If one looks at the dark to light transition, there is evidence that the immediate early gene c-*fos* is induced in the SCN at this time. In the mouse, there is also

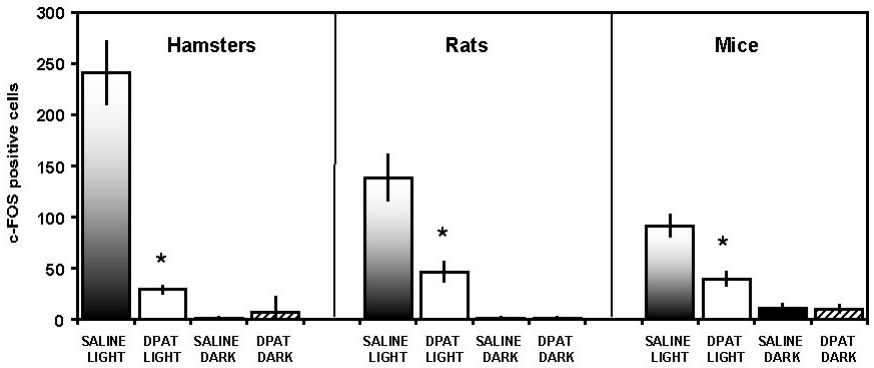

Figure 5. The effects of administration of the 5-$HT_{1A/7}$ receptor agonist, 8-OH-DPAT (5 mg/kg) or saline prior to a light pulse 6 hours after lights off, on the appearance of c-FOS in the SCN of rats, hamsters or mice. 8-OH-DPAT alone did not result in c-*fos* induction, but treatment 30 minutes before exposure to light, potently inhibited the induction of c-*fos* in all three species (Moyer, Pevet and Kennaway, unpublished results).

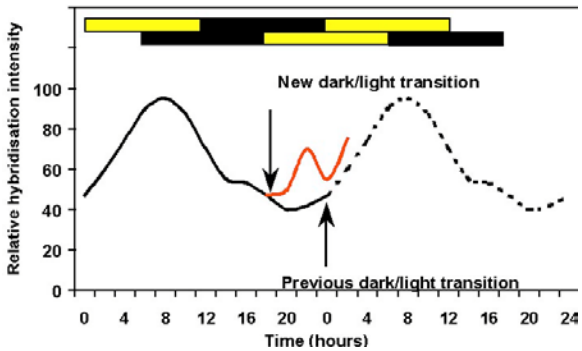

Figure 6. The effect of a shift of the light:dark cycle forward by 6 hours on the expression of *per1* in the mouse SCN. The incursion of light into the previous dark period resulted in the induction of *per1* and a shift in the rhythm of expression. The horizontal bars represent the old and new photoperiods. Redrawn from data in (30).

a steady increase in *per*1 and *per*2 expression in the SCN, but it clearly increases prior to light exposure (26). Studies involving an early exposure to light in the morning (a jet lag type manipulation involving a 6 hour phase advance of the lighting regime) showed that there was an induction of both *per*1 and *per*2 beginning at the commencement of the new light period (30) (figure 6). It was argued that this induction was linked to the rapid adjustment to the new photoperiod. This may be the case in this extreme example, but does not fit with normal entrainment.

6.2. Skeleton photoperiods

Since mice in particular are extremely photo phobic and yet forced to live in cages exposed to 12 hours of bright light each day during their "rest" period, perhaps the events are being masked. Entrainment of the mice to a skeleton photoperiod should overcome this situation. Schwartz et al (31) found that mice could be entrained to two 1-hour pulses separated by 13 hours of darkness. The mice responded to the light which preceded their onset of running by the induction of c-*fos* irrespective of whether they locked onto the pulse occurring before the 14 hours of dark or the 8 hours or darkness. We have observed a similar entrainment of activity rhythms to single 15 minute pulses (figure 7). Whatever is happening at the morning pulse would appear to be irrelevant for actual entrainment at least as far as the known clock genes are concerned.

There have been 2 recent studies that question simple light induction of clock genes being responsible for normal entrainment to a photoperiod. The *clock* mutant mouse was shown to entrain to daily 15 minute light pulses presented at the former dawn (32) despite this mutant producing a non functional CLOCK protein (4). Similarly, *per*1 and *per*2 mutants produced in the Weaver laboratory, could be entrained by a 1 hour pulse presented at the former dusk every 24 hours (33).

For rats the rhythm of clock gene expression is actually different from the mouse. The rhythms of *per*1 and *per*2 for example are late, peaking towards the time of the light /dark transition (34), particularly in the ventrolateral region (35). In these animals c-*fos* is induced in the SCN only at the dark/light transition and is presumably responsible for entrainment. It is not known whether clock genes are induced under these circumstances. In the case of rats, with a period >24 hours they would entrain to a short light pulse at the expected time of lights on. This is indeed the case, but interestingly c-*fos* is induced by both evening and morning pulses (36). There have been no reports of changes in clock gene expression in rats under these conditions.

7. CONCLUSION

It is clear that the generation of circadian rhythmicity is far more complex than originally thought. Instead of a simple negative feedback of one protein, PER on its own gene promoter as originally proposed, there are many genes operating in linked positive and negative feedback loops. The expression of these genes is fine tuned to the photic environment of the animal by neurotransmitters. The daily adjustments required to maintain entrainment to the prevailing photoperiod are very subtle. The techniques commonly used to gain an understanding of the mechanisms underlying resetting are far from subtle. Photophobic mice are routinely maintained

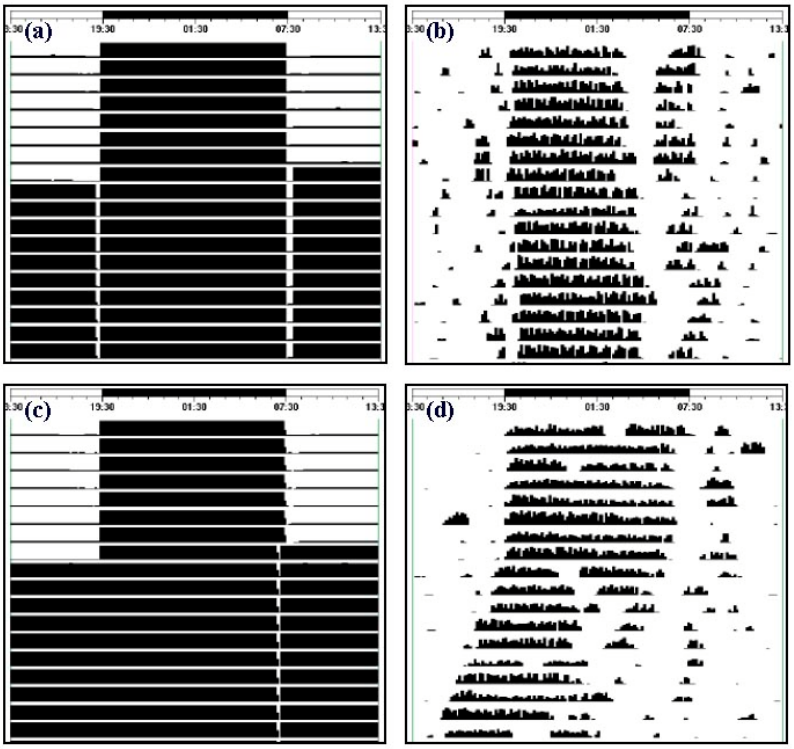

Figure 7. The effect of skeleton photoperiods on wheel running rhythmicity in the mouse. (a) The times of light and dark in a 2 pulse photoperiod where the pulses were 12 hours apart and set at the previous lights on and off. (b) The wheel running activity of a mouse receiving the 2 pulses per day. Note that the onset of running activity coincided with the onset of darkness and continued to be entrained to the pulses upon their introduction. (c) The times of light and dark in a 1 pulse photoperiod where the pulse occurred at the time of the previous lights on. (d) The wheel running activity of a mouse receiving one pulse per day. Note that the onset of running activity coincided with the onset of darkness until the time that the pulses commenced. After that the rhythm free ran, ie the morning pulse was apparently without effect. Data are from unpublished studies in my group.

in 12 or 14 hours of light per day and wheel running monitored. Or hamsters and mice are kept in constant darkness and moved to new wheels or pulsed for up to an hour or more with high intensity light during their rest period provoking arousal and possible fear/escape behaviours. To achieve significant changes in clock genes in rats, they must be pulsed with light intensities 100 fold higher, and for periods more than 100 times longer, than those required to shift hormonal rhythmicity. To fully understand entrainment, future studies must take into account the normal behaviours of the animals being used. There also needs to be an acceptance that even among what appear to be closely related species like rats, mice and hamsters, there are major anatomical, physiological and behavioural differences. We also need to recognise that the pharmacological responses to drugs may be very useful if they are translated across to humans, but they may not in fact be telling us a whole lot about the way the animal normally operated from day to day.

8. ACKNOWLEDGEMENTS

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9. REFERENCES

1. Reppert, S.M. and D. R. Weaver: Coordination of circadian timing in mammals. *Nature* 418, 935-941 (2002)
2. Albrecht, U. Invited review: regulation of mammalian circadian clock genes. *J Appl Physiol* 92, 1348-1355 (2002)
3. Shearman, L.P., S. Sriram, D. R. Weaver, E. S. Maywood, I. Chaves, B. Zheng, K. Kume, C. C. Lee, G. T. van der Horst, M. H. Hastings & S. M. Reppert: Interacting molecular loops in the mammalian circadian clock. *Science* 288, 1013-1019 (2000)
4. Gekakis, N., D. Staknis, H. B. Nguyen, F. C. Davis, L. D. Wilsbacher, D. P. King, J. S. Takahashi & C. J. Weitz: Role of the CLOCK protein in the mammalian circadian mechanism. *Science* 280, 1564-1569 (1998)
5. Preitner, N., F. Damiola, L. Lopez-Molina, J. Zakany, D. Duboule, U. Albrecht & U. Schibler: The orphan nuclear receptor REV-ERBalpha controls circadian transcription within the positive limb of the mammalian circadian oscillator. *Cell* 110, 251-260 (2002)
6. Honma, S., T. Kawamoto, Y. Takagi, K. Fujimoto, F. Sato, M. Noshiro, Y. Kato & K. Honma: Dec1 and Dec2 are regulators of the mammalian molecular clock. *Nature* 419, 841-844 (2002)

7. Inouye, S.T. and H. Kawamura: Persistence of circadian rhythmicity in a mammalian hypothalamic "island" containing the suprachiasmatic nucleus. *Proc Nat Acad Sci U S A* 76, 5962-5966 (1979)
8. Green, D.J. and R. Gillette: Circadian rhythm of firing rate recorded from single cells in the rat suprachiasmatic brain slice. *Brain Res* 245, 198-200 (1982)
9. Schwartz, W.J. and S. M. Reppert: Neural regulation of the circadian vasopressin rhythm in cerebrospinal fluid: a pre-eminent role for the suprachiasmatic nuclei. *J Neurosci* 5, 2771-2778 (1985)
10. Kramer, A., F. C. Yang, P. Snodgrass, X. D. Li, T. E. Scammell & F. C. Davis: Regulation of daily locomotor activity and sleep by hypothalamic EGF receptor signaling. *Science* 294, 2511-2515 (2001)
11. Cheng, M.Y., C. M. Bullock, C. Li, A. G. Lee, J. C. Bermak, J. Belluzzi, D. R. Weaver, F. M. Leslie & Q. Y. Zhou: Prokineticin 2 transmits the behavioural circadian rhythm of the suprachiasmatic nucleus. *Nature* 417, 405-410 (2002)
12. Buijs, R.M. and A. Kalsbeek: Hypothalamic integration of central and peripheral clocks. *Nat Rev Neurosci* 2, 521-526 (2001)
13. Kalkowski, A. and F. Wollnik: Local effects of the serotonin agonist quipazine on the suprachiasmatic nucleus of rats. *Neuroreport* 10, 3241-3246 (1999)
14. Kohler, M., A. Kalkowski & F. Wollnik: Serotonin agonist quipazine induces photic-like phase shifts of the circadian activity rhythm and c-*fos* expression in the rat suprachiasmatic nucleus. *J Biol Rhythms* 14, 131-140 (1999)
15. Kennaway, D.J., R. W. Moyer, A. Voultsios & T. J. Varcoe: Serotonin, excitatory amino acids and the photic control of melatonin rhythms and SCN c-FOS in the rat. *Brain Res* 897, 36-43 (2001)
16. Kennaway, D.J. and R. W. Moyer: Serotonin 5-HT_{2C} agonists mimic the effect of light pulses on circadian rhythms. *Brain Res* 806, 257-270 (1998)
17. Moyer, R.W., D. J. Kennaway, S. A. Ferguson & Y. P. Dijstelbloem: Quipazine and light have similar effects on c-*fos* induction in the rat suprachiasmatic nucleus. *Brain Res* 765, 337-342 (1997)
18. Prosser, R.A., J. D. Miller & H. C. Heller: A serotonin agonist phase-shifts the circadian clock in the suprachiasmatic nuclei *in vitro*. *Brain Res* 534, 336-339 (1990)
19. Prosser, R.A., H. C. Heller & J. D. Miller: Serotonergic phase shifts of the mammalian circadian clock: effects of tetrodotoxin and high Mg2+. *Brain Res* 573, 336-340 (1992)
20. Prosser, R.A., R. R. Dean, D. M. Edgar, H. C. Heller & J. D. Miller: Serotonin and the mammalian circadian system .1. *In vitro* phase shifts by serotonergic agonists and antagonists. *J Biol Rhythms* 8, 1-16 (1993)
21. Moyer, R.W. and D. J. Kennaway: Immunohistochemical localisation of serotonin receptors in the rat suprachiasmatic nucleus. *Neurosci Lett* 271, 147-150 (1999)
22. Prosser, R.A. *In vitro* circadian rhythms of the mammalian suprachiasmatic nuclei: comparison of multi-unit and single-unit neuronal activity recordings. *J Biol Rhythms* 13, 30-38 (1998)
23. Antle, M.C., M. D. Ogilvie, G. E. Pickard & R. E. Mistlberger: Response of the mouse circadian system to serotonin 1A/2/7 agonists in vivo: surprisingly little. *J Biol Rhythms* 18, 145-158 (2003)
24. Prosser, R.A. Serotonin phase-shifts the mouse suprachiasmatic circadian clock in vitro. *Brain Res* 966, 110-115 (2003)
25. Shearman, L.P., M. J. Zylka, D. R. Weaver, L. F. Kolakowski, Jr. & S. M. Reppert: Two period homologs: circadian expression and photic regulation in the suprachiasmatic nuclei. *Neuron* 19, 1261-1269 (1997)
26. Shigeyoshi, Y., K. Taguchi, S. Yamamoto, S. Takekida, L. Yan, H. Tei, T. Moriya, S. Shibata, J. J. Loros, J. C. Dunlap & H. Okamura: Light-induced resetting of a mammalian circadian clock is associated with rapid induction of the mPer1 transcript. *Cell* 91, 1043-1053 (1997)
27. Zylka, M.J., L. P. Shearman, D. R. Weaver & S. M. Reppert: Three period homologs in mammals: differential light responses in the suprachiasmatic circadian clock and oscillating transcripts outside of brain. *Neuron* 20, 1103-1110 (1998)
28. Albrecht, U., B. Zheng, D. Larkin, Z. S. Sun & C. C. Lee: MPer1 and mper2 are essential for normal resetting of the circadian clock. *J Biol Rhythms* 16, 100-104 (2001)
29. Daan, S., U. Albrecht, G. T. van der Horst, H. Illnerova, T. Roenneberg, T. A. Wehr & W. J. Schwartz: Assembling a clock for all seasons: are there M and E oscillators in the genes? *J Biol Rhythms* 16, 105-116 (2001)
30. Reddy, A.B., M. D. Field, E. S. Maywood & M. H. Hastings: Differential resynchronisation of circadian clock gene expression within the suprachiasmatic nuclei of mice subjected to experimental jet lag. *J Neurosci* 22, 7326-7330 (2002)
31. Schwartz, W.J., R. V. Peters, N. Aronin & M. R. Bennett: Unexpected c-fos gene expression in the suprachiasmatic nucleus of mice entrained to a skeleton photoperiod. *J Biol Rhythms* 11, 35-44 (1996)
32. Kennaway, D.J., A. Voultsios, T. J. Varcoe & R. W. Moyer: Melatonin and activity rhythm responses to light pulses in mice with the Clock mutation. *Am J Physiol* 284, R1231-1240 (2003)
33. Bae, K. and D. R. Weaver: Light-induced phase shifts in mice lacking mPer1 or mPer2. *J Biol Rhythms* 18, 123-133 (2003)
34. Sumova, A., M. Jac, M. Sladek, I. Sauman & H. Illnerova: Clock gene daily profiles and their phase relationship in the rat suprachiasmatic nucleus are affected by photoperiod. *J Biol Rhythms* 18, 134-144 (2003)
35. Yan, L. and H. Okamura: Gradients in the circadian expression of Per1 and Per2 genes in the rat suprachiasmatic nucleus. *Eur J Neurosci* 15, 1153-1162 (2002)
36. Schwartz, W.J., J. Takeuchi, W. Shannon, E. M. Davis & N. Aronin: Temporal regulation of light-induced Fos and Fos-like protein expression in the ventrolateral subdivision of the rat suprachiasmatic nucleus. *Neuroscience* 58, 573-583 (1994)

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