Research during the past two decades has led to the recognition that the mammalian circadian pacemaker (PG) plays a pivotal role in maintaining circadian rhythmicity. Such rhythmicity is central for the regulation of gene expression and rhythmic behaviors. In the mouse, for instance, the suprachiasmatic nucleus (SCN) appears to be the primary pacemaker that coordinate and synchronizes cellular processes in organs throughout the organism. This is reflected in changes in genome expression patterns, which can be monitored in tissues of the anterior pituitary, liver, and retina. These changes appear to be under the control of the SCN, which is an endogenous circadian pacemaker that persists in tissue explants in vitro. In this study, we examined the expression of a key clock gene, Per1, in the mouse SCN under conditions of constant darkness and using a real-time PCR approach. We found that the expression of Per1 was significantly increased in the SCN under conditions of constant darkness, consistent with previous findings. These results support the hypothesis that the SCN is a key pacemaker in the mammalian circadian system. 

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6.1 MEASUREMENTS OF ELECTRICAL COUPLING BETWEEN CIRCADIAN PACEMAKER CELLS OF THE BULLA EYE. Michael E. Osburn* and J. W. Koehn. School of Medicine, University of Virginia, Charlottesville, VA 22901.

A pacemaker in the eye of the newt Bulla bulla generates circadian rhythms in the frequency of compound action potentials (CAPs) in the optic nerve. The CAPs are produced by the synchronous firing of a population of at least 100 cells known as the basal retinal neurons (BRNs). The BRNs are believed to be electrically coupled because CAPs persist in media with low Ca concentrations and dye coupling between BRNs has been shown.

To assess the strength of the electrical coupling between the BRNs, simultaneous intracellular recordings were made from pairs of BRNs while subthreshold current pulses were injected into either cell. Coupling coefficients, measured using discontinuous current clamp, averaged 0.66 ± 0.15 S.D. (n = 12 pairs), indicating that coupling occurs. There was no obvious rectification when current was passed in either direction. Strong coupling was found during both the subjective day (9.15 ± 0.17 S.D.) and the subjective night, 0.64 ± 0.14 S.D. (n = 5).

Pair-wise recordings between BRNs and the H-type cells of the retina, which hyperpolarize transiently in response to light, did not show evidence of electrical coupling (n = 6). Between pairs of H-type cells, however, dye coupling was observed.

Treatments that block gap junctional conductances in other systems did not block electrical coupling between BRNs. CAPs and coupling persisted in 1 mM 1-octanol, or when extracellular pH was below 7.5. Coupling persisted in 1 mM heptanol at 15, although CAPs were blocked. Heptanol at 10 mM caused irreversible loss of CAPs. Both alcohols were administered in 0.5% by volume dimethyl sulfoxide. Supported by NS08806 to MEG and NS13524 to GDB.

6.2 DISSOCIATED PACEMAKER CELLS OF BULLA EXPRESS CIRCADIAN RHYTHM IN MEMBRANE CONDUCTANCE. S. Michel, H. Gotz, J. J. Ritz, and G. D. Block. NSF Center for Biological Timing, University of Virginia, Charlottesville, VA 22901.

The eyes of the marine snail Bulla bulla generate an intrarhythmic clock in the form of rapid changes in membrane conductance (mRNA). Such changes in membrane conductance appear to be a fundamental component of circadian rhythms.

Intracellular recordings from isolated neurons revealed a circadian rhythm. This rhythm is characterized by a decrease in conductance near subjective dawn and an increase near subjective dusk. The rhythm is present in all neurons examined, indicating that it is a fundamental property of the retina. The rhythm is not dependent on the presence of synaptic input, as it is also observed in isolated neurons. These results suggest that the circadian rhythm in membrane conductance is a fundamental component of circadian rhythms in the retina.


An antiserum against a 48kD protein specific to photoreceptors stains certain cells and fibers of the Aplysia retina, which contains a circadian pacemaker. This antiserum stains the optic nerve, the optic tract of the central ganglion and other central ganglia. Myelinated antiserum stains other specific fibers, neurons and cell bodies of the retina including several large neurons, whose serum project in the optic nerve to the optic tract of the central ganglion. The lateral terminus of the optic tract tracts with both antenna is a putative synaptic exchange area. In order to identify cells and fibers containing the antigens and preparatory to other types of analysis, the retinas were dissociated, using conventional enzymatic digestion, and cells were plated in cell culture using defined medium. Many cell types survived, grown neurites and interacted, including monopolar and bipolar neurons, large microvilli, large clubbed and small fission photoreceptors. The monopolar and bipolar neurons are putative pacemaker neurons, which produce the CAP activity that exhibits a circadian rhythm. They have cell bodies 12.1-18µm in diameter and survived for more than 2 weeks in culture. Under conditions that extend the normal light-dark cycle of Aplysia, the neurons grow neurites and interact with other cells by neurite association and putative synapse formation. Preliminary electrophysiological recordings revealed several voltage-sensitive characteristics of the neurons. Supported in part by NS 8107773; University California Vienna Station and Allen遗迹 Foundation Funds; thanks to University of California Neuroscience Faculty.