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## **Biological time-keeping mechanisms: A need for broader perspectives?**

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**Summary** Biological time-keeping mechanisms play fundamental roles in the regulation of behavior and physiology, disruption of which can lead to increased incidence of many disorders. Consequently, these mechanisms continue to be investigated intensively. For almost four decades they have been known to be able to store and program complex behaviors, and to be susceptible to the influences of light and day length. Yet present-day research concerns almost exclusively the means by which circadian and lesser time intervals are measured. Even within this narrow focus disagreements exist. Some early studies of small nocturnal rodents and primates running in exercise wheels illustrate the program-clock-like capabilities of endogenous time-keeping mechanisms and their degrees of susceptibility to external influences, such as artificial twilights and shortened light cycles. Broadening perspectives for research on biological clocks to take into account these often overlooked capabilities and susceptibilities could lead to a deeper understanding of them.

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Biological rhythms are fundamental regulators of many aspects of behavior and physiology relevant to human health, such as the sleep-wake cycle, blood pressure, body temperature, and

metabolism. Disruption of the rhythm can lead to increased incidence of many diseases, including mental illnesses, most notably bipolar disorder [1] and seasonal affective disorder (SAD; [2]). For instance, anecdotally, some people can awaken every morning at close to the desired time without need for an alarm clock, which implies the existence of an accurate endogenous time-keeping mechanism.

Circadian rhythms are present throughout the animal kingdom, usually in all body cells. Mammalian cells are believed to be entrained by signals from a central clock in the suprachiasmatic nucleus of the hypothalamus, which is reset daily by the natural light cycle [1]. Models for circadian clocks need to explain how to construct a feedback loop that takes about a day to close [3]. The clock mechanism of the fruit fly (*Drosophila melanogaster*) is also believed to be located in the hypothalamus, and one model considers the cellular transcription regulators PERIOD and TIMELESS as essential components of the diurnal cycle. A period of approximately 24 hours was thought to be the time needed for these transcription regulators to associate in the cell cytoplasm before entering the nucleus, where negative feedback loops are established [3]. The transcription regulators, however, have been found to be rapidly bound [4] and not to account for the time constant. Accordingly, despite continuing progress the genesis of the long time constant remains unclear [3].

In the domain of brief time intervals, no localized brain structure dedicated to track these intervals has yet been found [5]. There is increasing conviction that multiple brain cortices, including the posterior parietal and prefrontal regions, are implicated in assessing these intervals (milliseconds to minutes to hours). The question then arises as to how the distributed neurons measure time.

From the 1970s most researchers have assumed a “pacemaker-accumulator” model of time-keeping, by which some neurons release pulses of neurotransmitter(s) at periodic intervals. These

neurotransmitters were thought to accumulate in other neurons, with their quantities (recorded in long-term memory) corresponding to specific durations. In the mid 1990s, Meck, Malapani and others proposed that the brain's 'stopwatch' is located in the subcortical basal ganglia, with dopamine-secreting "pacemaker" neurons in the substantia nigra and "accumulator" neurons in the striatum (see [5]).

This model is now challenged as being too simplistic, and perhaps fundamentally flawed. A new, but contentious, "striatal beat-frequency" model, proposed by Matell and Meck [6], eliminates the need for an accumulator. This denies the concept of biological time flowing and collecting like sand in an hourglass. The new model instead proposes that the striatum reads out time intervals from a snapshot of activity across a network of cortical neurons whose firing rates oscillate with different frequencies to constitute the "clock input." Different times then are represented by different patterns of neuronal activity. In order to time an event, the cortical clock is reset by synchronization of neuronal firing in the network. The striatal neurons track the evolving network's activity until the end of a timed entity is marked by the arrival of reinforcement, say a pellet of food [5,6].

Although complex, these models remain narrowly focused, not probing all aspects of timing phenomena, which accomplish much more than just the measurement of time intervals. For example, almost four decades ago, it was discovered that some mammals, including White-footed Mice and members of several species of nocturnal primates and carnivores, possess timing mechanisms with program-clock-like capabilities [7,8]. These mechanisms appear to provide endogenous temporal signals, keep sequential records of the times at which specific events occur, can guide sequential 'replaying' of these events at the same points of time in another activity cycle and in many mammals can be reset by artificial twilights.

Instantaneous time, speed, and direction of running of mammals in exercise wheels can be recorded by point-prints every few sec on a moving 'center-zero' chart. The patterns of wheel-running performance (Figs. 1, 2) obtained in this manner may be even more complex and rich in detail than sonographs of birdsong. These complex patterns sometimes are almost exactly repeated from night to night: changes in speed, direction, and prevailing session lengths of running may begin and end at elapsed times differing only by seconds from the time of beginning activity, as if behavior were being programmed automatically by a 'clock.' [7,8].

For example, on Nov. 16, 1967, a White-footed Mouse (*Peromyscus polionotus*), on a regime employing natural dawns and artificial dusks, almost exactly repeated the pattern of Nov. 15 (Fig. 1). Running toward the east began shortly after the beginning of dusk on Nov. 15, but about 20 min later on Nov. 16. Notwithstanding the different starting times, the first reversal (to running westward) on both nights occurred within a few sec of 54 min afterwards. The second reversal (a return to running eastward) occurred within a few sec of 150 min afterwards. The third reversal was within 30 sec of 179.5 min, and the fourth within 120 sec of 248 min. Running ceased on both nights within 120 sec of 6 hr after starting.

Just as the start of running was about 20 min later during dusk on Nov. 16 (in much dimmer light than on Nov. 15), so also was the cessation about 20 min later during dawn (in much brighter light than on Nov. 15). This strengthens the conclusion that the beginning and cessation of running, and the directional changes that occurred in between, followed a persisting internal program rather than being determined by twilight light levels or other possible exogenous cues. Such pattern repeats usually occur during periods of adaptation to a new environment, which sometimes takes many weeks. Once fully adapted, however, most mammals subsequently display an underlying great conservativeness of behavior, generally running sustainedly in the same direction through much or all of their activity periods [9]. They more often than not orient

their running direction with respect to the artificial light source, and will change direction to maintain that orientation almost unfailingly every time the direction of the light source is changed

Artificial twilights, though not abrupt light changes, can have influences beyond merely resetting the internal clock. In some mammals artificial twilight can completely override the clock's program. Another species of White-footed Mouse was readily adapted to run only during twilights and the night phase of 22 consecutive cycles of 16-hr days [10; Fig. 2]. In similar circumstances, phase shifts by abrupt light changes greater than 2 or 3 hr are ineffective. A member of a third species followed 2-hr cycles, when artificial twilights were presented sequentially as 1-hr dusks and dawns in immediate succession, becoming active during each dusk and ceasing activity during each dawn [9; Fig. 9].

Next-night pattern repeats for four individuals of two species of nocturnal primates are shown in Fig. 2 [8; Fig. 1]. There is one example of a repeat for each of three bush babies (*Galago senegalensis*) and for a slow loris (*Nycticebus coucang*). The animals were studied for from 4 to 10 weeks, first outdoors and then indoors on 24-hr light cycles, the latter with artificial twilights. Under these conditions they were strictly nocturnal. Immediately following the last indoor 24-hr cycle, each animal was studied on around-the-clock 4-hr cycles for from one to three weeks. (the light cycles, consisted of approximately 1-hr each of artificial dusk, dim night light, artificial dawn, and bright day light), as indicated in Fig. 2.

Unlike the malleability of the mice, no individual primate entrained around-the-clock to the 4-hr periodicity. Rather, their activity continued to occur almost entirely during the night phase of the previous indoor 24-hr cycles. The records of Fig. 2 illustrate remarkable next-night repeats of each animal's activity pattern under these conditions. Those for the three bush babies are of particular interest. With all external conditions the same for them, their records revealed the

existence of great individual differences in their running responses. A similarity is that all three ran largely away from the light source (whereas the slow loris ran largely toward it). One unexpected difference between the three individuals is that bush baby #1 was appreciably active (but running more slowly) during the bright 'day' light phases in the second and third 4-hr cycles (Fig. 2, lower left,  $g_2$  and  $g_3$ ), whereas #2 and #3 were inactive then (Fig. 2, right, upper and lower). The findings also included an impressive pattern repeat for an owl monkey (*Aotus trivirgatus*), whose running pattern was more bidirectional (not shown).

A hint of further complexity and capabilities of mammalian behavioral sequencing mechanisms comes from recent studies of running by domestic rats. Replay of behavioral sequences in hippocampal place cells (involved in spatial navigation) occurs immediately after spatial experiences; however the replay occurs in reverse sequence [11].

Accordingly, proposed theories of the timing mechanisms possessed by some mammals might benefit from consideration and investigation of additional capabilities and susceptibilities, including those for extended memories and alterations by light programs. These are the findings that: (1) timing mechanisms can act continuously as sequence programmers for behavior; (2) the mechanisms can also retain records of sequences and timing of multifaceted activities of entire nights; (3) the activities of one night can bias toward specific behaviors occurring in the same sequence, and at the same elapsed times on the following night; (4) artificial twilights can reset the timing mechanisms of some mammals in some circumstances; and (5) among some of these mammals, the experimentalist can initiate or constrain activities almost at will using artificial twilights.

**Note added in proof:**

A recent finding of DeBruyne et al. [12] is pertinent to the theses of this article. Whole-mouse knockouts of CLOCK were generated. The CLOCK transcription factor is one of a pair believed to be essential components of the circadian clock, Nonetheless, the CLOCK-deficient mice continued to express circadian rhythms of locomotor activity, challenging the assignment of an essential role of CLOCK:BMAL1 heterodimers in clock function.

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### Figure legends

**Figure 1** Photographs of six running records of an Old-field Mouse on consecutive nights on a program employing natural dawns (upper arrows at right) and artificial dusks (lower arrows at right). Each dot records the time, speed, and direction of running (horizontal excursions to right or left on a linear scale) at 3-sec intervals. Dots to the right denote westward running; dots to the left, eastward running; central dots indicate a stationary wheel. Time is marked off in the margins at 30-min intervals progressing from bottom to top, as the chart advances downward (2.5 cm/hour). To facilitate comparisons, the 30-min marks begin when running began. The curved brackets at upper left indicate the high speed running before retiring during mid dawn.

**Figure 2** Photographs of next-night running pattern repeats of a slow loris and the three bush babies during around-the-clock indoor tests with 4-hr cycles. Recording techniques were as in Fig. 1. The horizontal scales are linear, with reference speeds of 0.5 m/sec (upper records) and 1.0 m/sec (lower records) indicated. The numbered 4-hr cycles are marked off between the paired records. Phases of the cycles are marked off on the centerline of each record and to the

right, with the phases of each first cycle labeled to the right. The letter-numeral labels mark bouts of activity (discussed only for bush baby #1).

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