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Biological Clocks

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Imagine for a moment that you have a pet hamster, a friendly fellow who quietly shares your bedroom. However, late one Thursday night you are studying for a test but finding it hard to concentrate because a hamster is most active during night hours, and his running wheel is squeaking. So, you place the hamster and his cage in your closet and continue to study. The next morning, you take the test and then leave for the weekend. On Monday night you are back, and you notice a squeaking in your closet. You have forgotten about the hamster. He has had plenty of food and water, but he has been in the dark for three days. As you retrieve him, you notice that he begins to run on his wheel at about the same time as he normally did.

How could he know what time it was? We attribute the ability to measure time without any obvious environmental cues to an internal, living clock. When any hamster is sequestered in the constant darkness and temperature of the laboratory so that each turn of its running wheel can be recorded automatically for months or even years, a record similar to the one shown in Figure 9.1 usually results. Notice that the hamster woke up almost exactly 12 minutes later each day during the entire study. Its bouts of activity alternate with rest with such regularity that it is often described as an activity rhythm. The ability to measure time is common not just in hamsters but also in most animals. In fact, biological clocks have been found in every eukary-

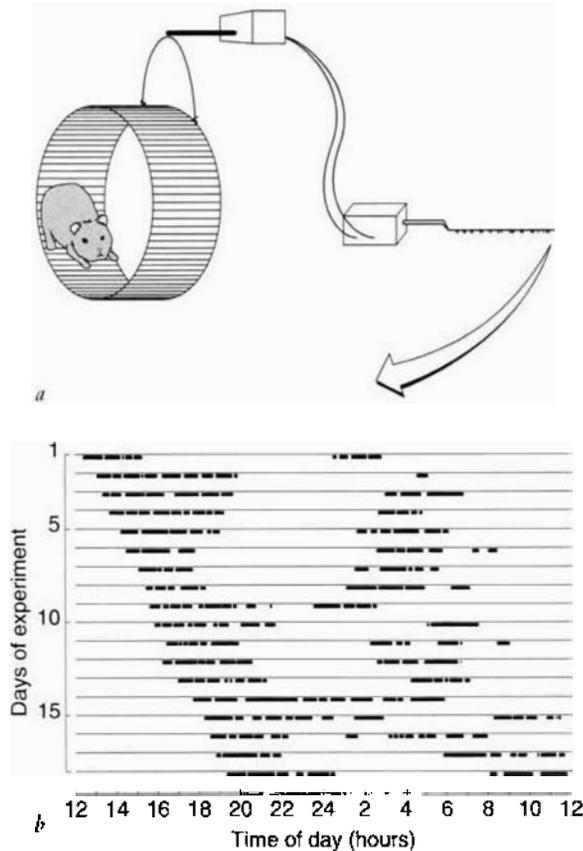


FIGURE 9.1 (a) A hamster in a running wheel equipped to record each turn of the wheel shows periodic bouts of activity that alternate with rest. (b) In constant dim light, the cycle length of this activity rhythm is slightly longer than 24 hours. For each rotation of the running wheel, a vertical line is automatically made on a chart at the time of day when the activity occurred. In this record the bouts of activity were so intense that the vertical lines appear to have fused, forming dark horizontal bands. Notice that although the animal had no light or temperature cycle as a cue to the time, it would awaken about 12 minutes later each day.

otic organism tested (Dunlap 1999), as well as in cyanobacteria (Golden, Johnson, and Kondo 1998).

The rhythmical nature of life may come as a surprise to some, although it should not in the light of evolutionary principles. Life evolved under cyclical conditions, and the differences in phases of the cycles are often so pronounced that they place a high adaptive value on being able to accommodate as specifically as possible to each phase.

Every living thing is subjected to the regularly varying environmental conditions on earth orchestrated by the relative movements of the heavenly bodies: the earth, the moon, and the sun. As the earth spins on its axis relative to the sun, life is exposed to rhythmic variations in light intensity, temperature, relative humidity, barometric pressure, geomagnetism, cosmic radiation,

and the electrostatic field. The earth also rotates relative to the moon once every lunar day (24.8 hours). The moon's gravitational pull draws the water on the earth's surface toward it, causing it to "pile up" and thus resulting in high tide. These tidal cycles cause dramatic changes in the environment of intertidal organisms—flooding followed by desiccation when exposed to air. The relative positions of the earth, moon, and sun result in the fortnightly alternation between spring and neap tides, as will be explained shortly. The moon revolves about the earth once every lunar month (29.5 days), generating changes in the intensity of nocturnal illumination and causing fluctuations in the earth's magnetic field. Finally, the earth, tilted on its axis, circles the sun, causing the progression of the seasons, with its sometimes dramatic alterations in photoperiod and temperature.

Although the environmental modifications may be extreme, they are generally predictable. Often it is advantageous to gear an activity to occur at a specific time relative to some rhythmic aspect of the environment. So, biological clocks are generally thought to have evolved as adaptations to these environmental cycles (Daan and Aschoff 1982; Enright 1970).

In this chapter we will see exemplified the various approaches to the study of behavior discussed in previous chapters. Rhythms are so pervasive that they have piqued the interest of those concerned with the adaptive value of behavior and its evolution; others are interested in its genetic roots, and still others have focused on the hormonal or the neural basis of biological timing. As we will see, their interactions have been fruitful. First we will describe some rhythmic behaviors and the properties of the clock that drives them.

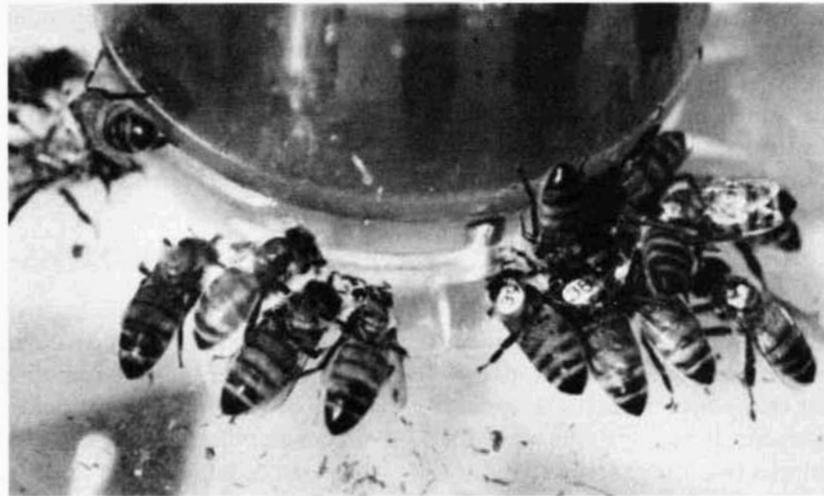
RHYTHMIC BEHAVIOR

Rhythmicity in behavior and physiology is so common that it must be considered by anyone studying animal behavior. An animal is not perpetually the same. Rather, its behavior may fluctuate so that it is appropriate to the time of day or the state of the tides or the phase of the moon or the season of the year. A description of a behavior at one point in a cycle may be totally inaccurate at another time. We will begin by describing a variety of biological rhythms.

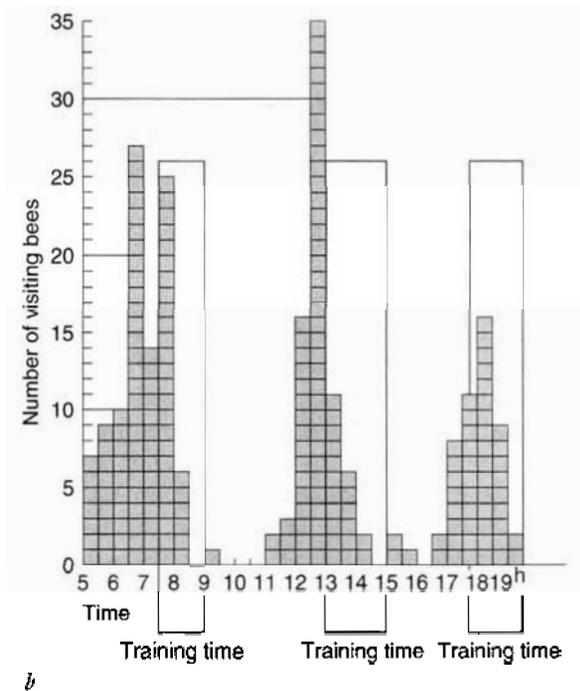
DAILY RHYTHMS

Most animals restrict their activity to a specific portion of the day. The hamster, for example, is busiest at night, as are cockroaches, bats, mice, and rats. Other species, songbirds and humans, for instance, are active during the day.

Among the best "timers" are bees. Their time sense was experimentally demonstrated during the early part



a



b

FIGURE 9.2 The time sense in bees. (a) Bees were marked for individual recognition and trained to come to a feeding dish only at the specific times at which food was made available. (b) After six days of training, the feeding dishes were left empty and the number of bees arriving throughout the day was recorded. The bees arrived at the feeding station only when food had been previously present. (Modified from Beling 1929.)

of this century by marking them for the purpose of individual recognition and offering them sugar water at a feeding station during a restricted time each day, between 10 A.M. and noon. After six to eight days of this training, most of the bees frequented the feeding station only during the learned hours. The real test, however, was on subsequent days, when no food was present at the feeding station. As seen in Figure 9.2, the greatest number of bees returned to the empty feeding station only at the time at which food had been previously available (Beling 1929). In subsequent tests, it was found that the bees' time sense is astonishingly accurate. Bees can be trained to go to nine different feeding stations at nine different times of the day. They are able to distinguish points in time separated by as few as 20

minutes (Koltermann 1971). The adaptiveness of such abilities for bees is clear. Flowers have a rhythm in nectar secretion, producing more at some times of the day than at others. The biological clock allows bees to time their visits to flowers so that they arrive when the flower is secreting nectar. This means that the bees can gather the maximum amount of food with the minimum effort.

Clocks are important in mating as well. For example, the fruit fly *Dacus tryoni* mates only during the evening twilight. Such a rhythm ensures reproductive synchrony between members of the same species, thereby increasing the chances of finding an appropriate mate. Other species of genus *Dacus* mate at different times of the day (Tychsen and Fletcher 1971). Thus, timing may also avoid matings

with other selected species that are mating at the same season, and time, energy, and gametes are not wasted on doomed reproductive efforts. Even in moth species in which the female entices a male by emitting a potent sex attractant, the biological clock plays an important role (Figure 9.3). Female moths of the genus *Hyalophora* produce a sex attractant pheromone that is attractive to both *H. cecropia* and *H. promethea* males. Costly reproductive mistakes are avoided because these species are active at different times of the day (Wilson and Bossert 1963).

Certain patterns of learned behavior may be under temporal control. When an electrode is placed into certain brain regions (areas of the hypothalamus or certain midbrain nuclei) of a rat, the animal will quickly learn to press a lever to get electrical stimulation (Figure 9.4a). An observer is tempted to conclude that the effect of the stimulation is the rat's equivalent of some form of ecstasy since animals with electrodes in these brain areas would rather stimulate themselves than eat, drink, or even copulate. Some will press the lever as often as 5000 times an hour. When the electrodes are first implanted, a rat will typically begin a 2-day marathon of bar pressing at a high rate, resting infrequently for intervals of only a few minutes. After this time, quiet periods—during which the rat may still respond but with lower frequency—alternate with periods of rapid pressing, and soon a 24-hour cycle appears (Figure 9.4b) (Terman and Terman 1970). The animals have apparently learned to press the bar to receive a reward, but the reward has more value at some times of the day than at others. Likewise, the operant behavior

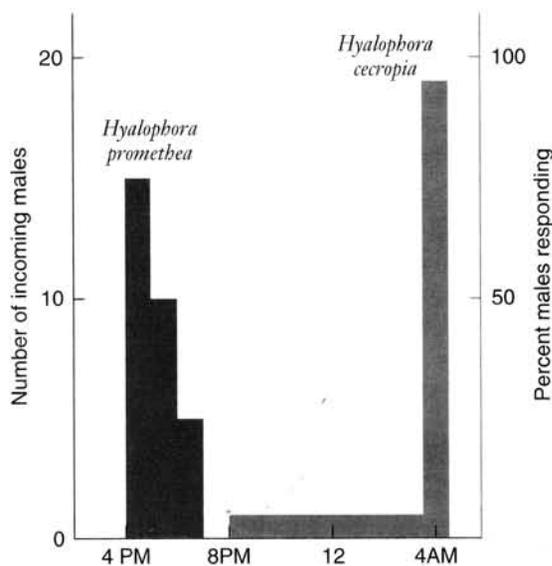


FIGURE 9.3 The flight times of moths of the species *Hyalophora cecropia* and *H. promethea*. The difference in the time of activity of these species helps prevent the males from mating with a female of the wrong species. (Modified from Wilson and Bossert 1963.)

of a chimpanzee that is responding for a food reinforcement has been shown to be rhythmic (Ternes, Farner, and Deavors 1967). It has been suggested that since identical stimuli may vary in their effectiveness as reinforcers with the hour of the day, the ease of learning should also fluctuate daily.

LUNAR DAY RHYTHMS

Although the 24-hour daily rhythm in light and dark is probably the most familiar environmental cycle, there are many others of importance. For example, the interaction of the gravitational fields of the moon and the sun create other pronounced environmental changes—those associated with the tides.

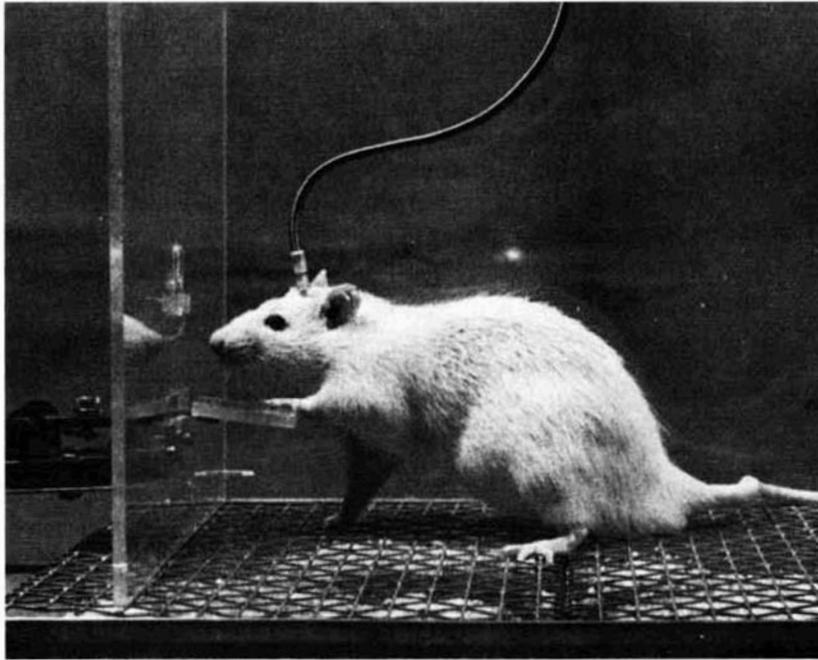
As the moon passes over the surface of the earth, its gravitational field draws up a bulge in the ocean waters. One bulge occurs beneath the moon and another on the opposite side of the earth. These bulges sweep across the seas as the earth rotates beneath the moon, thus causing high tides when they reach the shoreline. Since there are two “heaps” of water, there are usually two high tides each lunar day, one every 12.4 hours. The tides may cause some rather dramatic changes in the environment, particularly for organisms living on the seashore.

The activity of the fiddler crab, *Uca pugnax*, a resident of the intertidal zone, is synchronized with the tidal changes. Fiddler crabs can be seen scurrying along the marsh during low tide in search of food and mates. Before the sea floods the area, the crabs return to their burrows to wait out the inundation. When a fiddler crab is removed from the beach and sequestered in the laboratory, away from tidal changes, its behavior remains rhythmic. Periods of activity alternate with quiescence every 12.4 hours, the usual interval between high tides (Figure 9.5) (Palmer 1995).

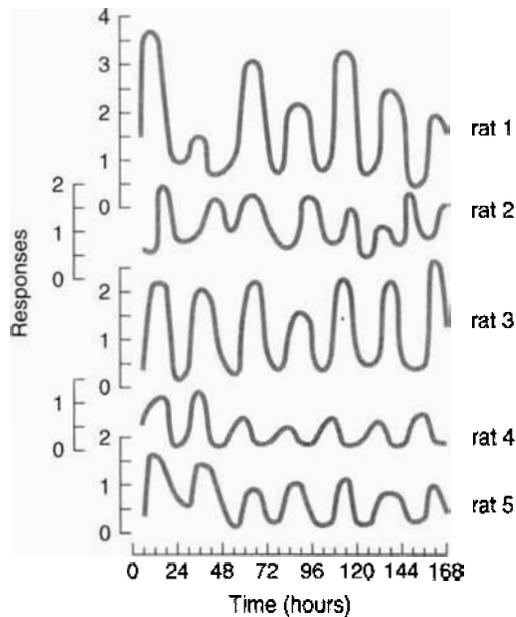
Living higher in the intertidal zone than the fiddler crab is a small isopod (a crustacean), *Excirologa chiltoni*. The open beach habitat of this organism is periodically exposed to the swirling surf. *Excirologa* is buried in the sand during low tide, but as the pounding waves reach its abode it emerges and begins swimming and feeding in the breaking waves. However, it is not simply that inundation presents an ultimatum of sink or swim. When the animals are brought into the laboratory and are maintained in a sandy-bottomed beaker of seawater, they begin to tread water only at the times of high tide on their local beaches (Figure 9.6). One isopod displayed a tidal rhythm for 65 days in constant conditions (Enright 1972).

SEMILUNAR RHYTHMS

The height of the tides is also influenced by the gravitational field of the sun. In fact, the highest tides are



a



b

FIGURE 9.4 (a) A rat with an electrode implanted in the “pleasure center” of the brain. When the rat presses the lever, the pleasure center is stimulated and a record is made of the action. (b) The cyclic self-stimulation of the pleasure center by rats. Notice that periods of rapid stimulation alternate with rest in cycles of approximately 24 hours even when the rat has no obvious time cues. (From Terman and Terman 1970.)

caused when the gravitational fields of the moon and the sun are operating together. At new and full moons, the earth, the moon, and the sun are in line, causing the gravitational fields of the sun and the moon to augment each other (Figure 9.7). Thus the earth experiences the highest high tides and lowest low tides at new and full moons. These periods of greatest tidal exchange are referred to as the spring tides. At the quarters of the moon, the gravitational fields of the moon and the sun are at right angles to each other. Since their pulls are now antagonistic, the tidal exchange is smaller than at

other times of the month. These periods of lowest high tides and highest low tides are called the neap tides. Some organisms possess a biological clock that allows them to predict the times of spring tides or neap tides and gear their activities to these regular changes.

The grunion, *Leuresthes tenuis*, a small, silvery fish living in the waters off the coasts of California, precisely times its reproductive activities to occur at fortnightly intervals synchronized with the spring tides. This fish is the only one in the sea to spawn on land. Beginning in late February and continuing until early September, for

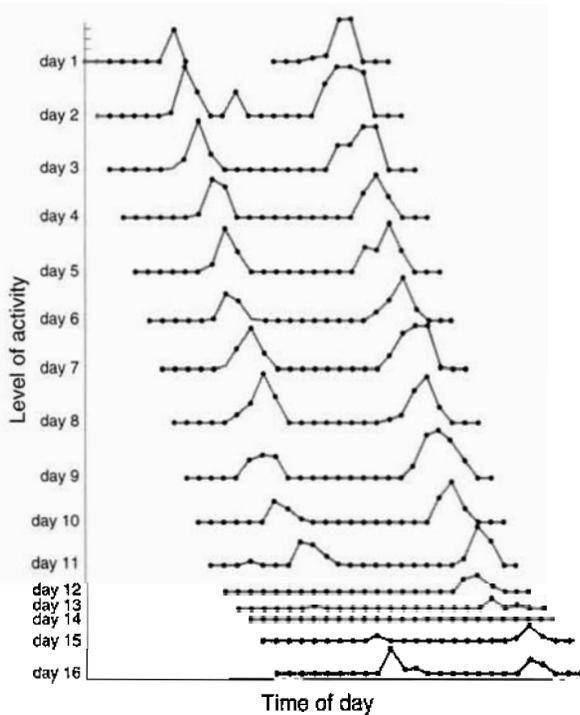


FIGURE 9.5 An activity rhythm of a fiddler crab (*Uca pugnax*). Although the crab was maintained in constant darkness and temperature (20°C), the animal was active at approximately the times of low tide at its home beach. (From Palmer 1990.)

three or four nights, right after the greatest spring tides, the adult grunion gather in the waters just offshore to wait until the tide has reached its peak and is just beginning to recede. Then they ride the waves ashore for a night of mating. At the height of the mating frenzy, the beach is carpeted with their quivering bodies. A female digs, tail first, into the sand so that only her head, from the gills up, is exposed. Several males may wrap around her and release sperm-containing milt that seeps through the sand and fertilizes the eggs discharged by the female (Figure 9.8). After mating, the fish catch a wave and disappear into the swirling sea, leaving the eggs buried in the sand to develop into the next generation.

The timing of this event is exquisite. Since the spring tides have the highest high tides of the fortnightly cycle, by waiting to mate until just after the spring tide, the grunion guarantee that unless there is a storm, their eggs will have about ten days to develop undisturbed in the sand before the pounding surf reaches them and triggers the hatching. If the fish mated before the peak of the spring tides, the next night's high tide would wash the eggs away. The same fate would meet the eggs if the grunion did not wait until the turn of the tide on their mating night (Ricciutti 1978).

Another example of a fortnightly rhythm is seen in the tiny chironomid midge, *Clunio marinus*. In *Clunio* it

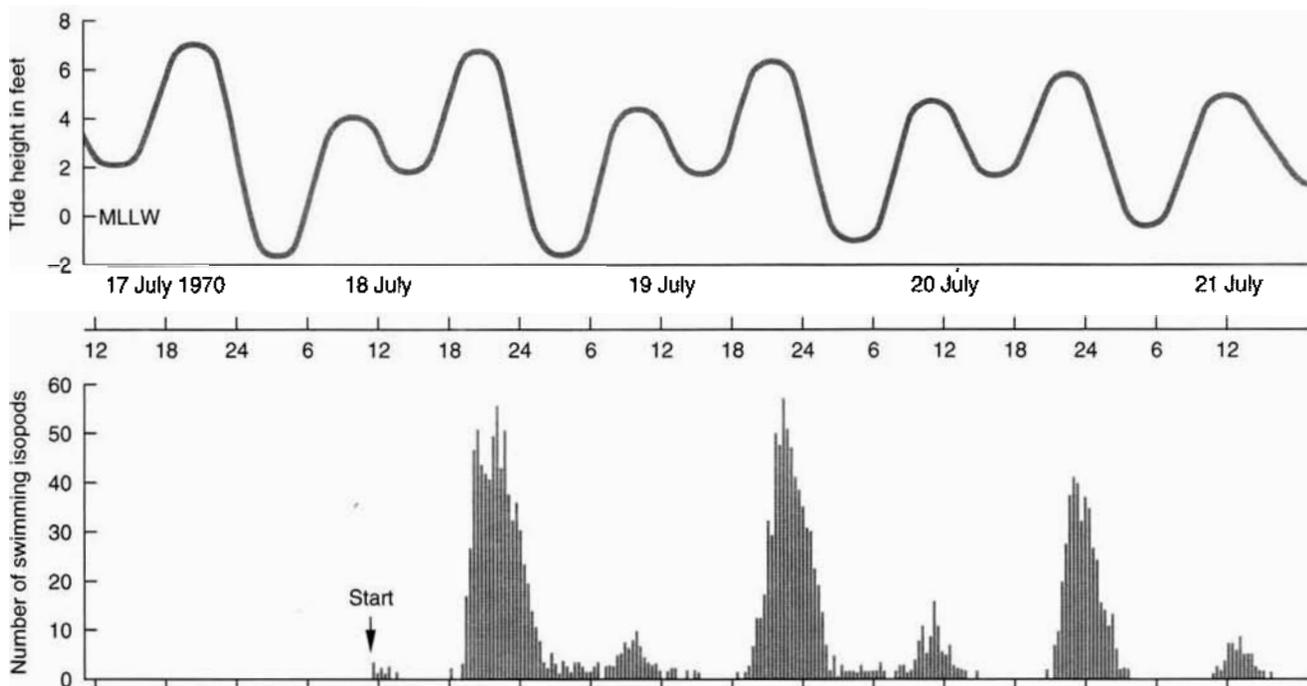


FIGURE 9.6 The activity pattern of isopods (*Excirolana chiltoni*) maintained in the laboratory. The pattern of activity mimics that of the height of the tide in the area of collection. (Modified from Klappow 1972.)

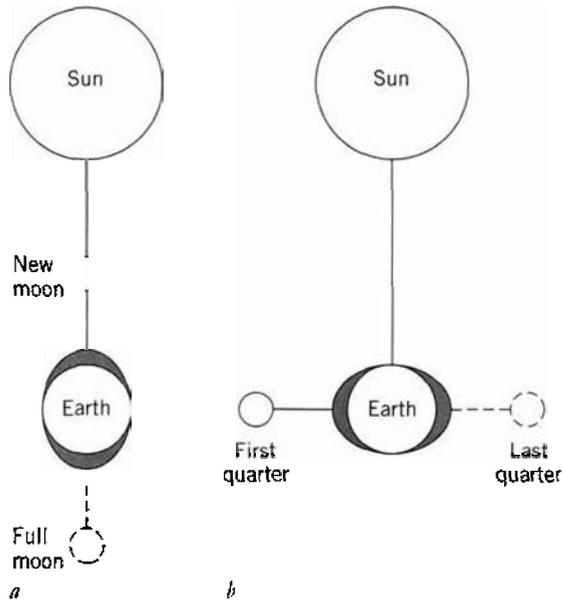


FIGURE 9.7 The effect of the relative positions of the earth, moon, and sun on the amplitude of tidal exchange. (a) At the times of new and full moons, the gravitational fields of the moon and the sun assist each other, causing the spring tides. (b) During the first and last quarters of the moon, the gravitational fields of the moon and the sun are perpendicular to each other. This results in the smallest tidal exchange, the neap tides.

is the end of development, the emergence of adults from their pupal cases, that is programmed to coincide with tidal changes. These insects live at the lowest extreme of the intertidal zone so that they are exposed to the air for only a few hours once every two weeks, during each spring low tide. When the tide recedes, the males are

first to break free of their puparia, the cases in which they developed. Each one locates a female and assists in her emergence. They have little time to waste, for their habitat will soon be submerged again, so copulation follows quickly. Then the winged male carries his mate to where she will lay her eggs. All these activities must be precisely timed so that they occur during the short, two-hour period during which the habitat is exposed.

Dietrich Neumann (1976) has found that if a population is brought into the laboratory and maintained in a light-dark cycle in which 12 hours of light alternate with 12 hours of darkness, emergence from the puparium is random. If, however, one simulates the light of full moon by leaving a dim (0.4 lux) light on for 4 consecutive nights, the emergence of adults from the puparia becomes synchronized. Now, just as in nature, emergence occurs at approximately fortnightly intervals for about 2 months.

MONTHLY RHYTHMS

The interval from full moon to full moon, a synodic lunar month (29.5 days), corresponds to the length of time it takes the moon to revolve once around the earth. Some organisms have a clock that allows them to program their activities to occur at specific times during this cycle.

An example of a clearly adaptive monthly rhythm is the timing of the reproductive activities of the marine polychaete *Eunice viridis*, the Samoan Palolo worm. Unlike the organisms previously mentioned, in which rhythms in reproduction are precisely synchronized with propitious environmental conditions, the Palolo worm restricts its procreation to a specific time so that



FIGURE 9.8 Mating grunion. The female buries her posterior end in the sand and releases her eggs while a male wraps himself around her and releases his sperm.

the population will release gametes simultaneously, increasing the probability of fertilization in the water.

The Palolo worm lives in crevices of the coral reefs off Samoa and Fiji. In preparation for reproduction, it elongates by budding new segments that become packed with gametes. This section of the worm, the epitoke, may be a foot long. Around sunrise on a day at or near the last quarter of the moon during October and November, the epitokes synchronously tear free from the rest of the body and rise to the surface, churning the water. Then they explode in unison, liberating gametes into the sea, where fertilization takes place. The timing of this self-destructive orgy is so predictable that the epicurean natives of Samoa and Fiji calculate the day of the event in advance and arrive at the coral reefs at dawn to await the frenetic swarming so that they can scoop up the epitokes, which they eat either raw or roasted (Figure 9.9) (Burrows 1945).

The ant lion (*Myrmeleon obscurus*) shows another sort of monthly rhythm. A lazy hunter, it builds a steep-sided conical pit in the sand and then lies in ambush at



FIGURE 9.9 A feast of Palolo worms. The parts of the Palolo worms (*Eunice viridis*) specialized for reproduction (epitokes) break free from the rest of the worm and swarm synchronously to the surface of the sea, releasing their gametes. The monthly rhythm in swarming increases the probability of fertilization and allows the local human population to predict the event in advance so that they can scoop up handfuls of epitokes to eat.

the base, with all but its immense mandibles covered with sand, waiting for some small arthropod, such as an ant, to slide into the pit toward its outstretched jaws. The ant lion then sucks out the prey's body fluids (Figure 9.10). Perhaps the most interesting observation on the ant lion's behavior is that it is different at full moon, similar to the pattern of blood-sucking vampires in fictional (one hopes) literature. It constructs larger pits at the time of full moon than at new moon. Careful daily measurements of the size of the pits of ant lions cloistered in constant conditions in the laboratory have revealed that this is a clock-controlled rhythm and not a simple response to some aspect of the environment such as the amount of moonlight (Youthed and Moran 1969).

ANNUAL RHYTHMS

The seasonal changes in the environment can be quite dramatic, especially in the temperate zone. As the days shorten and the temperature drops, plants and animals prepare themselves for severe and frigid weather. Some species avoid the cold and limited food of winter by migrating. An annual biological clock is important in timing migration. We see this, for example, in the activity of garden warblers, *Sylvia borin*. The bird's activity can be monitored by using microswitches mounted beneath its perch. The bird whose activity is shown in Figure 9.11 was maintained in the laboratory at a constant temperature and with an unvarying length of day (12 hours of light alternating with 12 hours of darkness), so that it was deprived of the most obvious cues for the onset of winter or of spring. Notice that during the summer and winter months, its activity was limited to the daylight hours. However, during the autumn and spring, when it would be migrating in nature, the caged bird also became somewhat active at night. This nocturnal activity, called *Zugunruhe*, or migratory restlessness, serves as an important trigger for the onset of migration.

This timing function of the annual clock is particularly important for birds that winter close to the equator, where there are few cues to the changing season. At the equator, the photoperiod is constant throughout the year, just as it is in the laboratory, and rainfall and food abundance are too variable from year to year to serve as reliable cues signaling the appropriate time to begin migrating.

An annual clock also physiologically readies birds for migration and reproduction. The bird gets fatter (indicated by body mass) during the winter, which helps provide fuel for the spring migration; it molts during the winter; and its testes enlarge for summer reproductive activity. These cycles continue for many years in constant conditions, and the length of the cycle is generally slightly longer or shorter than a year (Gwinner 1996).

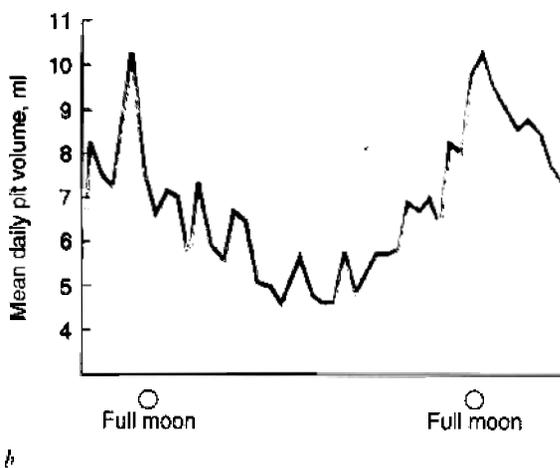


FIGURE 9.10 A monthly rhythm in the pit size of the predatory ant lion (*Myrmeleon obscurus*). (a) The ant lion waits at the bottom of a self-constructed pit with only its pincers exposed. When a small arthropod, such as this ant, slips into the pit, the ant lion sucks the prey's body juices. (b) Monthly rhythms in the pit size of 50 ant lions maintained under normal daylight conditions in the laboratory. Each of the predators was fed one ant a day. Larger pits were constructed at full moon than at new moon. (From Youthed and Moran 1969.)

The annual clocks of other species help them avoid the harsh conditions of winter by hibernating. It is especially useful for animals that hibernate in burrows or other locations where environmental cues are not available. For example, when the golden-mantled ground squirrel, *Citellus lateralis*, is maintained in the laboratory at a constant temperature and with an unvarying length of day (12 hours of light alternating with 12 hours of darkness), it will still enter a period of hibernation at approximately yearly intervals. This was true even when laboratory-born animals were subject to constant cold (3°C) and darkness; such animals still showed an annual rhythm of alternating activity and hibernation. Some of them even continued the pattern for three years.

Associated with hibernation but separate from it is a seasonal cycle in feeding. If a ground squirrel is subjected to a relentless "summer" by maintaining the laboratory temperature at an unvarying and torrid 35°C (95°F), it cannot enter hibernation. However, it will still reduce its food and water consumption during the assumed winter and begin to eat again and gain weight in the spring (Pengelley 1975).

One must be cautious in describing a behavior or physiological process that fluctuates annually as one that is controlled by an annual clock. In animals with short life spans, many of the seasonal changes in behavior are controlled by the changing photoperiod, the shortening of days during the winter months and the increasing daylight of the spring and summer. A daily

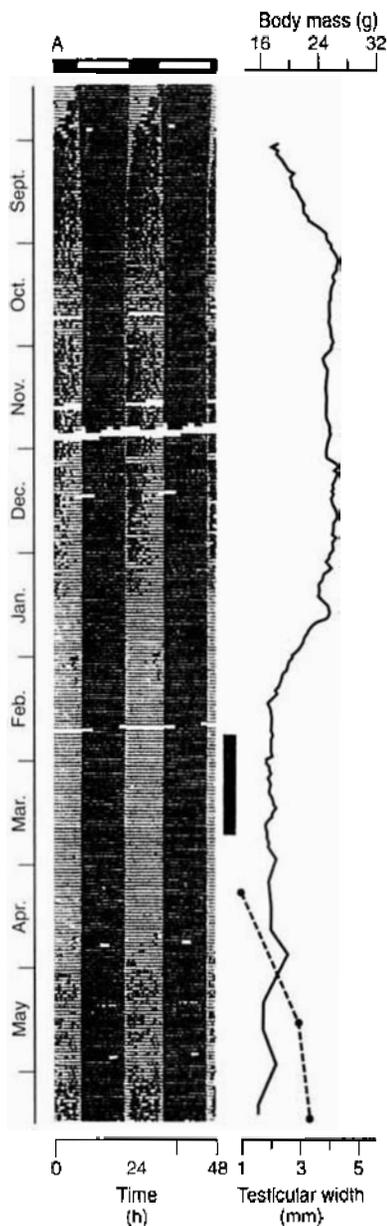


FIGURE 9.11 Annual cycles in migratory restlessness, body weight, testis size, and molting in a garden warbler held in a constant light-dark cycle (12 hours of light alternating with 12 hours of darkness) and at a constant temperature. Activity was measured with a microswitch mounted under the perch. Successive days are mounted underneath each other. The original record (0–24) is repeated on the right (24–48). Most of the bird's perch-hopping activity occurred during the day. When birds in nature are migrating, in the autumn and spring, the caged bird showed increased activity (migratory restlessness) during the night. The body weight changes throughout the year such that the bird fattens during the winter. These energy stores will increase the chances of successful spring migration. The testes enlarge during the spring in preparation for summer breeding. The molt (indicated by the vertical black bar) occurred in late February to March. (Data from Gwinner 1996.)

clock appears to be involved in measuring the interval of darkness to regulate photoperiodically controlled behaviors and processes. Unlike a response governed by photoperiod alone, a rhythm that is controlled by an annual clock will continue to be rhythmic even in the absence of changing day length.

THE CLOCK VERSUS THE HANDS OF THE CLOCK

When we study biological rhythms, we actually look at the rhythmic processes and make inferences about the clock itself. However, it is important to remember that the biological clock is separate from the processes it drives. Perhaps an analogy to a more familiar timepiece will emphasize this important point. The clock mechanism of an alarm clock is distinct from the hands of the clock, although it is responsible for their movements. If you are particularly vindictive after being awakened from a dream by the clamor of your alarm and tear the hands from the face of your clock, the internal gears will continue to run undaunted. And so it is with internal clocks. You can alter the rhythmic process without affecting the mechanism.

David Welsh and his colleagues (1995) performed the biological equivalent of tearing the hands from the clock. The suprachiasmatic nuclei (SCN) in the brain of a mammal are a “master” biological clock that drives rhythms in other processes. Welsh removed neurons from the SCN of a newborn rat and grew them in tissue culture. The spontaneous rate of firing of these single neurons varies regularly during each day, even in tissue culture, and so we can assume that the rhythm is driven by an internal cellular clock. This nerve firing was completely stopped by the addition of tetrodotoxin, a chemical that prevents action potentials that require sodium (Na^+) ions. However, 2.5 days later, when the tetrodotoxin was washed out of the cells, the rhythm reappeared with a phase predicted by the initial cycles (Figure 9.12). This suggests that although nerve firing had been halted, the clock was running accurately the entire time. Therefore, like the hands of a clock, the rhythmic process—nerve firing in this case—is separate from the clock mechanism. Processes are made to be rhythmic because they are coupled to and driven by a biological clock.

CLOCK PROPERTIES

Like any good clock, biological clocks measure time at the same rate under nearly all conditions, and they have mechanisms that reset them as needed to keep them synchronized with environmental cycles.

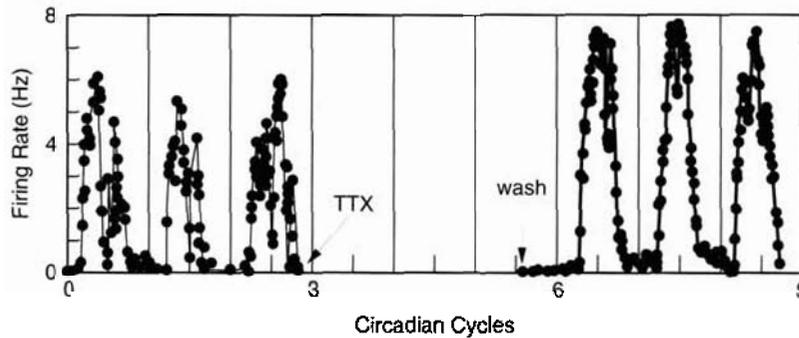


FIGURE 9.12 The rhythm in spontaneous electrical activity in isolated neurons from rat SCN. Sodium-dependent action potentials are blocked for 2.5 cycles by tetrodotoxin (TTX). When the inhibitor is washed out, the rhythm in spontaneous nerve firing returns with a phase predicted by the initial cycles. This demonstrates that the biological clock is separate from the rhythmic process it drives. (From Welsh et al. 1995.)

PERSISTENCE IN CONSTANT CONDITIONS

A defining property of clock-controlled rhythms is that cycles continue in the absence of environmental cues such as light-dark and temperature cycles. This means that the external day-night cycles in light or temperature are not causing the rhythms. Instead we attribute the ability to keep time without external cues to an internal, biological clock.

However, in the constancy of the laboratory, the period (the interval between two identical points in the cycle) of the rhythm is rarely exactly what it was in nature; that is, it becomes slightly longer or shorter. This change in the period is described with the prefix *circa*. So, a daily rhythm, one that is 24 hours in nature, is described as being circadian—*circa*, “about”; *diem*, “a day” (Figure 9.13). A lunar day (tidal) rhythm is described as being circalunidian; a monthly rhythm, circamonthly; and an annual rhythm, circannual. In other words, a laboratory hamster kept in constant conditions may begin to run a little later every night. If it starts to run 10 minutes later in each cycle, after two weeks its activity will be about 2.5 hours out of phase with the actual daily cycle.

When an animal is kept in constant conditions, the period length of its rhythms generally deviates from that observed in nature. An assumption is made that the period length is a reflection of the rate at which the clock is running. Sometimes this point is emphasized by describing the circadian period length in constant conditions as free-running, implying that it is no longer manipulated by environmental cycles.

STABILITY OF PERIOD LENGTH

If any clock is to be useful, it must be precise, and the biological clock is no exception. When an animal is cloistered in unvarying conditions and the free-running period length of its activity rhythm is determined on successive days of several months, the measurements are usually found to be extremely consistent. For some

animals the precision is astounding. For example, the biological clock of the flying squirrel, *Glaucomys volans*, measures a day to within minutes without external time cues. In fact, the precision of the clock in most animals is greater than one might expect. The daily variability in the free-running period length is frequently no more than 15 minutes and is almost always less than 1 hour (Saunders 1977).

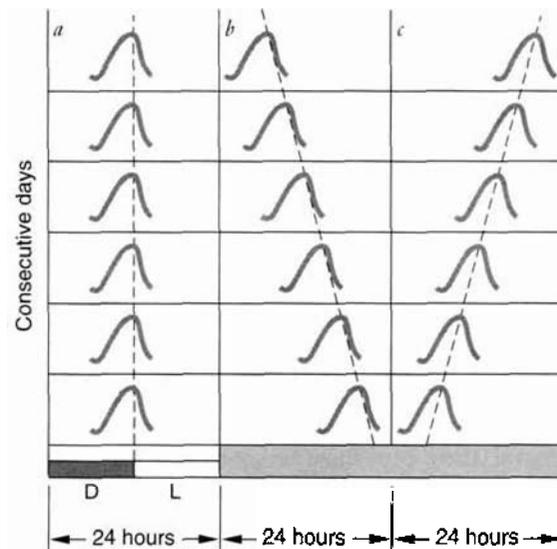


FIGURE 9.13 Diagram illustrating a biological rhythm in the entrained and free-running state. (a) The clock is entrained to the light-dark cycle indicated by the bars at the bottom of the column. Entrainment is the establishment of a stable phase relationship between the rhythm and the light-dark cycle, thus ensuring that the activities programmed by the clock occur at the appropriate times. When an organism is placed in constant conditions, the period length of its rhythms is seldom exactly 24 hours. Depending on the organism, the light intensity, and the temperature, the period length may be slightly longer than 24 hours (b) or slightly shorter than 24 hours (c). The adjective *circadian* is used to describe this change in period. (From Brown, Hastings, and Palmer 1970.)

ENTRAINMENT BY ENVIRONMENTAL CYCLES

The properties we have described so far are generally true for biological clocks of all cycle lengths. Although it is also true that tidal, fortnightly, monthly, and annual rhythms are set by environmental cycles, the nature of the environmental cycle varies for clocks of different period lengths. So, here we will focus on the environmental cycles that set circadian clocks.

Period Control: Daily Adjustment of the Free-running Period to the Natural Day-Night Cycle

Isn't it annoying when your watch runs slowly? If you did not reset it every day, your schedule would soon become a shambles. And so it is with biological clocks.

As we have just seen, the clocks themselves do not run on a precise 24-hour cycle. In nature, however, the period length of biological rhythms is strictly 24 hours. This is possible because the clock is entrained by (locked onto) the natural light-dark cycle. The clock is reset during each day by changes in light intensity as the sun rises and sets. For example, if the free-running period length of a mouse housed in constant darkness were 24 hours and 15 minutes, its clock would have to be reset by a quarter of an hour every day, or it would awaken progressively later each evening. These adjustments must be made so that the onset of activity, or any event programmed by the clock, will occur at the appropriate time each day.

Phase Control: Adjustment to a New Light-Dark Cycle

We have seen that one function of the biological clock is to time certain activities so that they occur at the best point of some predictable cycle in the environment. To be useful, then, they must be set to local time. But some species travel great distances during their lives. Clearly there must be a way to set the clock during long-distance treks, or those activities would occur at inappropriate times. In other words, biological clocks, like any clock, cannot be useful unless there is some way to set them (adjust the phase). If you were to fly from Cape Cod, Massachusetts, across three time zones to Big Sur, California, the first thing that you would want to do is to set your watch to the local time. It is obvious that if your biological clock is to gear your activities to the appropriate time of day in the new locale, it too must be reset.

When you first step off the plane after any flight across time zones, your biological clock is still set to the local time of your home. The clock will gradually adjust to the day-night cycle in the new locale (Figure 9.14). However, this shift cannot occur immediately; it may take several days. The length of time required for the

biological clock to be reset to the new local time increases with the number of time zones traversed. To make matters worse, not all your body functions re-adjust at the same rate, so the normal phase relationship among physiological processes is upset. Therefore, for a few days after longitudinal travel, your body time is out of phase with local time and your rhythms may be peaking at inappropriate times relative to one another. During this time you often suffer psychological and physiological disturbances. The syndrome of effects, which frequently includes a decrease in mental alertness and an increase in gastric distress, is referred to as jet lag.

For circadian rhythms the most powerful phase-setting agent is a light-dark cycle, although temperature cycles are also effective in plants and poikilothermic animals (those whose body temperature tends to be near that of their surroundings, commonly called cold-blooded). With a few exceptions, temperature cycles are generally not very effective in setting the clocks of birds or mammals (Hastings, Rusak, and Boulos 1991). When a rhythm becomes locked onto (synchronized with) an environmental cycle such as light-dark changes, we say that it is entrained to the cycle.

In the laboratory the biological clock can be reset at will by manipulating the light-dark cycle. If a hamster is kept in a cycle of 12 hours of light alternating with 12 hours of darkness such that the light is turned off at 6 P.M. real time, its activity begins shortly after 6 each evening. The light-dark cycle might then be changed so that darkness begins at midnight. Over the next few days the clock would be gradually reset so that at the end of about five days, the activity would begin shortly after midnight real time. If, after several weeks of this lighting regime, the hamster is returned to constant conditions, its activity rhythm would have a period that approximates 24 hours, and more important, the rhythm would initially be in phase with the second light-dark cycle.

Phase resetting occurs because a cue, a change from dark to light, for instance, affects the clock differently depending on when in the clock's cycle it occurs. Although the rhythms are separate from the clock itself, we assume that they indicate what time the clock is signaling. If an organism is kept in constant dark, its rhythms will free-run and we refer to points in the cycle as circadian time. For example, a hamster is active at night. So, when the hamster is kept in constant darkness, we refer to the time when it begins activity as early circadian nighttime. If a brief light pulse interrupts the darkness during early circadian night, it causes a phase delay. In other words, it resets the clock such that the hamster will become active later than expected on the next day. On the other hand, if a brief light pulse interrupts darkness during the late circadian night, it causes a phase advance: The animal becomes active sooner than expected in the next cycle. In most

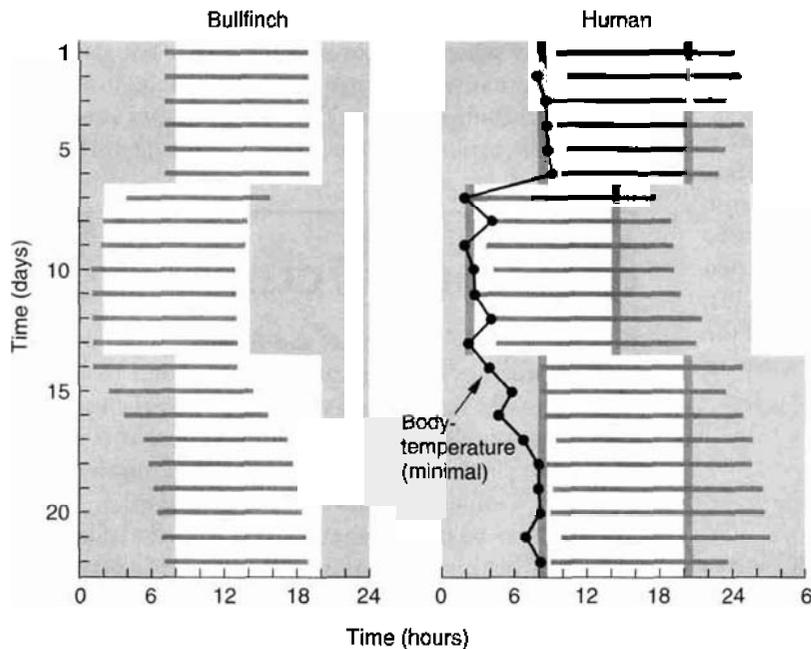


FIGURE 9.14 The resetting of the biological clock by light-dark cycles. Immediately following a trip across time zones, the biological clock is still set to home time rather than that of the vacation locale. It may take several days for the clock to be reset so that it has the proper phase relationship with the new light-dark cycle. In addition, various rhythms (clocks) may rephase at different rates. During the interval of readjustment, the individual suffers from jet lag and may not feel well. (From Aschoff 1967.)

animals, a brief light pulse during circadian daytime has little or no effect. In nature, the clock is reset by light at dawn and dusk each day so that it keeps accurate time and is set to local time.

TEMPERATURE COMPENSATION

The biological clock remains accurate in spite of large changes in environmental temperature. This is somewhat surprising if one assumes that the timing mechanism is rooted in the cell's biochemistry. As a general rule, chemical reactions double or triple in rate for each 10°C change in temperature. However, the effect of an equal temperature rise on the rate at which the clock runs is usually minor, rarely as large as 20%. This effect may be described by a temperature coefficient, or Q_{10} value (calculated as the period at T° divided by the period at $T^\circ + 10^\circ$).^{*} Typically, the Q_{10} values for the effect of temperature on the clock fall between 0.8 and 1.04. In contrast, the temperature coefficients for most chemical reactions are typically in the range of 2.0 to +.0. This insensitivity to the effects of temperature suggests that the clock somehow compensates for them. It should be apparent that if the clock were as sensitive to temperature changes as most other chemical reactions are, it would function as a thermometer, indicating the ambient temperature by its rate of running, rather than as a timepiece.

^{*}Remember that if the clock runs faster, it measures a cycle in a shorter amount of time. Thus the period length decreases as the rate increases.

ADVANTAGES OF BIOLOGICAL CLOCKS

We have seen that there are many behavioral rhythms that match the prominent geophysical cycles—a day, a lunar day, a lunar month, and a year. The geophysical cycles generate rhythmic changes in environmental conditions. One might wonder, then, why biological clocks exist at all. If the clocks cause changes that are correlated with environmental cues, why not just respond to the cues themselves?

ANTICIPATION OF ENVIRONMENTAL CHANGE

One reason for timing an event with a biological clock rather than responding directly to periodic environmental fluctuations is that it lets an animal anticipate the change and allow adequate time for the preparation of the behavior. For example, in nature, adult fruit flies (*Drosophila*) emerge from their pupal cases during a short interval around dawn. At this time the atmosphere is cool and moist, allowing the flies an opportunity to expand their wings with a minimal loss of water through the still permeable cuticle. This procedure takes several hours to complete. However, the relative humidity drops rapidly after the sun rises. If the flies waited until there was a change in light intensity, temperature, or relative humidity before beginning the preparations for emergence, they would emerge later in the day, when the water loss to the arid air could prevent the wings from expanding properly.

SYNCHRONIZATION OF BEHAVIOR WITH AN EVENT THAT CANNOT BE SENSED DIRECTLY

Another advantage of the clock's control of an event is that it allows a behavior to be synchronized with a factor in the environment that the animal cannot sense directly. An example is the timing of bee flights to patches of flowers that the bees have learned are open only during restricted times of the day (Figure 9.15). The flowers visited for nectar may be far away from the hive, and so the bee could not use vision or olfaction to determine whether the flowers were open.

CONTINUOUS MEASUREMENT OF TIME

Sometimes an animal may consult its clock to determine what time it is. As we have seen, this information is necessary to anticipate periodic environmental changes or to synchronize behavior with other events. However, at other times a clock is consulted to measure an interval of time. This, then, is a third benefit of a biological clock.

The ability to measure the passage of time continuously is crucial to an animal's time-compensated orientation. For example, a worker honeybee (*Apis* spp.) indicates the direction to a nectar source to recruit bees through a dance that tells them of the proper flight bearing relative to the sun. Since the sun is a moving

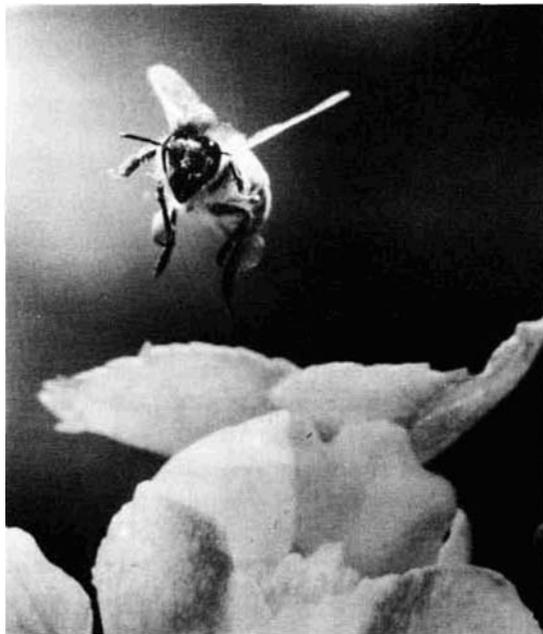


FIGURE 9.15 Honeybees can use their biological clock to time their visits to distant patches of flowers so that they arrive when the flowers are open and nectar is available.

reference point, the honeybee must know not only the time of day when it discovered the nectar but also how much time has passed since then. The biological clock provides this information. The use of the sun as a compass will be explored in more detail in Chapter 10.

ADAPTIVENESS OF BIOLOGICAL CLOCKS

Keeping these advantages of the clock in mind, we may wonder whether there is any evidence that a biological clock actually does increase fitness. Surprisingly few people have addressed this question. There is evidence that the clock enhances fitness in cyanobacteria (Johnson, Golden, and Kondo 1998; Yan et al. 1998), but that may be of marginal interest to those interested in animal behavior. However, Patricia DeCoursey and her colleagues have begun gathering evidence that the clock is indeed adaptive for certain rodents. They destroyed the SCN of some animals and compared their survival rate outdoors to that of intact animals. Since the SCN is the master biological clock in mammals, this procedure allowed them to compare the survival of animals with and without clocks. In a preliminary study, 12 intact control animals and 10 SCN-lesioned antelope ground squirrels (*Ammospermophilus leucurus*) were monitored in a desert enclosure. Their activity was continuously monitored in several ways, including a motion detector and a video camera. All the ground squirrels were primarily active during daylight.

However, an important difference in the behavior of the two groups is that the SCN-lesioned animals were more likely to be active on the ground surface of the enclosure during the nighttime than were intact animals. Whereas the amount of activity occurring during the night in SCN-lesioned animals ranged from 16% to 52.1%, nighttime activity represented no more than 1.3% of the activity of intact animals. Nine of the 12 control animals were active only in the day. This difference in nighttime activity had unfortunate consequences for the SCN-lesioned animals. One night, when 7 control animals and 5 SCN-lesioned animals had been introduced to the enclosure, a feral cat treated the enclosure as a kitty-convenience store. The videotape recorded the cat picking off ground squirrels that were active that night. As a result, the cat killed 60% of the SCN-lesioned animals, but only 29% of the intact controls (DeCoursey et al. 1997). Thus, it seems that an important function of the clock for these ground squirrels may be to reduce activity at dangerous times of the day.

DeCoursey then asked whether a biological clock enhances survival in eastern chipmunks, *Tamias striatus*. Animals were captured in the wild and taken to the laboratory for surgery. The SCN was destroyed in ten animals, and five others were given sham lesions; that is,

they were anesthetized but not lesioned. The sham-lesioned animals served as surgical controls because they were removed from their habitat and suffered the consequences of removal, such as the possible takeover of their dens by other animals, but did not undergo surgery. The survival of these two groups of animals was compared with that of 13 intact controls. Survival was only 60% for SCN-lesioned animals but was 100% for surgical controls and 84.6% for intact controls. Thus, there was not a significant difference in survival between surgical control animals and intact controls. However, the survival of these two control groups was significantly greater than that of the SCN-lesioned chipmunks. The lack of a biological clock did not seem to affect the chipmunks' ability to obtain food because the annual body weight patterns for all the animals were similar. It would be interesting to know whether the clock enhances reproductive success, but those data are difficult to obtain. Most chipmunks in all groups were reproductively active during the fall and spring. However, the genetic studies necessary to determine the parents of surviving young have not been done (DeCoursey and Krulas 1998).

ORGANIZATION OF CIRCADIAN CLOCKS

Single cells may contain the necessary equipment for biological timing. Unicellular organisms have biological clocks, and the cells that make up tissues and organs often have their own independent clocks. Thus, a complex nervous system or endocrine system is not an essential component of the biological clock.

MULTIPLE CLOCKS

Since the clock can exist in single cells, does this mean that every cell in a multicellular organism has its per-

sonal timepiece? Not necessarily. One way to demonstrate that a group of cells has its own clock is to remove the tissue, grow it in culture, and see whether the rhythm persists. For example, when the adrenal glands of a hamster are grown in tissue culture, they continue to secrete their hormone, corticosterone, rhythmically for ten days (Shiotsuka, Jovonovich, and Jovonovich 1974). Likewise, pineal glands of chickens that are organ-cultured in continuous darkness display a circadian rhythm in the production of the hormone melatonin (Takahashi, Hamm, and Menaker 1980) and in N-acetyl transferase, the enzyme that controls the melatonin rhythm (Deguchi 1979; Kasal, Menaker, and Perez-Polo 1979). In fact, the chick pineal cells can be separated from one another and not only will the rhythm in melatonin continue but also each can be entrained by a light-dark cycle (Robertson and Takahashi 1988a, b). If different glands or organs have clocks that continue running even when removed from the body, it follows that a multicellular organism must have several clocks.

Another way to demonstrate that an organism may have more than one clock is to somehow get different clocks in one individual to run independently, each at its own rate. Occasionally this happens when an animal is kept in constant conditions. When humans lived in underground shelters without any time cues, about 15% of them had a sleep-wakefulness rhythm with a different period length than their body temperature rhythm (Aschoff 1965). Notice in Figure 9.16 that one person's body temperature rhythm had a period length of 24.7 hours, whereas the sleep-wakefulness rhythm had a period of 32.6 hours. If we follow the reasoning that the period length of a rhythm in constant conditions is a reflection of the rate at which the clock is running, this may be taken as evidence that these processes are controlled by different clocks.

More recently, it has been demonstrated that fruit flies (*Drosophila*) have a multitude of independent clocks through their bodies and that these clocks respond to

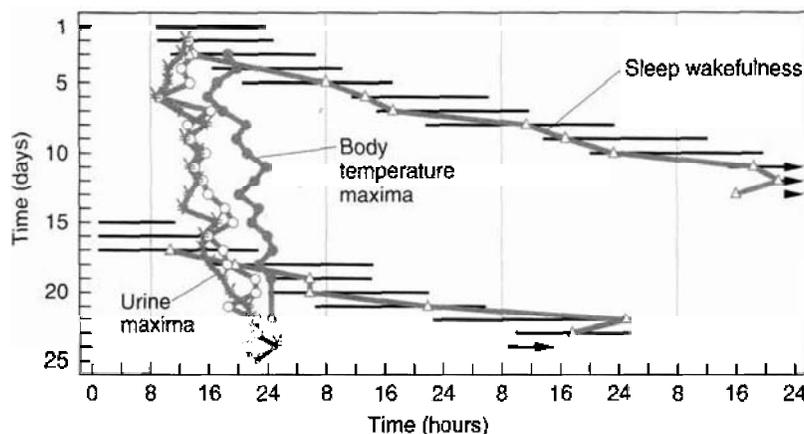


FIGURE 9.16 Desynchronization of rhythms of a man living in constant conditions. The maxima of body temperature (closed circles), urine volume (open circles), and potassium excretion (crosses) occurred at approximately 24.7-hour intervals, whereas the sleep-wakefulness rhythm (lines) and calcium excretion (open triangles) had a period of 32.6 hours. The fact that these rhythms had different period lengths is evidence that the processes are controlled by different clocks. (From Aschoff 1965.)

changes in light-dark cycles without any help from the head (Plautz, Kaneko, Hall, and Kay 1997; Plautz et al. 1997). This was shown by using an interesting technique that has since proved to be a valuable tool; it has advanced the study of rhythms because it allows researchers to observe the molecular activity of important clock genes in a single, living, intact animal. Before this, clock gene activity had to be studied by synchronizing the clocks of members of a large population of fruit flies with a light-dark or temperature cycle and then periodically selecting a group of flies from the population, grinding them up, and testing for gene activity.

Many researchers investigating the fruit fly's clock focus their efforts on the *period* (*per*) gene, which is an integral part of the clock mechanism (as discussed shortly). To monitor the clock's activity, the research groups headed by Jeffrey Hall and by Steve Kay genetically engineered fruit flies to contain the firefly luciferase gene. Luciferase is an enzyme that acts on luciferin to produce light, allowing the firefly to glow. The firefly luciferase gene was placed in the fruit fly's chromosome in the promoter region that turns on the clock gene, *per*. Then, whenever the *per* promoter turned on the *per* gene, it also switched on the luciferase gene, causing luciferase to be produced. Because the flies' diets were laced with luciferin, they glowed whenever luciferase was present. Thus, whenever the fly glowed, it meant that the *per* gene was turned on. Special cameras and video equipment measured the glow, and computers traced and recorded the glow pattern.

These glow rhythms will synchronize with light-dark cycles and will continue in constant darkness with a free-running period length. Not only do intact flies glow rhythmically, but so will cultures of head, thorax, or abdomen. Furthermore, separate cultures of body parts exposed to the same light-dark cycle will glow in unison, showing that each piece of cultured tissue has its own independent clocks and that these clocks have their own photoreceptors. Moreover, this raises the possibility that the insect's brain is not required as a master clock to synchronize rhythms throughout the body.

The glow rhythm of a fly kept in constant darkness gradually decreases in amplitude because the clocks in different cells run at slightly different rates without a light-dark cycle; thus the independent clocks gradually become asynchronous. It is interesting to note that the head is the only body part in which the clocks remain synchronized in the prolonged absence of light. But, when exposed to a new light-dark cycle, the clocks throughout the fly entrain within one cycle and the glow becomes rhythmic again. In nature, asynchrony among peripheral clocks is not a problem: Fruit flies almost always have an environmental light cycle that is able to synchronize their many independent clocks because each has its own photoreceptor, as we will see shortly (Figure 9.17).

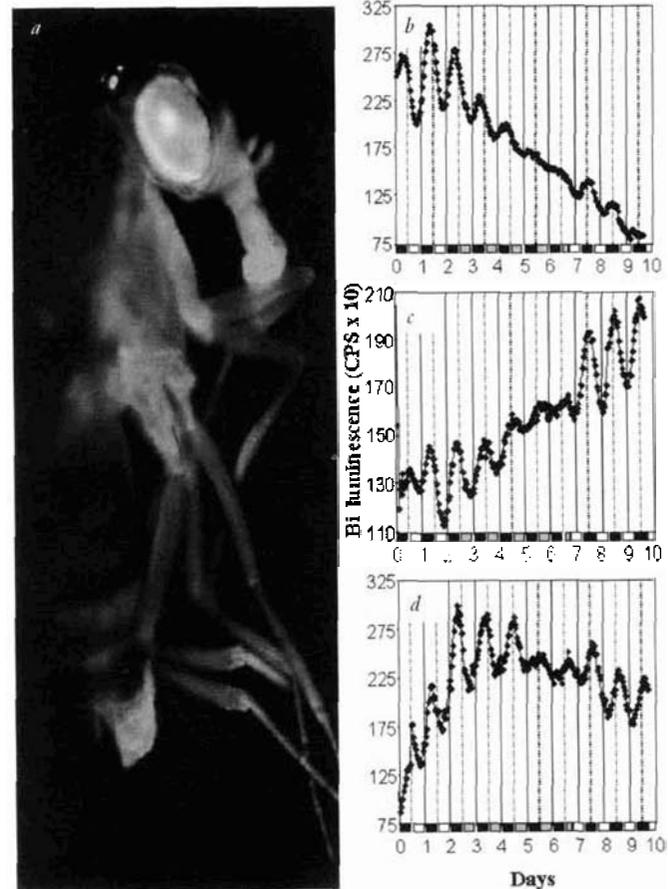


FIGURE 9.17 Biological clocks are found throughout fruit flies, not just in the brain. The *period* (*per*) gene is thought to be an integral part of the clock's mechanism. To measure *per* activity, the firefly gene for luciferase was linked to the *per* promoter, which turns on the gene. Luciferase is the enzyme that causes a firefly to glow in the presence of luciferin. As a result, the fruit fly glowed with an eerie green color whenever the *per* gene was turned on (a). Computers measured and recorded the pattern of glow. The glow rhythm persisted in constant darkness for several cycles and could be synchronized with light-dark cycles. When parts of the fly were cultured separately, the cultured segments continued to glow rhythmically and could still be set by light-dark cycles. Rhythmic glow can be seen in separately cultured heads (b), thoraxes (c), and abdomens (d). Thus, these peripheral clocks do not require input from the brain. (From Plautz, Kaneko, Hall, and Kay 1997.)

A HIERARCHY OF CLOCKS

Most rhythmic organisms have a multitude of independent peripheral clocks in cells throughout the body. However, information about environmental cycles may not reach each clock directly. How, then, are an individual's many clocks synchronized so that all the rhythmic processes occur at the appropriate time relative to one another and the environment's cycles? It appears that there are one or more "master" clocks in the brain

that are entrained by the light-dark cycle and regulate other clocks through the nervous and/or endocrine system. Therefore, we can consider three questions: (1) What photoreceptors are responsible for entrainment? (2) Where is the master clock? (3) How does the master clock regulate the other clocks in the body? Table 9.1 summarizes what is known about the location of circadian clocks in selected animals. We will approach these questions by focusing on the "clockshops" of silk moths, cockroaches, birds, and rodents.

Circadian Organization in Silk Moths

In silk moths, the photoreceptor for entrainment and the clock that controls eclosion (emergence of the adult from the pupal case) are located in the brain, which controls eclosion by producing a hormone. A series of elegant experiments revealed the details of the neuroendocrine control of the circadian eclosion rhythm (Truman and Riddiford 1970). The moths (*Hyalophora cecropia*) emerged at the usual time of day, just after sunrise, even if the nerves that connect the eyes and the brain were cut and certain parts of the nervous system (such as the subesophageal ganglion, corpora cardiaca, and corpora allata) or the developing compound eyes were removed. Apparently this treatment did not stop the clock or blind it to the light-dark cycle. However, when the brains were

removed from pupae, the adults emerged at random times throughout the day. When a brain was implanted in the abdomen of a pupa whose brain had been removed, the adult emerged at the customary time of day. So, the brain is needed if the clock is to tick; but how does it "know" when the sun rises or sets?

The search for the photoreceptor for entrainment involved removing the brains from 20 pupae and implanting a brain into the head end of 10 of these individuals and into the abdomen of the remaining 10. The pupae were then inserted through holes in an opaque board so that the two ends of each pupa could be presented with light-dark cycles that were 12 hours out of phase with one another. In other words, both ends of the pupae experienced 12 hours of light alternating with 12 hours of darkness, but the anterior end experienced light (day) while the posterior end was exposed to dark (night) and vice versa. The time of eclosion was determined by the light-dark cycle that the brain "saw," whether it had been implanted into the head or abdomen. So we see that the brain is the photoreceptor for the clock, and since there were no longer any nerves connected to the brains in these pupae, the brain must be hormonally linked to the eclosion process.

Although the previous experiments demonstrated that the brain is essential for rhythmicity, they did not conclusively show that it was the clock. An alternative explanation for these results is that the brain is responding to a signal from the clock by releasing a hormone necessary to initiate eclosion. However, if implantation of a brain transferred some clock property, such as a characteristic period or phase, this would be evidence that the brain is the clock. This was done by exchanging the brains of two species of moths, *H. cecropia*, which ecloses just after dawn, and *Antheraea pernyi*, which emerges just before sunset. The moths emerged at the time of day appropriate for the species whose brain they possessed. Since it is the clock that determines the appropriate time for eclosion, the transplantation of this phase information indicates that the brain contains the clock. Later experiments point to the cerebral lobes of the brain as the more specific site of the clock and the medial neurosecretory cell cluster as the source of the hormone that initiates eclosion (Truman 1972).

TABLE 9.1 Known Circadian Clocks in Selected Invertebrates and Vertebrates

	SCN	Pineal	Eyes	Optic Lobe	Brain
Silk moths					X
Fruit flies					X
Cockroaches				X	
Crickets				X	
Sea hares			X		
Lampreys		X	Some		
Fish		X	Some		
Lizards		X	Some		
House sparrows	X	X			
Java sparrows	X	X			
Quail		X	X		
Pigeons		X	X		
Chickens		X	X		
Hamsters	X		X		
Rats	X				
Ground squirrels	X				
Humans	X				

Circadian Organization in Cockroaches

The circadian system in cockroaches is somewhat different from that in moths. Unlike moths, in which the brain is the photoreceptor for entrainment, in cockroaches, such as *Leucophaea maderae* and *Periplaneta americana*, light information reaches the clock through the compound eyes (S. Roberts 1965). The location of the biological clock has been pinpointed to a region of the optic lobes of the brain. It is perhaps more precise to say that the cockroach has two clocks since there are

two optic lobes and each can keep time independently (Page 1985; S. Roberts 1974). The clock regulates activity in the cockroach via nerves, not hormones as in the moths. We know this because cutting the nerves leading from the optic lobes causes the cockroach's activity to become arrhythmic. However, the neural connections will re-form within about 40 days if they have been cut or if optic lobes have been transplanted into a cockroach, and activity once again becomes rhythmic (Page 1983).

Circadian Organization in Birds

There is a good deal of redundancy in the circadian system of vertebrates. In birds, for example, the eyes, the pineal, and as yet unidentified photoreceptors in the brain can provide information about lighting conditions to the clock. Furthermore, birds have three interacting clocks: the pineal gland, the suprachiasmatic nuclei of the hypothalamus (SCN), and the eyes. The importance of each of these clocks varies among species. This system of interacting clocks controls other clocks within the body through the pineal's rhythmic output of the hormone melatonin (Binkley 1993).

Although the eyes can provide lighting information to a bird's clock, there are also photoreceptors in the brain of a bird that can cause entrainment (McMillan, Keatts, and Menaker 1975). Light, it seems, can penetrate through skin, skull, and brain tissue to reach photoreceptors within the brain itself (Figure 9.18). In one experiment, a blinded house sparrow (*Passer domesticus*) was exposed to cycles of 12 hours of dim light (0.2 lux) alternating with 12 hours of darkness. The light was so



FIGURE 9.18 Flesh is amazingly transparent to light. The brain of some species contains photoreceptors that help coordinate the biological rhythms of some species of birds with the day-night cycle.

faint that most birds did not entrain to this cycle. However, when the feathers were plucked from a bird's head so that the amount of light reaching the brain was increased by several orders of magnitude, the bald bird entrained to the light-dark cycle. As the feathers grew back, entrainment failed. Replucking restored entrainment until India ink was injected beneath the skin of the head. The ink allowed only one-tenth of the light to reach the brain, and entrainment was lost. Sensitivity to the light cycle was regained when some of the scalp and the underlying India ink were scraped off. From the results of this experiment (shown in Figure 9.19), one can conclude that direct illumination of the brain can cause entrainment (Menaker 1968).

Where in the brain are the photoreceptors? We know that the pineal gland of at least some birds is sensitive to light because the rhythm in the hormone melatonin from pineals removed from chickens and kept alive in tissue culture will still entrain to light-dark cycles (Takahashi and Menaker 1979). But there is more to the story than this. The perch-hopping and body temperature rhythms of house sparrows (*P. domesticus*) whose pineals and eyes have been removed still entrain to light-dark cycles (Menaker 1968). So, there must be photoreceptors outside the pineal. Studies have tried to find these photoreceptors, but most have met with frustration because even the most focused light beam spreads outward within the brain tissue. Nonetheless, there do seem to be receptors within the hypothalamus and nearby regions (Silver et al. 1988). We still do not know whether the eyes, pineal, and other brain photoreceptors are doing the same job or are playing different roles in conveying light information to the clock.

We now know that there are several interacting clocks in the circadian system of birds: the pineal, the SCN, and the eyes. The search for the site of the circadian pacemaker began with the discovery that the pineal gland is an important master clock in some species. When the pineal is surgically removed from the brain of a house sparrow or a white-crowned sparrow (*Zonotrichia leucophrys*), the bird's perch-hopping activity and body temperature are no longer rhythmic in constant darkness (Gaston and Menaker 1968). This demonstrates only that the pineal is necessary for the expression of rhythmicity. But a later experiment indicated that the pineal is a circadian pacemaker. Two groups of donor house sparrows were entrained to light-dark cycles that were ten hours out of phase. The pineal glands of these sparrows were transplanted into the anterior chamber of the eye of arrhythmic pinealectomized sparrows that had been maintained in continuous darkness. Within a few days of a successful transplant, the recipients, still housed in constant darkness, became rhythmic. The phase of the newly instilled rhythm was that of the donor bird. This strongly sug-

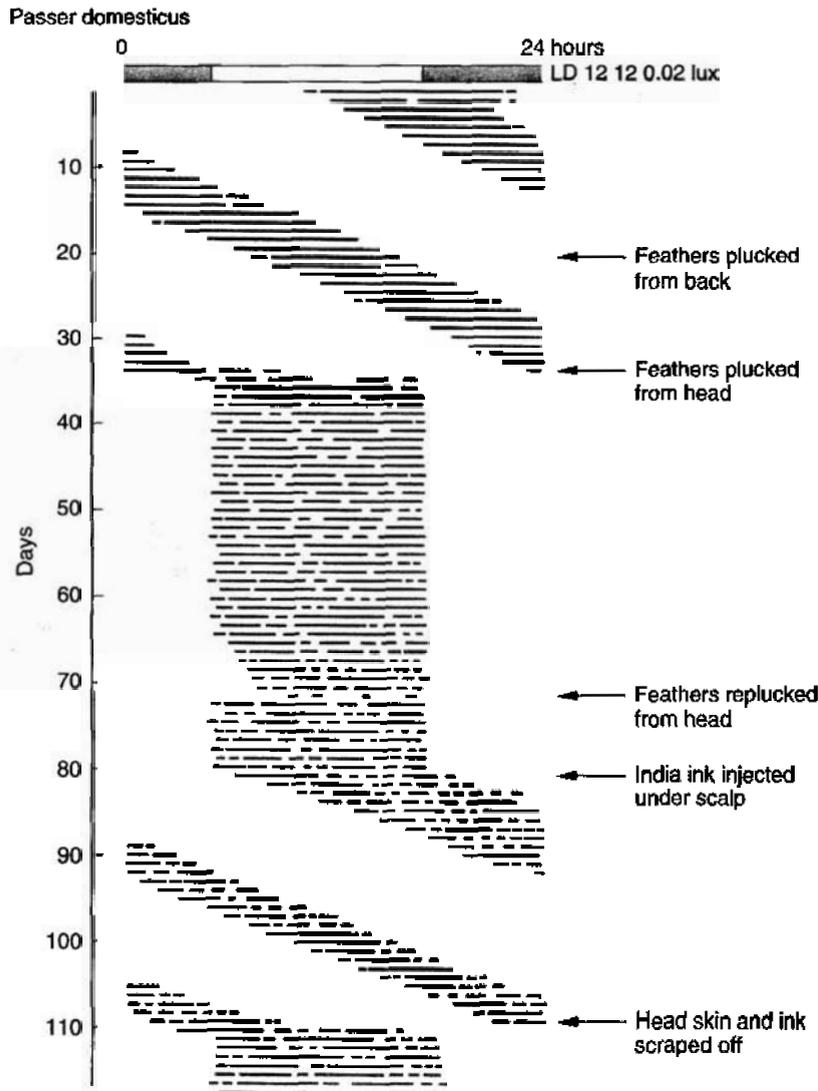


FIGURE 9.19 Activity rhythm of a blind house sparrow kept in a 24-hour light-dark cycle with 12 hours of dim (0.2 lux) light. The time of darkness is indicated by the shaded bars at the top of the figure. Activity is represented by the solid black horizontal bars. When the feathers were plucked from the bird's back, there was no effect on the rhythm. The bird was not entrained to the light-dark cycle. Then the feathers were plucked from the bird's head, thereby increasing the light that reached the brain. This resulted in entrainment until the feathers began to grow back. When the feathers were plucked once again, entrainment was reestablished. Next, India ink was injected under the skin of the scalp to decrease the light intensity that reached the brain. Entrainment was lost until the ink and the skin of the scalp were scraped off. (Modified from Menaker 1968.)

gests that the pineal gland of house sparrows contains a clock essential for the persistence of rhythmicity in the absence of external time cues (Zimmerman and Menaker 1979).

The importance of the pineal gland in the circadian system depends on the species of bird. We have seen its importance as a major clock in house sparrows, and the same is true in European starlings, *Sturnus vulgaris* (Gwinner 1978). However, it is not as important in the circadian system of Japanese quail, *Coturnix coturnix japonica* (Simpson and Follett 1981; Underwood and Siopes 1984).

In some species of birds, the SCN has been shown to be an important biological clock. The perch-hopping activity of the house sparrow, the Java sparrow (*Padda oryzivora*), and Japanese quail is severely disrupted following the destruction of the SCN (Ebihara and Kawamura 1981; Simpson and Follett 1981; Takahashi and Menaker 1979). The birds in which at

least 80% of the SCN was destroyed were arrhythmic in constant conditions. Nonetheless, they did entrain to light-dark cycles. Lesions that spared the SCN, that only partially destroyed them, or that were unilateral did not abolish the free-running activity rhythm.

The eyes may also be involved in the circadian system in some species. Quail, for instance, remain rhythmic after pinealectomy, unless they are also blinded (Underwood and Siopes 1984). Blinding also affects rhythmicity in pigeons, *Columba livia* (Ebihara, Uchiyama, and Oshima 1984), but not in sparrows or chickens (Hastings, Rusak, and Boulos 1991). In Japanese quail, the eye has been shown to be a clock in its own right (Underwood, Barrett, and Siopes 1990b).

How do the clocks in a bird's circadian system interact? The SCN is thought to communicate with the pineal gland over a neural pathway. The pineal may "talk back" to the SCN through its rhythmic production of melatonin. The SCN is known to bind mela-

tonin, which is a good indication that it responds to the hormone (Vaněček, Pavlik, and Illnerová 1987). It may be that the eyes also play a role in the circadian system by rhythmically secreting melatonin, much in the same way as the pineal gland. The eyes of chickens, quail, and sparrows have rhythms in melatonin production (Binkley 1988). The eyes of quail produce about a third of the melatonin in the blood, and the pineal produces the rest. In spite of this, the link between a quail's eyes and the rest of its circadian system is a neural pathway, not the rhythmic melatonin secretion. Continuous administration of melatonin does not affect the free-running period of activity (Simpson and Follett 1981). Furthermore, cutting the optic nerve does not disrupt the rhythmic production of melatonin, but it does have a dramatic effect on the activity rhythm. In fact, the effect of cutting the optic nerve on the activity rhythm is the same as blinding the bird (Underwood, Barrett, and Siopes 1990a).

How does this multiclock system exert its control over the rest of the circadian system? The experiment in which the pineal placed into the anterior chamber of the eye restored rhythmicity to a pinealectomized host demonstrated that the pineal must exert its influence via hormones because the nerve connections of the gland were obviously eliminated.

Melatonin, a hormone produced by the pineal in greater amounts during the night than during the day, appears to be the hormone through which the pineal exerts its effect. If a capsule that continually releases melatonin is implanted in an intact house sparrow, either the bird becomes continuously active or the period length of its rhythm is shortened. In other words, when melatonin release is continuous rather than periodic (as it normally is), circadian rhythmicity is disturbed (Turek, McMillan, and Menaker 1976).

Furthermore, periodic release of melatonin can entrain the activity rhythm of a bird. This has been demonstrated by pinealectomizing a group of European starlings, *S. vulgaris*, and giving half the birds a daily injection of sesame oil (to ensure that any effect on the clock was caused by the hormone and not the injection). Almost all, 21 out of 22, of the starlings receiving melatonin injections entrained, whereas only 1 of the 10 control birds did. The daily injections seem to have mimicked the effect of the rhythmic release of melatonin by the pineal gland (Gwinner and Benzinger 1978).

Circadian Organization in Mammals

The circadian organization in rodents differs from that in birds in several ways: Mammals seem to lack extraretinal photoreceptors for entrainment, the pineal gland is not an independent clock, and the master clock seems to exert its influence through nerves and chemicals (Figure 9.20). However, as in birds, the SCN and the retina, the light-sensitive portion of the eye, are

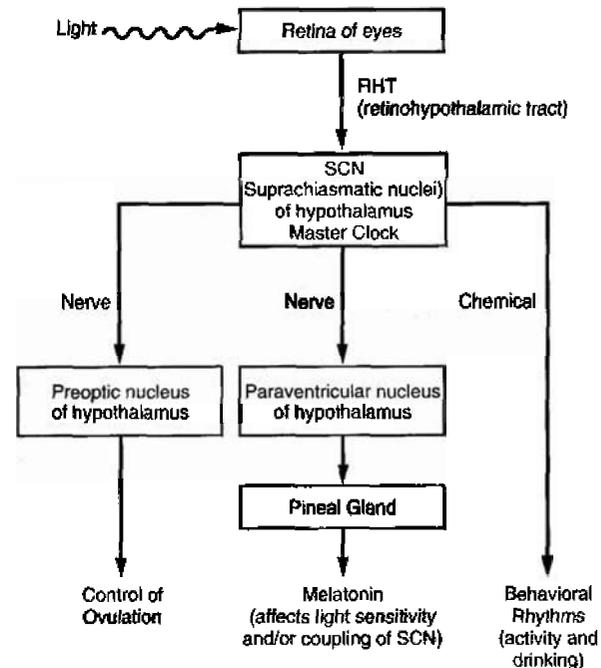


FIGURE 9.20 The circadian organization of mammals.

independent, self-sustaining clocks (Hastings 1997; Tosini and Menaker 1996). These two clocks interact to produce the observed characteristics of the circadian system (Lupi et al. 1999).

The eyes contain the photoreceptors for light entrainment in mammals. However, these are in a different part of the retina than the photoreceptors involved in vision (as discussed shortly). The information about the lighting conditions reaches the clock through the retinohypothalamic tract (RHT), a bundle of nerve fibers connecting the retina with the hypothalamus (R. Y. Moore 1979).

There is evidence that the SCN is a master clock in rodents and holds several "slave" clocks in the proper phase relationship. The initial studies in which the SCN was destroyed showed that the SCN is necessary for most rhythms in the rat (Moore and Eichler 1972; Raisman and Brown-Grant 1977) and the hamster (Stetson and Watson-Whitmyre 1976). Comparable destruction elsewhere in the brain has not so far destroyed rhythmicity.

The next step was to show that the SCN contains an autonomous clock. In one experiment, the SCN was isolated from neural input while still in place in the brain, and its activity remained rhythmic. Neural activity in the SCN and in one of several other brain locations was recorded simultaneously to be certain that the observations were specific to the SCN. In an intact animal, neural activity in all regions of the brain was always rhythmic. Then, a knife cut was made around the SCN, creating an isolated island of hypothalamic tissue that included the SCN. Following this treatment, the neural activity in the hypothalamic island remained

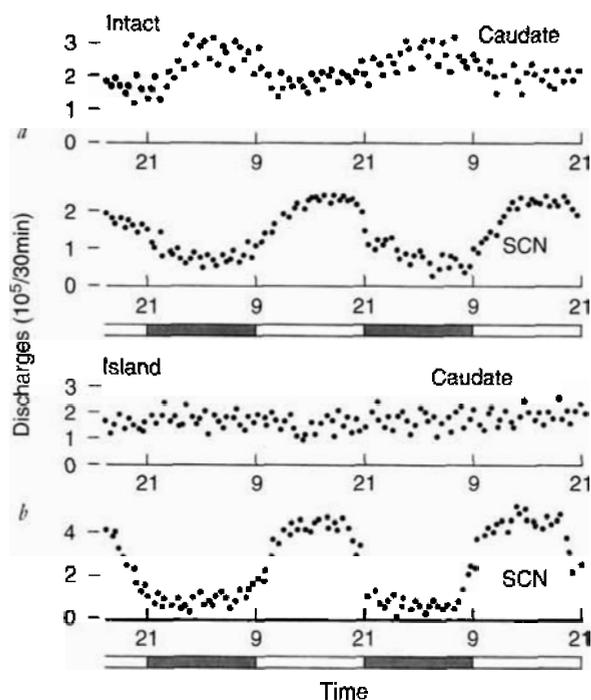


FIGURE 9.21 Nerve activity in specific brain regions (the caudate and the suprachiasmatic nuclei) of a rat before and after isolating an “island” of brain tissue containing the suprachiasmatic nuclei. (a) The activity in a normal, intact rat is rhythmic in both regions of the brain. (b) After a region of the hypothalamus that contains the suprachiasmatic nuclei was isolated as an island, the nerve activity was rhythmic within the SCN but not outside the island in the caudate region. This supports the idea that the suprachiasmatic nuclei are self-sustaining oscillators. (Courtesy of S. T. Inouye.)

rhythmic in constant darkness but activity in other brain regions was continuous (Figure 9.21). This strongly suggests that the SCN is a self-sustaining oscillator that instills rhythmicity in other brain regions through neural connections (Inouye and Kawamura 1979).

The activity of the SCN remains rhythmic in tissue culture, confirming that it is an independent clock (Inouye and Shibata 1994; Shibata and Moore 1988). Indeed, individual neurons within the SCN have their own clock. When cultured SCN neurons are separated, the spontaneous electrical firing of individual neurons is rhythmic in constant conditions, each of them with a slightly different period (Welsh et al. 1995).

The SCN was finally established as the primary clock in mammals by transplantation studies. When an SCN is transplanted into the brains of rats or hamsters that have been made arrhythmic by destroying their own SCN, their activity becomes rhythmic once again. The transplanted tissue is from fetal or newborn animals, so new neural connections may form with the host's brain. In those individuals whose activity rhythms

were restored after they received a new SCN, some neural connections had formed. As a result, the grafted SCN received input from the eyes and could communicate with other regions of the brain (Lehman et al. 1987; Sawaki, Nihonmatsu, and Kawamura 1984). It is important that the period length of the restored activity rhythm matches that of the transplanted SCN rather than the period length previously displayed by the recipient (Ralph et al. 1990). This result is what one would expect if the SCN were the clock that was providing timing information and not just a component needed to make the host's clock function.

As we have learned more about the SCN, we have begun to see that it contains many clocks, and this has raised new questions about how these clocks are linked together, or coupled, so that they function as a single unit. First, there is a suprachiasmatic nucleus in each half of the brain. There is evidence supporting the hypothesis that each of these centers is an independent oscillator (or contains a population of oscillators). In a normal hamster the two nuclei are mutually coupled and produce an activity rhythm with a single, well-defined band of activity during each cycle. When a nocturnal animal such as the hamster is maintained in constant light for an extended period, its activity pattern occasionally splits into two components that have different free-running periods until they become stabilized approximately 180° out of phase with each other. Some researchers have interpreted the splitting phenomenon as evidence for two circadian oscillators that are controlling activity and have become uncoupled as a result of the continuous illumination. There is some experimental support for this interpretation. When one of the suprachiasmatic nuclei in hamsters whose activity pattern had become split was destroyed, the split in the activity was eliminated and replaced by a new, single activity rhythm, presumably the one controlled by the remaining SCN (Pickard and Turek 1982).

There are other reasons for thinking of the SCN as a multiclock system. We now know, for instance, that each SCN has two structural components—an inner core and an outer shell. These differences in structure suggest that the core and shell may have different functions, but we don't know yet what these might be (Moore and Silver 1998; Weaver 1998). Recall also that individual neurons within the SCN have their own clocks. What synchronizes them? We have ideas but no answers (J. D. Miller 1998).

We have seen that the SCN is the primary clock in mammals that drives rhythms in behavior and physiology. Whereas some signals from the SCN are sent over neural pathways, others are sent by small, diffusible molecules (LeSauter and Silver 1998).

The SCN has at least two neural output pathways that affect rhythms. One of these is a pathway to the preoptic nucleus of the hypothalamus, and this seems to control the rhythm in ovulation but does not affect the

activity rhythm. The second neural pathway leads to the paraventricular nucleus in the hypothalamus and then to the pineal. This pathway controls the pineal's production of melatonin by affecting transcription (Foulkes et al. 1997). Melatonin synthesis occurs only in the dark, and it is rhythmic.

In contrast to the effects of melatonin in birds, it has a subtle effect on the circadian system of mammals. Melatonin affects the mammalian SCN in some way, perhaps by decreasing the sensitivity of the circadian system to light or by altering the coupling between the SCN and its output (Cassone 1998). There are two other roles for melatonin in mammals. First, it serves as an entraining agent during development. Maternal melatonin cycles synchronize the clocks of fetuses with one another and with the mother. Second, it has become important in timing the seasonal reproduction of some species (Menaker 1997).

Activity rhythms, on the other hand, are thought to be caused by the SCN's release of a chemical signal to other parts of the brain without the help of neural connections. Rae Silver and her colleagues (1996) demonstrated this through transplant experiments similar to those described earlier, but with one important difference. The donor SCN tissue was enclosed within a capsule that allowed nutrients and diffusible molecules to flow between the host and graft tissue but did not allow neural processes to grow (Figure 9.22a). As in previous transplant experiments, both SCN of the host were destroyed prior to transplant, making the animal arrhythmic. The transplants were made between hamsters whose clocks ran at different rates because some carried the *tau* mutation, which alters the period length observed in constant conditions. The implanted SCN was the wild type, which has a period length of 24 hours in constant dark. Before SCN destruction, the activity rhythms of the *tau* mutant hosts were either 22 hours or 20 hours, depending on whether they were heterozygous or homozygous for the *tau* mutation. The encapsulated grafts restored the activity rhythm with the period length characteristic of the donor SCN (Figure 9.22b).

It is interesting that the chemical control of rhythms by the SCN seems to be unique to behavioral rhythms, such as activity and drinking. Reproductive responses to the length of day, which depend on melatonin from the pineal, and endocrine rhythms are not restored. These require neural connections.

THE MOLECULAR BASIS OF SELECTED CIRCADIAN CLOCKS

In recent years, we have learned a great deal about the molecular gears that make the clock tick and how it entrains to environmental light cycles.

THE GENETIC BASIS OF THE CLOCK MECHANISM

The molecular bases of the clocks investigated so far involve rhythmic gene activity. The products of one gene or set of genes activate or inhibit the activity of other genes, which in turn affect the activity of the first genes. This creates a self-regulated feedback loop of gene activity that measures an approximately 24-hour interval.

If your knowledge of genetics is rusty, you may want to refer to Chapter 3. In brief, however, genes consist of DNA. When a gene becomes active it is transcribed to mRNA, which is then translated to protein. Besides providing a more thorough discussion of gene activity, Chapter 3 discusses additional aspects of the *period* (*per*) gene that is known to be part of a gear in the clock of the fruit fly (*Drosophila*).

Fruit Fly

The starting point in the circadian cycle is arbitrary. We'll begin around noon, circadian time (Figure 9.23). Two clock genes, *period* (*per*) and *timeless* (*tim*), begin to be transcribed to mRNA (C. B. Green 1998; M. W. Young 2000). These genes have been turned on by a complex that forms between the protein products of two other clock genes, *clock* (*clk*) and *cycle* (*cyc*). The CLOCK-CYCLE complex binds to the promoter region of *per* and *tim* and turns on gene activity (Allada et al. 1998; Darlington et al. 1998). The *per* and *tim* mRNA slowly accumulate, and shortly after sunset their translation to proteins begins (C. B. Green 1998; M. W. Young 2000).

The PER protein forms a complex with the TIM protein. The level of the PER-TIM complex peaks around four hours before dawn. As a complex, PER-TIM is able to move into the nucleus, where it prevents the CLOCK-CYCLE complex from turning on the transcription of *per* and *tim*. In this way, PER and TIM shut down their own production (Darlington et al. 1998).

A time delay is built into the system because the PER-TIM complex can form only when there are high levels of *per* and *tim* mRNA, and these take time to accumulate (M. W. Young 1998). In addition, the product of another gene, called *double-time* (*dbt*), adds a phosphate group to PER, which causes it to be destroyed. Thus, phosphorylation of PER protein controls its level. After awhile, however, the level of TIM is high enough that TIM binds to PER before a phosphate can be added. In this way, *dbt* adds a time lag between the transcription of *per* and *tim* and the accumulation of the PER-TIM complex (Price et al. 1998).

The TIM protein is broken down by light; so with the light of dawn, TIM degradation begins (M. W. Young 1998). PER is then released from the complex, and because of phosphorylation, it too is broken down.

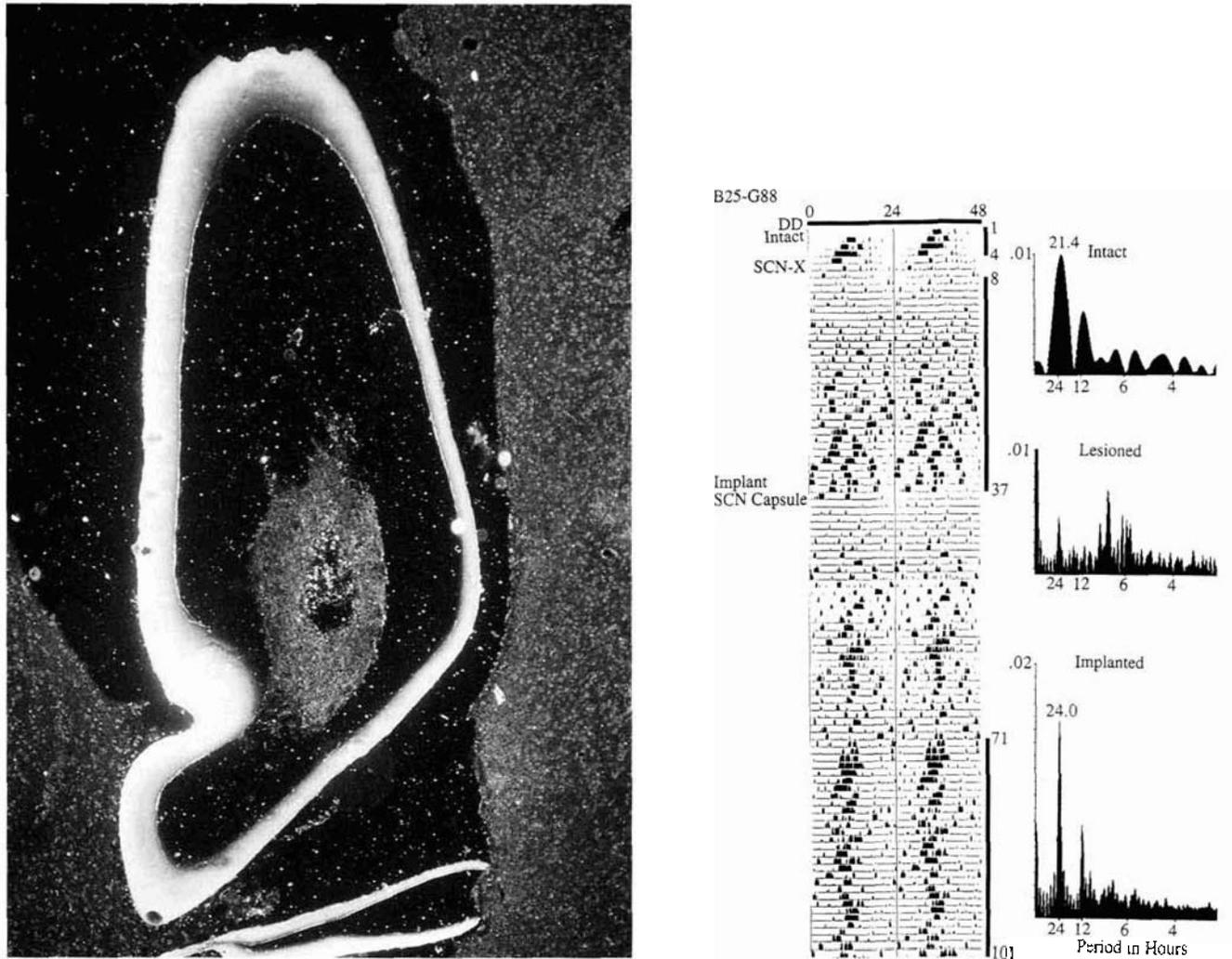


FIGURE 9.22 Encapsulated donor SCN can reinstall rhythmic activity in SCN-lesioned hamsters. (a) The capsule, shown here in white, surrounds the donor SCN. Although the capsule allowed nutrients and small molecules to diffuse between the host and graft tissue, it prevented the formation of neural connections. (b) The activity rhythm of the intact host hamster (wild-type/ τ) had a period length of 21.4 hours in constant darkness. After the SCN was destroyed, the animal was arrhythmic. Implantation of a capsule containing wild-type SCN (period length of 24 hours) restored the activity rhythm with a period characteristic of the implant. (Data from Silver et al. 1996)

When the level of the PER-TIM complex falls, it can no longer prevent CLOCK-CYCLE from activating *per* and *tim*. The inhibition of transcription of *per* and *tim* is lifted, and they become active once again (Darlington et al. 1998).

Mammals

Less is known about the genetic basis of the clock of mammals than about the clock of fruit flies. But, based on what is known, it seems that the mechanisms of both clocks are based on self-regulated feedback loops in

which the products of certain genes regulate the activity of other genes (Figure 9.24). The cast of molecular players in the clock of mammals is similar to those in the fruit fly. The names of some of these genes are familiar—*per*, *tim*, and *clk*. Their names are often distinguished from the *Drosophila* forms of the genes by the prefix *m*, for “mouse.” The mouse has three different *per* genes, *mPer1*, *mPer2*, and *mPer3*. The proteins produced by these genes have regions that will allow them to form complexes with other specific proteins, but they do not have regions for binding to DNA. The protein product of another gene, *bmal1*, forms a com-

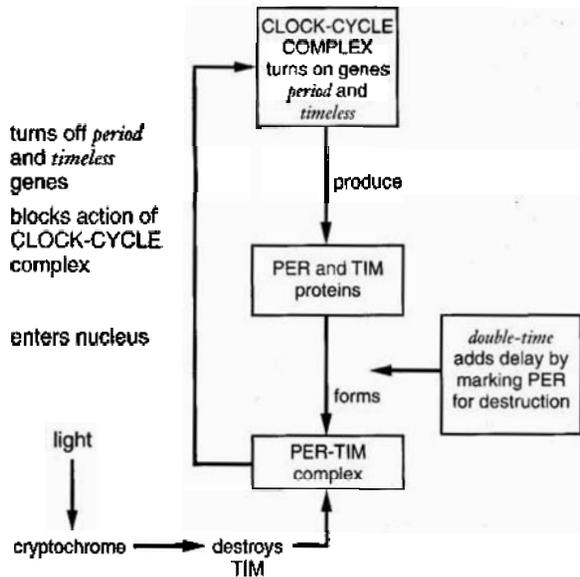


FIGURE 9.23 A model of gene activity thought to underlie circadian timing in the fruit fly.

plex with the CLOCK protein in much the same manner as CYCLE does in the fruit fly.

Two additional proteins—mCRYPTOCHROME1 (mCRY1) and mCRYPTOCHROME2 (mCRY2)—are also known to be involved in the circadian feedback loop of mammals. When mice are genetically engineered so that the *mCry1* and *mCry2* genes are knocked out (made nonfunctional), the animals are totally arrhythmic in constant darkness. However, if only one of these genes is disabled, the clock still functions in constant darkness but runs at a different rate, either faster or slower than

usual. Thus the *mCry* genes are necessary for clock functioning (van der Horst et al. 1999).

A cycle begins when the mCLOCK/BMAL1 complex binds the promoter region of *mPer1* and turns on gene activity, producing *mPer1* mRNA (Gekakis and Witz 1998). Soon afterward, *mPer3* and then *mPer2* become active. It is thought that the difference in the timing of gene activity might mean that factors in addition to the CLOCK/BMAL1 complex are affecting gene activity. Based on their role in the fruit fly's clock, it is suspected that the mPER proteins alone or in a complex with another clock protein enter the nucleus and turn off the activity of their own genes. The CRY proteins play two important roles in this feedback loop. First, the movement of the mPER proteins into the nucleus is promoted by CRY1 and CRY2. Second, once in the nucleus, CRY1 and CRY2 prevent the CLOCK/BMAL1 complex from turning on the *per* genes. The inhibition of the CLOCK/BMAL1 caused by the CRY proteins is stronger than that caused by any of the mPER proteins or by mTIM (Griffin, Staknis, and Weitz 1999; Kume et al. 1999). The proteins in the mPER complex then become phosphorylated, which causes them to break down. CLOCK and BMAL1 are still present, so as the level of mPER falls, they will once again turn on the *mPer* gene.

The role of mTIM in the mouse clock is unclear (Dunlap 1999). It might function along with the mPER proteins and the mCRY proteins in inhibiting the CLOCK/BMAL1 complex, thereby preventing it from turning on the activity of the *per* genes (Hardin and Glossop 1999).

ENTRAINMENT

If the clock is to be adaptive, it must be synchronized to environmental cycles, such as the light-dark cycles caused by the rising and setting of the sun. We are beginning to understand how this synchronization might occur.

Fruit Fly

We will describe the events involved in entrainment in the fruit fly. Recall that the TIM protein is broken down by light. (Cryptochrome is the pigment that absorbs light and causes TIM destruction, as we will see shortly.) The destruction of TIM is responsible for the changes in the phase of rhythms in response to light (Suri et al. 1998).

A light pulse can cause a phase delay or a phase advance, depending on the part of the cycle at which it occurs. In the early part of the evening, a light pulse causes a delay in the cycle. At this point in the cycle, the PER-TIM complex is still in the cytoplasm. The destruction of TIM by light will cause a delay until TIM can be replenished and the complex restored.

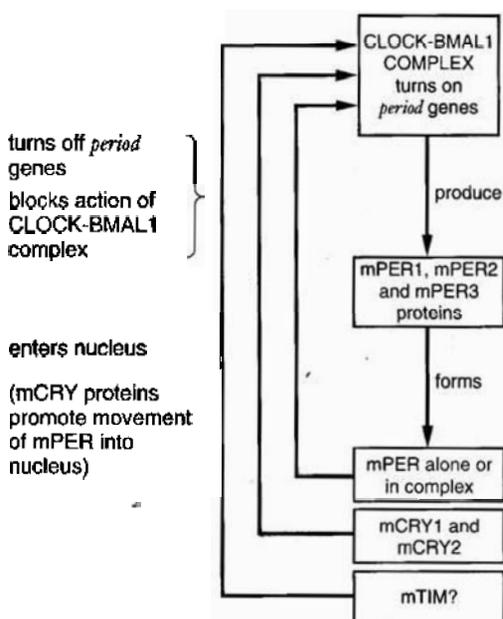


FIGURE 9.24 A model of gene activity thought to underlie circadian timing in mammals.

Only then can it move into the nucleus and shut off the transcription of *per* and *tim*. So, *per* and *tim* remain active longer than usual, causing a delay in the cycle.

A light pulse in the late night causes a phase advance. Late at night the PER-TIM complex is in the nucleus, preventing the CLOCK-CYCLE complex from activating the *per* and *tim* genes. At this point in the cycle, the light-induced destruction of TIM destroys the PER-TIM complex, allowing the CLOCK-CYCLE complex to turn on the *per* and *tim* genes sooner than usual. The early activation of *per* and *tim* advances the cycle. During the daytime portion of the cycle, light pulses have no effect because there is so little TIM present that further breakdown by light has no effect (Lee et al. 1996).

How does light information reach the clock? Before it can have biological effects, light must first be absorbed by a pigment. The pigments involved in vision are called opsins. Although the opsins play some role in the clock's response to light, we now know that they are not the most important way in which the clock senses light.

In fruit flies, the clock's primary light-sensing pigments are cryptochromes (CRY), which are blue light receptors. When cryptochrome absorbs light, it triggers a cascade of events, including the destruction of TIM, that synchronizes the biological clock to the environmental light-dark cycle.

The importance of cryptochrome in entrainment in fruit flies has been examined by looking at the effect of a mutation, *cry(b)*, that prevents cryptochrome production. As we describe the effects of this mutation, keep two things in mind. First, fruit flies have clocks throughout their bodies, and these clocks respond to changes in light-dark cycles without any help from the head. Second, cryptochrome is found in nearly all tissues (Plautz, Kaneko, Hall, and Kay 1997; Plautz et al. 1997).

Cryptochrome is the only photoreceptor for the peripheral clocks. This was demonstrated by observing the glow rhythm in fruit flies with the *cry(b)* mutation. (Recall that the glow rhythm can be observed when the *per* promoter is fused with the firefly luciferase gene. This procedure creates a fly that glows whenever the *per* gene is active.) The glow of mutant *cry(b)* flies is not rhythmic in light-dark cycles or in constant darkness. However, if *cry(b)* flies are exposed to a temperature cycle while they are in constant darkness, PER and TIM proteins are produced rhythmically. The *cry(b)* flies cannot produce cryptochrome, and without it the independent clocks cannot be synchronized to a light-dark cycle.

However, the clocks in a fruit fly's brain can receive information about light from visual pigments, as well as from cryptochrome. These clocks, specifically a group of cells on each side of the brain called the lateral neurons, drive the locomotor activity rhythm (Kaneko 1998). The locomotor activity rhythm of *cry(b)* flies is

nearly normal in both light-dark cycles and constant darkness. We know that the clock in the lateral neurons is still running in *cry(b)* flies and that it can be set by light because the level of PER and TIM proteins cycle rhythmically in these neurons in a light-dark cycle.

Light can reset the brain clocks because light information reaches them by two pathways: one involving visual pigments and the other involving cryptochrome. In *cry(b)* flies, the visual pigments supply light information to the lateral neurons. However, the light responses aren't completely normal without cryptochrome. The clock cannot be reset by brief pulses of light, and light cannot cause large phase shifts in *cry(b)* flies (Stanewsky et al. 1998).

Light causes a change in CRY that allows it to form a complex with TIM both in the nucleus and in the cytoplasm. As a result of this interaction, the PER-TIM complex is unable to block the action of the CLOCK-CYCLE complex. Thus the *per* and *tim* genes remain turned on. Light enables CRY to block the action of the PER-TIM complex even if TIM is not broken down. Thus, TIM destruction must be a later consequence of the interaction between light and CRY. It is thought, then, that CRY acts as the photoreceptor for the circadian clock by interacting directly with a component of the clock mechanism, TIM. However, CRY is not thought to function as a component of the clock mechanism itself (Ceriani et al. 1999).

Mammals

In mice, information about the presence of light is sent to the SCN, where it turns on a set of immediate early genes (IEGs). You may recall that IEGs produce factors that turn on the activity of other genes. We know that light also turns on two clock genes, *mPer1* and *mPer2*, but we don't know whether IEGs play a role in this. It is interesting that the two clock genes are not activated simultaneously: *mPer1* is activated within 30 minutes, but *mPer2* is activated after about 3 hours (Shearman et al. 1997; Shigeyoshi et al. 1997). The activation of *mPer1* by light is essential for phase-shifting the clock (Akiyama et al. 1999). A phase delay results when light is present during the early night because it causes the levels of *mPer1* and *mPer2* mRNAs to remain high for a prolonged length of time. However, when light is present during the late evening, *mPer1* and *mPer2* are transcribed prematurely, causing a phase advance (Hardin and Glossop 1999).

What is the photoreceptor for the mammalian circadian clock? When cryptochrome was shown to be the photoreceptor for the clock in fruit flies, there was wide speculation that it would play a similar role in mammals. It does not.

Although the cryptochromes are central clock components in mammals, it seems that they are not

involved in photoreception for the clock. Recall that light shifts the phase of the clock by turning on the *mPer* genes. Light still has this effect in mice that have been genetically engineered to lack both CRY1 and CRY2. Since the CRY proteins are not needed for light-induced phase shifting, light information must reach the clock through a different photoreceptor (Okamura et al. 1999). We know that the photoreceptor is present in the eyes, but it is not one of the visual pigments in the rods or cones that are important in vision (Freedman et al. 1999).

SUMMARY

Life evolved in a cyclic environment caused by the relative movements of the earth, sun, and moon. Often it is advantageous to gear an activity to occur at a specific time relative to some rhythmic aspect of the environment. Thus clocks evolved as adaptations to these environmental cycles.

There are many examples of rhythmic processes in animals that match the basic geophysical periods: a day (24 hours), the tides (12.4 hours), a lunar day (24.8 hours), a fortnight (14 days), a lunar month (29.5 days), and a year (365 days). Many of these processes remain rhythmic when the individual is isolated from the obvious environmental cycles that might be thought to provide time cues. For instance, many daily rhythms persist when the individual is kept in the laboratory without light-dark or temperature cycles. Therefore, we say that the rhythms are caused by an internal biological clock.

The biological clock is separate from the rhythms it drives. Processes become rhythmic when they are coupled to the biological clock.

In the constancy of the laboratory, the period length of biological rhythms may deviate slightly from the one displayed in nature. For this reason, periods are described with the prefix *circa*, meaning "about," and are called circadian, circalunidian, or circannual. The period length in constant conditions is described as the free-running period and is assumed to reflect the rate at which the clock is running. The free-running period is generally kept constant, which indicates that the biological clock is very accurate.

Although the period length of a biological rhythm is "circa" in the constancy of the laboratory, in nature it matches that of the geophysical cycle exactly because the clock is entrained to (locked onto) an environmen-

tal cycle. Entrainment adjusts both the period length and the phase of the rhythm. Daily rhythms can be entrained to light-dark cycles, and in some species, to temperature cycles.

Environmental temperature has only a slight effect on the rate at which the clock runs. This property is called temperature compensation.

There are several reasons why it may be advantageous to have a biological clock to measure time rather than responding directly to environmental changes: (1) anticipation of the environmental changes with enough time to prepare for the behavior, (2) synchronization of the behavior with some event that cannot be sensed directly, and (3) continuous measurement of time so that time-compensated orientation is possible. There is now some evidence that a functional clock enhances survival.

Biological clocks exist in single cells, and there may be many clocks in a single individual. It seems that there is a hierarchy of clocks, with one or more master clocks regulating the activities of other, slave clocks.

We are beginning to understand the circadian system in several species. A silk moth has photoreceptors for entrainment and a clock located in its brain, which then regulates eclosion by producing a hormone. In cockroaches, light information reaches the clock through the compound eyes. Two biological clocks, one in each optic lobe, regulate activity through nerves.

Birds have photoreceptors in their brains capable of providing information to the clock about lighting conditions. The circadian system in birds seems to contain three interacting clocks: the pineal gland, the suprachiasmatic nuclei of the hypothalamus, and the eyes. The importance of each of these clocks varies among species. This system of interacting clocks controls other clocks in the body through the pineal's rhythmic output of the hormone melatonin.

The photoreceptors for entrainment in mammals are the eyes. The information reaches the suprachiasmatic nucleus via nerves (the retinohypothalamic tract). The master clock control for certain rhythms is in the SCN, which regulates activity through nerves and chemicals.

The molecular basis of circadian clocks involves self-regulated feedback loops in which the products of one or more genes affect the activity of other genes. The pigment cryptochrome is important in bringing information about light to the clock of fruit flies but not to that of mammals.