Setting a Biological Stopwatch

The body's interval timer—a short-term timepiece separate from the better known circadian clock—used to be treated like a UFO, but the neurobiological evidence is getting harder to ignore

For male and female ring doves, which incubate their eggs in alternating shifts, domestic bliss is a matter of timing. Males arrive for their stint in the morning and give way to females when the mothers resume nesting duties in the late afternoon. But delay the male's arrival by a few hours in a lab experiment, and the team falls apart. The male refuses to leave, and the two act like a violent version of Ralph and Alice Kramden: pecking and kicking each other—because two biological clocks, the circadian and interval timer, are no longer in synch.

The conflict is between a sundial and a stopwatch. The circadian clock is reset every day by sunlight and governs such things as hormonal rhythms and sleep; it also tells a female dove that it's late in the day and time to get into the nest. The interval timing clock, on the other hand, gauges the passing of seconds or minutes and tells creatures how to time their movements when hunting or evading predators. It's also the clock that tells a male dove how long it's been sitting on its eggs. "Animals use time all the time, and they do it with these two clocks," explains John Gibbon, chief of biopsychology at the New York State Psychiatric Institute in New York City and—with Rae Silver of Barnard College—one of the scientists behind the doves' domestic discord. Yet while the circadian clock has been studied in great biological detail for decades (see box), the interval timer has been something of a black box. With no anatomical structures linked to it, some researchers have wondered whether such a timer really exists.

But this week at the annual meeting of the American Association for the Advancement of Science (AAAS, which publishes *Science*) in Baltimore, Maryland, Gibbon and his colleagues presented new neurological data locating the interval timing clock firmly in structures of the brain called striato-cortical loops. They also described clues about how the clock is ticking in humans as well as animals—and how it is impaired in Parkinson's disease patients, who suffer damage to some of these same brain regions.

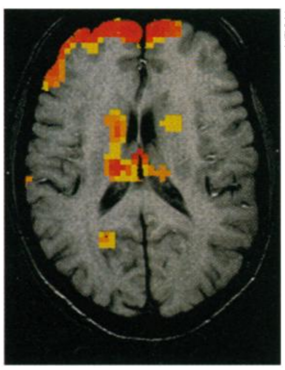
"This timing work is terribly important and interesting; it's going to open a lot of new doors," says Charles Stevens, a neurobiologist at the Salk Institute in La Jolla, California. It should give researchers "insights we don't have now into the brain's mechanisms for doing computations, and should demystify many animal behaviors, since an animal wouldn't work without this clock."

Shift change. With their internal clocks out of synch, doves fight over whose turn it is to mind the nest.

R. SILVER

The first clues pointing to an interval timer grew out of the conditioned learning studies started by B. F. Skinner in the 1930s. He and other researchers noticed that the animals in their experiments were extremely sensitive to the time intervals between food rewards. Such observations led to laboratory studies of birds, such as Gibbon's work on the doves, which support the idea that animals keep track of time. And work on starlings, presented at the AAAS meeting by Alex Kacelnik, a behavioral ecologist at Oxford University in the United Kingdom, has added finer details.

Kacelnik gave the starlings a choice between two colored keys. Each time they pecked at one key, they got a food pellet after a fixed time interval; the other key delivered the pellets after either a shorter or longer interval. Similar experiments varied the food amount. The pattern of pecks indicated that the birds could determine which key provided the best payoff: the most food over the shortest amount of time. "They divide the prey-size by the amount of time they waited for each prey, and then average these ratios," says Kacelnik. "This ... shows that the animals are capable of measuring time very accurately."



Clock watching. Functional MRI image of a human brain shows that the thalamus, caudate-putamen, and frontal cortex *(colored areas)* are active during an interval timing task.

S. HINTON

Looking for a watch

But while such research illustrates that animals can measure time, it doesn't explain how their stopwatch works. At the meeting, Warren Meck, a Duke University cognitive neuroscientist, revealed some of the clockworks. "You used to hear about the interval timing clock the way you hear reports about extraterrestrials," says Meck. " 'Does it actually exist as a structure?' And the answer is, 'Yes.' " That structure is in the basal ganglia, a region of the brain that coordinates voluntary muscle movements. Researchers suspected this part of the brain might play a role when Russell Church, an experimental psychologist at Brown University, and his students discovered in the 1980s that drugs that regulate the level of the neurotransmitter dopamine could either speed up or slow down an animal's sense of timing. "That suggested that you should be looking for neurons that secrete dopamine," says Meck. Such a population of neurons occurs in the substantia nigra, a structure with extensive neural inputs to the basal ganglia.

Together, Church, Gibbon, and Meck hypothesized that the clock would actually function like a stopwatch—or an hourglass. Thus, the substantia nigra serves as the hourglass's metaphorical reservoir of sand; the sand grains—pulses of dopamine—fall into an accumulator—the caudate-putamen, a major part of the basal ganglia. For an animal to read these pulses, they thought, the clock had to be connected to the cerebral cortex (that part of the higher brain that integrates perception, memory, and movement control). And the basal ganglia are, in fact, linked to the cortex in neural pathways known as striato-cortical loops. If these structures do make up the clock mechanism, damaging any part of the pathway should impair an animal's ability to measure time.

Meck set out to test this idea. He first trained rats to press a lever for food in response to signals of specific durations. Once they mastered that task, he lesioned the rats' caudate-putamen, substantia nigra, or frontal cortex. Lesions in the caudate-putamen and substantia nigra stopped the clock cold: The rats were no longer able to discriminate among the temporal signals they had previously learned. Lesions of the frontal cortex, on the other hand, made it impossible for the

Evolving Rhythms

While some researchers are beginning to tease out the physiology of the interval timing clock (see main text), others have spent the last 25 years identifying the pieces of the brain's light-sensitive timepiece, the circadian clock, which regulates such things as hormone and sleep cycles. And at the American Association for the Advancement of Science meeting, investigators reported insights into how and when those pieces fit together. In vertebrates, for instance, the whole clock system arose more than 450 million years ago and may have persisted because of some remarkable flexibility. Experiments in mammals highlight that flexibility: One clock piece, the suprachiasmatic nucleus (SCN), may communicate with the rest of the mammal brain in a novel way.

In vertebrates, the SCN is but one part of the so-called circadian axis; the other two components are the pineal gland (which produces the hormone melatonin) and the retina. But finding out when this interconnected clock started ticking isn't easy, because these soft tissues don't fossilize. So Michael Menaker, a neurobiologist at the University of Virginia, decided to look for the clock's origins in an existing primitive vertebrate, the lamprey, an eellike fish with a circular mouth and large eyes. "We hypothesized that because the last common ancestor of lampreys and all other vertebrates dates to 400 million years ago, then if lampreys and sparrows, a more modern vertebrate, have the same circadian system, that correspondence would indicate that the system was very old," he says.

Menaker and his colleague Gianluca Tosini compared the lampreys to birds and lizards. They grew pineal glands from all three groups of animals in culture to see if they rhythmically produce melatonin; they also made the animals arrhythmic by lesioning their SCN and pineal glands, and conducted other tests as well. The similar responses, Menaker says, indicate that the circadian axis is as old as the overall vertebrate lineage, which arose more than 400 million years ago. "It's unlikely that the similarities in biochemistry, morphology, physiology, and behavior would have arisen by convergent evolution," he says. "It seems more reasonable to think the system evolved once and has then been adapted to animals' specific needs."

ANIMALS ANIMALS

Ticking through time. Similarities between circadian clocks of the lamprey *(above)* and of birds indicate the mechanism is widespread in vertebrates, and thus very old.

One such adaptation is seen in the lamprey. While in many vertebrates the clock is always on, the lamprey switches its pineal gland on during its larval stage, turns it off during its subsequent parasitic stage, and then on again as the parasite turns into a spawning adult. "This shows just how easily and quickly the system can be modified" as animals adapt to changing circumstances, Menaker says, "and perhaps that high plasticity explains why it has endured."

In mammals, that plasticity is also evident. While the regulation of circadian rhythms is divided among the three parts of the circadian axis in other vertebrates, in mammals, the SCN serves as the master driver—and ongoing transplant studies may soon reveal how it communicates its directives. At the meeting, Rae Silver, a neuroscientist at Barnard College in New York, reported that she's been transplanting the SCN of fetal hamsters into adult hamsters whose nuclei had been removed. The transplanted SCNs don't seem to form many neuronal connections, yet the recipients recover at least part of their circadian rhythms. "People have assumed that it has to be a point-to-point connection for the SCN to work," says Silver, "but it's also possible that the SCN's cells are sending out a diffusible chemical signal that restores the clock's response."

She notes, however, that her research and that of Patricia Sollars at the University of Pennsylvania in Philadelphia have shown some neuronal regrowth, so to sort this out, Silver plans to place fetal SCNs in plastic capsules before transplanting them, making it impossible for the SCN to physically connect with the recipient's brain. "It's a brilliant, heroic effort," comments Gary Pickard, a neuroscientist at the University of Pennsylvania, although he doubts the results will be positive, as there is a lot of research pointing to the necessity of synaptic connections. "It's the ultimate way to address this question, but the results are going to have to be strong, since [the idea] goes against the grain of traditional brain research dogma."

–V.M.

rats to respond—with excitement or arousal—to events gauged by clock speed.

Gibbon has seen a similar phenomenon in his work with Parkinson's disease patients. People who suffer from this disease lose dopamine-producing neurons in the substantia nigra, and they also have trouble keeping track of time. "You can ask them to remember signals of 8 and 21 second durations," says Gibbon. "But if you ask them to reproduce the 8-second signal, they can't do it; they'll give you 10 seconds instead; and conversely, if you ask for 21 seconds, they'll give you 17." A control group was more accurate. But the drug L-DOPA, which increases the amount of dopamine in the brain, can restore time to Parkinson's patients, Gibbon found. And Meck found a similar drug effect in rats that had lesions in their substantia nigra. "That makes sense because the substantia nigra generates the pulses of dopamine, which are needed for timing," says Meck.

Additional support for the resemblance of the human and animal clocks came when Meck made functional magnetic resonance images (fMRI) of healthy people performing specific interval-timing tasks. Meck isolated the striato-cortical loops in these images as the circuit involved in time perception. "Overall, that's one of the most striking things to us," says Meck, "the surprising similarity between what we've seen in the animals and what we're seeing in humans."

Clocks and memories

The next question for studies of interval timing, say Gibbon and his colleague Randy Gallistel, a behavioral neuroscientist at the University of California, Los Angeles, is how animals and people remember the time intervals that their clock measures. "It's actually what permits classical Pavlovian conditioning," says Gibbon. Pavlov's dog, he and Gallistel argue, salivated when it heard a bell because it had learned the interval between the ringing and the food. "What all this [interval timing] research shows is that animals are storing the value of variables; that they know how long an interval has lasted and they are writing that to memory, in much the same way that a computer does," says Gallistel. "What remains to be found is the cellular or molecular mechanism" in the brain that allows that memory to be encoded and later retrieved. And that discovery, researchers say, is only a matter of time.

–Virginia Morell