Circadian clock may be critical for remembering what you learn

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Without circadian rhythms, Siberian hamsters did not recognize things they had investigated before. Courtesy of Indiana University

(PhysOrg.com) -- The circadian rhythm that quietly pulses inside us all, guiding our daily cycle from sleep to wakefulness and back to sleep again, may be doing much more than just that simple metronomic task, according to Stanford researchers.

Working with Siberian hamsters, biologist Norman Ruby has shown that having a functioning circadian system is critical to the hamsters' ability to remember what they have learned. Without it, he said, "They can't remember anything."

Though not known for their academic prowess, Siberian hamsters nonetheless normally develop what amounts to street smarts about their environment, as do all animals. But hamsters whose circadian system was disabled by a new technique Ruby and his colleagues developed consistently failed to demonstrate the same evidence of remembering their environment as hamsters with normally functioning circadian systems.

Until now, it has never been shown that the circadian system is crucial to learning and memory. The finding has implications for diseases that include problems with learning or memory deficits, such as Down syndrome or Alzheimer's disease. The work is described in a paper published Oct. 1 online in the early edition of the *Proceedings of the National Academy of Sciences*. Ruby is lead author on the paper. Siberian hamsters, also known as dwarf hamsters, are about the size of a mouse.

The change in learning retention appears to hinge on the amount of a neurochemical called GABA, which acts to inhibit brain activity. All mammal brains function according to the balance between neurochemicals that excite the brain and those that calm it. The circadian clock controls the daily cycle of sleep and wakefulness by inhibiting different parts of the brain by releasing GABA.

But if the hippocampus - the part of the brain where memories are stored - is overly inhibited, then the circuits responsible for memory storage don't function properly. "Those circuits need to be excited to strengthen and encode the memories at a molecular level," Ruby said.

"What I thought was happening was that our animals were having chronically high levels of GABA because they had lost their circadian rhythm," Ruby said. "So instead of rhythmic GABA, it is just constant

GABA output."

To test that idea, Ruby and his colleagues gave the circadian-deficient hamsters a GABA antagonist called pentylenetetrazole, or PTZ, which blocks GABA from binding to synapses, thereby allowing the synapses to continue firing and keeping the brain in a more excited state. It worked. The learning-impaired hamsters caught up with their intact peers to exhibit the same level of learning retention.

Research on people with Down syndrome has shown that one reason they don't perform well on cognitive tests is that they grow up with what amounts to an over-inhibited brain. Studies on mice that exhibit symptoms of Down syndrome have demonstrated that when given PTZ, the mice demonstrate improved learning and memory. That research, conducted by Fabian Fernandez, then a graduate student in the lab of Craig Garner, a professor of psychiatry and behavioral sciences at Stanford, prompted Ruby to investigate whether using PTZ to reduce GABA levels would improve memory function in the hamsters.

Other researchers working with mouse models of Alzheimer's disease have reported similar findings. When those mice were given GABA antagonists, their ability to learn was restored, suggesting a possible link with their circadian system.

Ruby's findings may also have implications for the decline in memory function that older adults in general experience.

"In aging humans, one of the big things that happens is the circadian system starts to degrade and break down," Ruby said. "When you get older, of course, a lot of things break down, but if the circadian system is a player in memory function, it might be that the degradation of circadian rhythms in elderly people may contribute to their short-term memory problems," he said. "There are a lot of things that could cause memory to fail, but the idea would be that in terms of developing therapeutic treatments, here is a new angle.

"This is also important because it is one of the first lines of evidence that shows losing your circadian timing actually does cost you something," Ruby said. "It makes it hard to learn things. And the underlying mechanism is that you have too much GABA."

Ruby said researchers have known since the early '70s that the circadian system modulates learning in humans and other animals, but no one knew what the effect would be on learning if the system was completely wiped out. Laboratory animals-rats, mice and hamsters-whose circadian systems have been disabled as part of a study typically live long and healthy lives.

"We thought it might be possible to wipe out circadian rhythms and eliminate the rhythm in learning, but that the animals could still learn something," Ruby said. "But they don't. That is what was so surprising. They actually can't remember anything. Losing their rhythms costs them a lot."

The researchers knocked the hamsters' circadian systems out of commission using a new noninvasive technique they developed involving manipulating the hamsters' exposure to light. The hamsters were first exposed to two hours of bright light late at night. Then the next day the researchers delayed the usual light/dark cycle by three hours. "It is like sending them west three time zones," Ruby said.

After the treatment, the normal light/dark cycle is resumed, but that one-time treatment is enough to wipe out their circadian system.

To assess the effect of the treatment, Ruby's team conducted a standard test called a novel object recognition task that takes advantage of animals' innate tendency to explore their environment. Using a box roughly 2 feet square, the researchers put two identical objects in adjacent corners, such as two saltshakers or two shot glasses. The hamster is then placed in the box, on the opposite side from the objects. As it explores the box and the objects, the hamster spends approximately equal amounts of time on each of the two identical objects.

After 5 minutes, the hamster is removed from the box, and one of the objects is replaced with a new, different object. After a span of time-in Ruby's study, the time was varied between 20 minutes and an hour-the hamster is put back in the box.

"A normal animal will spend time with both objects, but it will spend easily twice as much time with the new one," Ruby said. "It understands that it has seen the other one before."

But when a hamster that lacks circadian rhythms is put back in the box, it's as if it is a whole new world for the hamster. Whether the hamster is out of the box for an hour or as short a time as 20 minutes, it spends the same amount of time with each object, Ruby said.

"What that means is they don't remember the object that was in there before," he said.

The finding is even more striking when you consider that when a hamster loses its circadian system, it gets even more sleep than usual.

"What our data are showing is that these animals still performed terribly on a simple learning task, even though they're getting loads of sleep," Ruby said. "What this says is that the circadian system really is necessary for something that is deeply important: learning."

Provided by Stanford University