SOUTHWESTERN NEWS

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Brain-mapping technique aids understanding of sleep, wakefulness

DALLAS – April 21, 2005 – The power of a new technique to map connections among nerve cells in the brain has a UT Southwestern Medical Center scientist dreaming of solving the mysteries of sleep.

By tracking which nerve cells in the mouse brain stimulate others, researchers in Japan and at UT Southwestern found that a type of neuron responsible for keeping animals awake receives inhibitory signals from neurons active only during sleep, as well as reinforcing, positive signals from nerve cells that are very active during wakefulness.

The findings, available online and appearing in today's issue of the journal *Neuron*, shed light on the complex mechanisms involved in sleep regulation and may help to explain why once a person wakes up and moves around, he tends to stay awake.

"We all know subjectively and objectively that there is a very strong force regulating sleep, but there is very little knowledge about the actual biological mechanism controlling sleep," said Dr. Masashi Yanagisawa, professor of molecular genetics at UT Southwestern and senior author of the study. "Eventually my dream is to elucidate all the pathways regulating sleep."

Dr. Yanagisawa and his colleagues focused on neurons in the brain that produce the protein orexin, which helps keep animals awake. In humans, a lack of, or deficiency in, orexin causes narcolepsy, a rare disease in which people uncontrollably fall asleep, have excessive daytime sleepiness and experience sudden muscle weakness called cataplexy.

Because orexin-producing neurons play such a key role in regulating sleep, determining how they are connected to other neurons in the brain is an important step toward understanding how and why we sleep.

Mapping the neurons to which orexin neurons send signals has been relatively easy, Dr. Yanagisawa said, but determining which neurons send signals to orexin neurons has been a challenge. A brain-mapping technique developed recently by French scientists provided Dr. Yanagisawa and his research group new hope for navigating the neural network.

The technique involves genetically engineering mice to produce a "tracer" protein only in a certain population of neurons. The tracer, a nontoxic fragment of the tetanus toxin, transfers itself from one neuron in retrograde fashion to neurons "upstream" – those from which the tracer-producing neuron receives signals. Researchers then track the tracer to map the upstream neurons.

Dr. Yanagisawa and his colleagues are the first to apply the technique in order to study a specific neural pathway. They introduced the tracer into mice so that it was expressed only in orexin neurons, which are found in a part of the brain called the lateral hypothalamus. The researchers found that some

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of the upstream neurons in the anterior hypothalamus, known to be active only during sleep, send inhibitory signals to orexin neurons preventing them from releasing orexin.

"Neurons producing orexin keep you awake, and they also stabilize wakefulness," Dr. Yanagisawa said. "In order to fall sleep, you somehow must inhibit these orexin neurons. It appears that sleep-active neurons in a specific region of the brain are doing just that, keeping the animals asleep."

The researchers also found that during wakefulness, orexin neurons activate cells in other parts of the brain that negatively feed back to the sleep-active neurons in the anterior hypothalamus, keeping them from becoming active.

"This makes perfect sense," said Dr. Yanagisawa, a Howard Hughes Medical Institute investigator at UT Southwestern. "When one group of neurons is active, the other group must be inactive, and vice versa. It's a seesaw, or flip-flop, mechanism. That's important because you don't want to be half-asleep. You want to be either completely awake or completely asleep."

Another finding from the *Neuron* study may help explain why once you're awake and moving around, you tend to stay awake, a process called activity induced consolidation of wakefulness.

Some neurons in the basal forebrain produce the chemical acetylcholine. These cells are very active during wakefulness when an animal or human is aroused and attentive. Previous studies have shown that orexin neurons project to these cells, sending positive signals downstream to help keep them active. The new study, however, shows that these acetylcholine-producing neurons, or cholinergic cells, are upstream of the orexin neurons as well – the two send positive, reinforcing signals back and forth.

"By being active, animals fire up their cholinergic cells, which feed back to orexin neurons positively, and orexin neurons feed back to cholinergic cells," Dr. Yanagisawa said. "This creates a self-reinforcing loop, a positive feedback system that's very important to consolidating wakefulness."

Dr. Yanagisawa said the trigger that causes the sleep "seesaw" to tip from one side to the other and maintain a proper balance between time spent awake and time spent asleep is still a big mystery in brain science. By shedding light on the network of neural connections involved in sleep, he said, his and other studies will help unravel the mechanisms involved in sleep homoeostasis.

In addition to his laboratory at UT Southwestern, Dr. Yanagisawa also directs the ERATO Yanagisawa Orphan Receptor Project in Japan, where these studies were carried out. He and his colleague, Dr. Takeshi Sakurai of the University of Tsukuba, lead author on the study, discovered orexin in 1998 when Dr. Sakurai was a postdoctoral researcher in Dr. Yanagisawa's UT Southwestern lab.

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