Negative-Stiffness Vibration Isolation Facilitates Neuronal Research into Animal Learning and Memory

The technology is capable of attenuating vibrations down to 0.7 Hz.

University of Texas
Austin, TX

CLM's JEOL 1400-transmission electron microscope positioned on negative-stiffness isolators.

Micro- and nano-level microscopy, whether used in academic laboratories or industry, is susceptible to vibrations from the environment, requiring these instruments to employ vibration isolation systems. When measuring a very few angstroms, nanometers, or microns of displacement, an absolutely stable surface must be maintained to support the instrument. Any vibrations that are transferred into the mechanical structure of the instrument will cause vertical and horizontal noise, compromising data sets and limiting the ability to measure high-resolution features.

Traditionally, air tables have been the isolators used for microscopy equipment. The ubiquitous passive-system air tables, adequate up until a decade ago, are now being seriously challenged by the need for more refined imaging requirements. Air systems provide limited isolation vertically and very little isolation horizontally. Yet, high-resolution microscopy demands vibration isolation requirements that are unparalleled in both the vertical and horizontal axes. This has posed a significant challenge for many researchers.

Center for Learning and Memory

Such was the case with the Center for Learning and Memory (CLM), part of the Department of Neuroscience at the University of Texas at Austin, a multidisciplinary group studying the mechanisms governing the processes of learning and memory in animals.

Research in one of the CLM laboratories is primarily directed to understanding the cellular and molecular mechanisms of synaptic integration and long-term plasticity of neurons in the animal medial temporal lobe. The lab focuses attention on the hippocampus, subiculum, and prefrontal cortex areas of the brain that play important roles in learning and memory. These regions are also of interest because they have a low seizure threshold and are implicated in several forms of human epilepsy.

Neurons are electrically excitable cells that process and transmit information through electrical and chemical signals in a process known as neurotransmission, also called synaptic transmission. The fundamental process that triggers the release of neurotransmitters is the action potential, a propagating electrical signal that is generated by exploiting the electrically excitable membrane of the neuron.

These signals between neurons occur via synapses, specialized connections with other cells. Transmitter-activated channels mediate conduction across the synapses, and underlying the nerve impulses are ion channels. These are pore-forming membrane proteins whose functions include establishing a resting membrane potential, shaping action potentials and other electrical signals by gating the flow of ions across the cell membrane. They are often described as narrow, water-filled tunnels that allow only ions of a certain size and/or charge to pass through, called selective permeability.

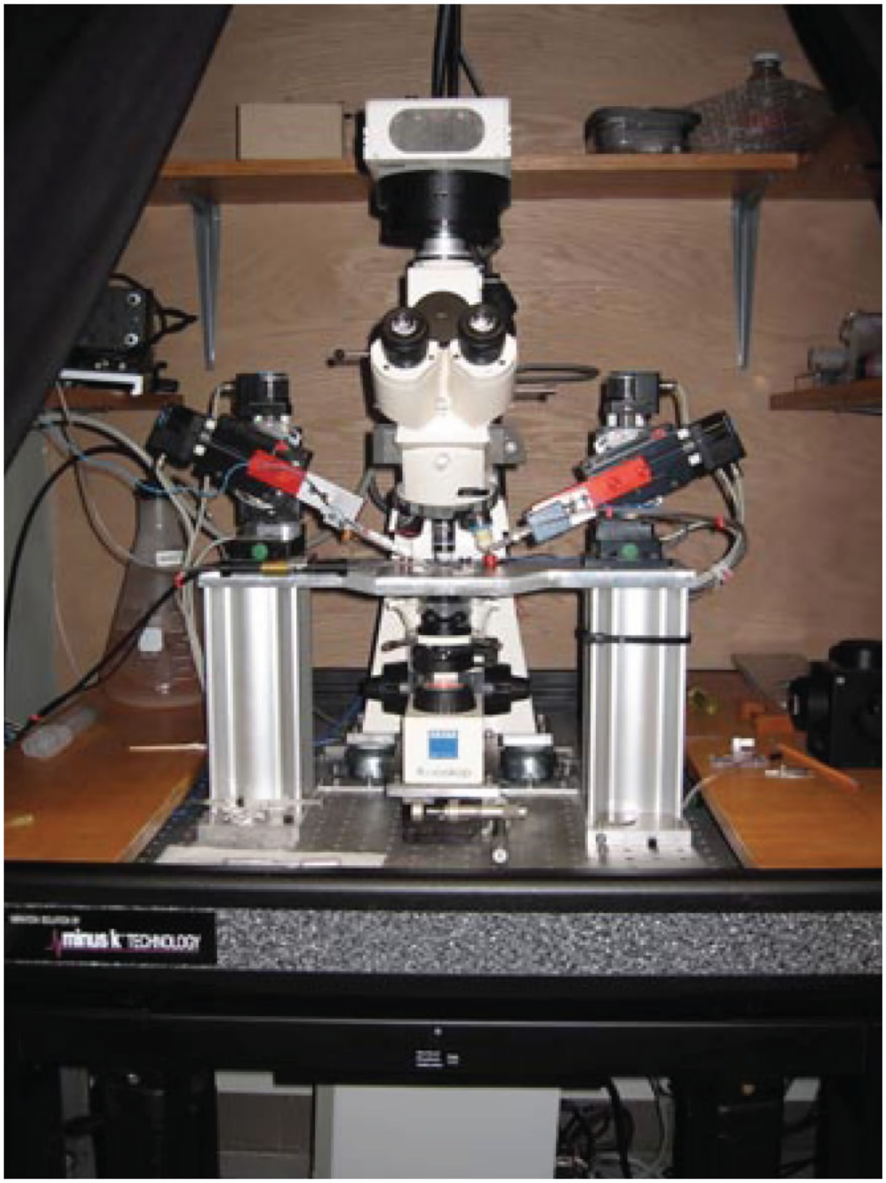
A typical neuron possesses a cell body (soma), dendrites, and an axon. Dendrites are thin structures that arise from the cell body, often extending for hundreds of microns and branching multiple times, giving rise to a complex dendritic tree. An axon is a special cellular extension that arises from the cell body and travels for a distance, as far as one meter in humans. The cell body of a neuron frequently gives rise to multiple dendrites.

One team at CLM employs quantitative electrophysiological, optical imaging, and computer modeling techniques to study how synapses, and the dendrites they contact, change during learning.

The CLM team uses high-resolution light microscopy to identify and study dendrites that are on the order of 1–2 μm in diameter. A glass needle electrode (called a *patch electrode*) is positioned under the light microscope to make contact with a dendritic branch. Any significant vibrations would make this impossible.

Vibration Problem

"We had always used fairly traditional air tables to dampen vibrations," says Daniel Johnston, PhD, director of CLM at the University of Texas at Austin. "But when we moved into [our current]



One of CLM's 13 high-resolution microscopy and micro-manipulator workstations, located on a negative-stiffness vibration isolator.

building, on the fourth floor, we found that the vibrations were too severe to perform our work using the traditional air isolation table."

"The vibration frequencies were very low, and that was the problem" says Johnston. "The sway of the building, air conditioning blowers — these were some of the causes of the low-frequency vibrations. Air tables do better at isolating the higher frequencies than they do the lower frequencies. We are working with real-time electrical measurements on a very small scale, so vibrations were a big problem for us, as they would be for anybody in this field."

This CLM team's research involves making brain slices from rodents, the cells of which, and their cell connections, are kept alive for approximately 12 hours in a preparation. Electrical stimulation is then applied to an individual neuronal cell to mimic the kinds of patterns and activity that might occur in the animal during a behavioral task.

Recordings are taken from single ion channels along the cell's dendrite tree. Each neuron connects to 10,000–20,000 other neurons, and every time a behavioral event occurs some pattern of neurons become active. The electrical changes that take place in a single neuron, as relating to learning and behavioral patterns, are then studied and recorded in detail.

The neuronal stimulation is done with a probe, a tiny glass needle, connected to a stimulation device that outputs electrical pulses. The needle is attached to a micro-manipulator, which is a motorized mechanical device that can make very fine movements advancing the needle into a cell. With high-powered light microscopy, both the needle and the cell can be viewed, so that the needle can make precise contact with the cell. The micro-manipulator is attached to the table that the microscope is sitting upon. Any vibrations that come in from the building get magnified through the micro-manipulator and the protruding needle.

"Imagine having a brain slice of hundreds of thousands of neurons, magnified to a micron level, then bringing in a small electrical probe to touch one part of that cell," explains Johnston. "Vibration would have to be at an absolute minimum, otherwise the probe would be vibrating too much, far more than the diameter of the dendrite we are trying to touch. Without extreme measures to suppress these ambient vibrations, we would have no hope of making these micro-measurements."

Vibration Isolation Solution

The vibration isolation solution selected by this CLM team was negative-stiffness vibration isolation, engineered by Minus K Technology. Because of its very high vibration isolation efficiencies, particularly at the low frequencies, negative-stiffness vibration isolation systems enable vibration-sensitive instruments to operate in severe low-frequency vibration environments that would not be practical with top-performance air tables and other vibration-mitigation technologies.

The CLM team also tested an active electronic force cancellation workstation, but chose the negative-stiffness systems because of their superior performance. Thirteen negative-stiffness Model MK26 workstations were installed in the CLM laboratory to support both the microscopes and the micro-manipulators.

Transmission Electron Microscope

Another CLM research group uses a JEOL-1400 transmission electron microscope (TEM) to identify and study functional units smaller than 250 nm within nerve cells. Transmission electron microscopy is a technique in which a beam of electrons is transmitted through an ultra-thin specimen, interacting with the specimen as it passes through. An image is formed from the interaction of the electrons transmitted through the specimen. The image is magnified and focused onto an imaging device, such as a fluorescent screen, a layer of photographic film, or to be detected by a sensor such as a CCD camera. TEMs are capable of imaging at a significantly higher resolution than light microscopes. This enables the instrument's user to examine fine detail — even as small as a single column of atoms.

"This was a very difficult vibration isolation problem," says David Platus, PhD, president of Minus K Technology. "The floor vibrations in the horizontal (X/Y vectors) were out of the manufacturer's specifications in the very low frequency range of about 1.5–2.5 Hz. A 0.4 Hz custom negative-stiffness vibration isolation floor platform was designed for CLM that brought the TEM vibrations well into the required vibration specifications."

Negative-Stiffness Isolators

Negative-stiffness isolators employ a unique and completely mechanical concept in low-frequency vibration isolation. They do not require electricity or compressed air. There are no motors, pumps, or chambers, and no maintenance because there is nothing to wear out. They operate purely in a passive mechanical mode.

"In this vibration isolation system, vertical-motion isolation is provided by a stiff spring that supports a weight load, combined with a negative-stiffness mechanism," explains Platus. "The net vertical stiffness is made very low without affecting the static load-supporting capability of the spring. Beam-columns connected in series with the vertical-motion isolator provide horizontal-motion isolation. A beam-column behaves as a spring combined with a negative-stiffness mechanism."

The isolator provides 0.5 Hz isolation performance vertical and horizontal, using a totally passive mechanical system — no air or electricity required. Note that for an isolation system with a 0.5 Hz natural frequency, isolation begins at about 0.7 Hz and improves with increase in the vibration frequency. The natural frequency is more commonly used to describe the system performance.

Negative-stiffness isolators resonate at 0.5 Hz. At this frequency there is almost no energy present. It would be very unusual to find a significant vibration at 0.5 Hz. Vibrations with frequencies above 0.7 Hz (where negative-stiffness isolators begin isolating) are rapidly attenuated with increase in frequency.

Transmissibility with negative-stiffness isolators is substantially improved over air systems. Transmissibility is a measure of the vibrations that are transmitted through the isolator relative to the input vibrations.

The negative-stiffness isolators, when adjusted to 0.5 Hz, achieve 93 percent isolation efficiency at 2 Hz; 99 percent at 5 Hz; and 99.7 percent at 10 Hz. Negative-stiffness isolators deliver very high performance, as measured by a transmissibility curve.

"The negative-stiffness vibration isolation system turned out to be a lifesaver for our research," says Johnston.

This article was written by Jim McMahon, who writes on advancements in instrumentation technology. For more information, contact Dr. Daniel Johnston, PhD, Director, Center for Learning and Memory, University of Texas at Austin, ***djohnston@mail.clm.utexas.edu.***