Laser Focus World – January 2024

Negative-Stiffness Vibration Isolation Improves Patch Clamping in Electrophysiology Research

Unique vibration isolation solution improves quality of microscopy data sets, and preserves and protects glass micropipettes in electrophysiological research at the McCloskey Laboratory, College of Staten Island, City University of New York.

By Jim McMahon

The McCloskey Laboratory's research at the College of Staten Island, City University of New York (CUNY) is focused on understanding the ways that neurons in the naked mole-rat brain participate in networks to produce complex behaviors such as learning and memory, as well as to examine the effect of hypoxia in brain cells. The Lab uses electrophysiological tools to monitor neuron function, from the level of the isolated single cell to the whole brain. Its methods range from patch clamp measures on cultured neurons through single- and multi-ion channel recording of acutely isolated brain regions, to electroencephalograms (EEGs) in awake and moving animals.

Using low-powered USB/computer-connected microscopy for targeting and patch clamping, and higher powered confocal microscopy for imaging, the McCloskey Laboratory has relied upon air tables for vibration isolation, a critical component for micron-level neuronal electrophysiological microscopy. But the Lab's air table vibration isolation has had limitations, particularly isolating vibrations at lower frequencies below 5 Hz, caused by the building's HVAC system, closing of doors, and researchers bumping into tables and equipment. This has introduced excessive noise into data, and at times interrupted research to replace fragile micron-scale glass electrodes which were damaged by vibration.

Air systems provide limited isolation vertically and very little isolation horizontally. Yet, electrophysiology microscopy demands vibration isolation requirements that are able to isolate very low-frequency vibrations in both the vertical and horizontal axes.

Targeting Neurons

"Electrophysiology is very sensitive," said Michael Zions, a researcher and doctoral candidate with the McCloskey Laboratory. "Our research involves making thin brain slices, about 300 microns in thickness, from the hippocampus of naked mole-rats, which we then keep alive in a bath of artificial cerebral spinal fluid. Once the sample is in the fluid, we now have a complete neuronal network that can pass signals along intact neurons. But these slices are also extremely delicate. We then insert a glass micropipette into a singular neuronal cell, or line up alongside one, and electrical stimulation is applied to mimic normal signaling or isolate a particular feature of the cell. A multichannel electrode array can also be applied to the slice, to provide an overview of the organization and to track signal propagation through the network."

"We are working with real-time electrical measurements on a very small scale with delicate cells and fragile glass instruments, so vibrations are a big problem for us," continued Zions. "If the pipette shifts just a little bit, it will at best miss the target. Often the electrode just shatters instead, spoiling that area and compromising the sample. We can replace an electrode, but it takes time and the samples are only viable for a short while. Limiting vibration reduces our vulnerability to disruption."

Crashing Micropipette Electrodes

The micropipette electrode is inserted into the neuron to a depth of 75 to 100 microns from the top surface, leaving approximately 200 microns of space to the bottom of the dish, which is holding the brain specimen in the cerebral spinal fluid. Once embedded, sideways shear can crack the electrode, and vertical shock will crash it into the floor of the chamber.

A crashed single electrode takes fifteen minutes to replace. If the electrode was silylated or specially drawn, the project might be done for the day, and the hippocampal slices will need to be replaced for the next experiment. This adds hours and cost. And if the crashed electrode was a multichannel array, the delay stretches to weeks and several thousand dollars. Unfortunately, this became such a common event that a more effective answer needed to be found.

"We needed a vibration isolation solution that was compact, not electrically noisy, and could contend with challenges along any axis and across broad frequencies in the damaging ranges we had encountered" explained Zions. "Ideally, we could just swap it in for our existing air table."

Negative-Stiffness Vibration Isolation

The vibration isolation solution selected by the McCloskey Laboratory was Negative-Stiffness vibration isolation, engineered by Minus K Technology. Because of its very high vibration isolation efficiencies, particularly in 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-isolation technologies.

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," explained Vice President of Engineer of Minus K Technology. "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. *(*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 approximately 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.

Accelerated Analytics

"The McCloskey Laboratory employs a mix of electrophysiological, optical-imaging and computer-modeling techniques to conduct our research," Said Zions. "We track our animals' movement and behavior in their colony housing, and model and interpret social results at a high-performance computing center, then investigate neural correlates in the wet lab."

"Using microscope imaging and immunohistochemistry, the Lab can quantify structures of interest in the study of the mole-rats' social behavior and epilepsy," continued Zions. "But each particular hippocampal preparation matters and we cannot afford to replace unique samples, or spend time endlessly replacing delicate sensors."

"Our recordings are significantly cleaner now," added Zions. "We have eliminated all of the artifacts that we were experiencing prior. We have also stopped breaking pipettes because of vibration-related causes. This has been a huge cost savings and time savings for the Lab."

About the McCloskey Laboratory

The McCloskey Laboratory's research at the College of Staten Island is focused on understanding the ways that neurons in the naked mole-rat brain participate in networks to produce complex behaviors such as learning and memory.

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