Hippocampal calcium imaging in rats with nVista™

**Introduction**

The hippocampus, a large, bilateral brain structure of the mammalian species, forms part of the limbic system, and plays a critical role in numerous functions underlying memory formation, cognition and emotion\(^1\text{-}^3\). In rats, the hippocampus has been extensively studied as a model system for spatial circuit responses of place cell activity\(^1\), long-term potentiation as a model for neural plasticity\(^4\), and theta oscillations underlying arousal state control\(^5\). Because the hippocampus is thought to play a central role in memory, Alzheimer’s and other age-related dementias are of great interest to researchers seeking to understand neural circuit dysfunction caused by hippocampal deterioration. With the advent of new viral vectors enabling successful expression of calcium indicators in rat brain\(^6\), it’s now possible to selectively target neurons for calcium imaging. These novel viral tools, used in combination with our miniaturized imaging system nVista™ and an active commutator to facilitate free behavior in the rat, enable scientists to address novel neural circuit questions relevant to hippocampal-driven behaviors.

**In vivo Ca\(^{2+}\) imaging during freely moving behavior**

*In vivo* calcium imaging allows you to capture neural activity information at single cell resolution within specified cell types. nVista imaging enables new ways of studying spatial coding, ensemble dynamics during learning and memory, and drug effects on hippocampal physiology, in diverse behavioral paradigms\(^7\text{-}^9,^{10}\). The nVista system can now be combined with the Inscopix commutator (*Figure 1*) to more efficiently capture Ca\(^{2+}\) signals repeatedly from large-scale neuronal populations with minimal supervision during active rodent behavior.

**nVista materials and accessories**

*In vivo* Ca\(^{2+}\) imaging can be achieved in many brain regions of awake behaving rats when the nVista system is paired with an implantable lens probe. We now offer a 13 mm lens probe designed specifically for targeting deep brain regions in rats (a variety of sizes are available).

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*Figure 1.* The nVista imaging system (top) is composed of a miniaturized fluorescent microscope, data acquisition box, nVista acquisition and data processing software, and hardware accessories (not shown). The Inscopix commutator (bottom) is designed to facilitate high-speed video transmission in freely-moving animals, minimizing the need to supervise your imaging sessions. Images not to scale.
This Application Highlight will outline surgical methods (Figure 2) and data acquisition procedures using the Inscopix commutator along with results collected using the nVista Ca$^{2+}$ imaging system in the CA1 hippocampus of freely behaving rats. Some additional materials needed:

- Stereotax with manipulator
- Microinjection pump
- Approved rodent survival surgery tools and anesthetics
- Skull screws
- Dummy microscope
- GCaMP virus
- Wildtype rat
- Dental cement or cyanoacrylate adhesive
- Data cable sheath & application tool
- Inscopix data acquisition and data processing softwares

* ProView lens probe selection dependent on target brain region depth, and single or multi-layer imaging; 5 lenses included with nVista system purchase.

**General experimental workflow**

**A. Viral Injection: label neurons in target brain region with Ca$^{2+}$ indicator**
1. Obtain the optimal virus, brain coordinates, volume and injection rate to label cell type of interest. Confirm viral expression and location with histology.
2. Prepare animal for standard survival surgery procedures.
3. Inject virus into target brain region with the aid of a stereotax and micropump.
4. Suture skin and conduct postoperative recovery procedures.
5. Wait approximately 1-2 weeks for viral expression.

**B. Enable optical access: implant the lens probe**
1. Sterilize the lens probe and prepare animal for standard survival stereotaxic surgery.
2. Open a craniotomy large enough to permit lens probe access. Install 3-6 skull screws.
3. Use the stereotax manipulator arm to securely hold the lens probe using a ProView holder; slowly lower the probe through the opening into the brain until the desired depth is reached. Aspiration or a guide needle tract may be needed depending on the target brain region.
4. Affix lens probe and screws securely to the skull by evenly coating with metabond.
5. Build headcap with dental acrylic to protect the lens probe and implant site. Cover the lens top with Kwik cast to protect and keep it clean.
6. Return the animal to a clean home cage. Administer postoperative analgesics (per your institution’s guidelines) and allow for 1-2 weeks of recovery.

**C. Install nVista: securing the baseplate docking system**
1. Prepare animal for standard stereotaxic surgery (note: this step is not an invasive procedure).
2. Use stereotax and gripper arm to hold the nVista microscope with attached baseplate above implanted lens probe imaging face. Ensure the objective lens and the lens probe imaging face are parallel.
3. Connect the nVista system to a computer, and start the Inscopix Data Acquisition Software. Turn on the nVista microscope LED.

*Figure 2.* Schematic illustrating abbreviated workflow methods for implanting a lens probe and installing nVista.
4. Observe brain tissue using Inscopix Data Acquisition Software while advancing the nVista objective towards the imaging face of the lens via the stereotax. When tissue is in focus through the lens probe, the nVista microscope is at the proper location for optimal focus.

5. Use adhesive to cement the baseplate at this optimal location. Carefully adhere the baseplate only (not the microscope).

6. Once the adhesive and baseplate are securely fixed to the skull, remove the nVista microscope, and put on a baseplate cover.

7. Return the rat to its homecage for recovery from anesthesia.

**D. Acquire in vivo Ca^{2+} imaging data**

1. Mount the Inscopix commutator over your behavioral arena (exact placement of commutator is dependent on experimental design and laboratory setup).

2. Connect the commutator cable to the DAQ box.

3. Plug the DAQ box into your power supply and turn on.

4. Apply the cable sheath to microscope cable ensuring that it fully protects the cable nearest to the microscope, and plug in the microscope to the commutator connector labeled “microscope”.

5. Adjust the length of the microscope cable to provide free movement of the animal in the arena. Wrap excess length of microscope cable around the cable harness of the commutator, clip cable to set final length; adjust slack as needed (Figure 3).

6. Briefly awake restrain the animal and replace the baseplate cover with the microscope. Place animal in the behavioral arena.

7. Turn the commutator on to “Automatic” mode using the Inscopix Data Acquisition Software (Figure 4), allowing the commutator to react to animal’s movement.

8. Allow the animal to recover briefly from handling, and begin your imaging experiment.

**Abbreviated from detailed experimental and surgical methods in accordance with institution’s guidelines.**

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**Figure 3.** Awake behaving rat in an open field environment wearing the nVista microscope and connected to the Inscopix Commutator and DAQ box.

**Figure 4.** Panel shows the Inscopix Acquisition Software settings under Configure and Record Devices, where either Automatic Mode or Manual Mode is selected to enable how the Commutator communicates with the nVista system.
Results
The animal was expressing GCaMP6f and imaged while freely moving in an open arena and foraging for food. Various straight and prism lens probes are available to target different regions of the rat brain, with the capability of either imaging single layers of neurons as imaged in CA1 hippocampus, or obtaining data from multiple layers simultaneously (e.g. cortex). The same cell population can be imaged repeatedly for up to several weeks, based on the preparation and specific experimental protocol. This allows for within- and between-cohort comparisons of neuronal activity data over days.

Data Analysis
After image acquisition, nVista files can be imported into the Inscopix Data Processing Software, a specialized image processing software platform designed to preprocess the imaging data along with PCA-ICA analysis to detect cellular signals and extract Ca\(^{2+}\) dynamics of individual cells. The software’s intuitive workflow allows you to process your nVista datasets in a straightforward manner, where steps such as correcting for brain motion, calculating ∆F/F, denoising images, and overlaying multiple days of datasets (longitudinal registration) and other such manipulations are easily managed without scripting expertise. Figure 5A illustrates a map of neurons identified through PCA-ICA from representative rat hippocampal CA1 movies acquired with nVista. Traces of highlighted neurons, following cell extraction with Inscopix Data Processing Software are shown in panel B below.

Discussion
The ability to conduct neuronal population studies using rigorous and biologically relevant behavioral paradigms in behaviorally powerful animal models is vital to gaining a better understanding of brain function in health and disease.

The newest nVista platform combined with the Inscopix commutator expands experimental horizons and allows scientists to ask exciting new questions about how the brain works in an important and well-established animal model.

References