Deep brain imaging with nVista™

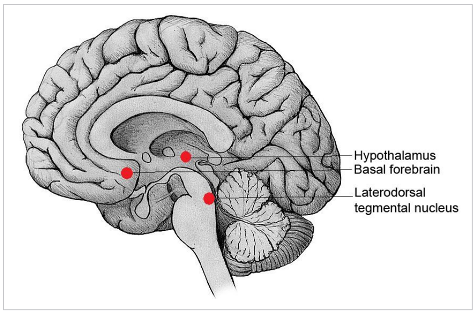

Figure 1. Lateral view of human brain showing deep brain regions in red.

Introduction

Deep brain regions like the hypothalamus (LH), basal forebrain (BF) and laterodorsal tegmental nucleus (LDT) regulate multiple essential physiological functions such as emotion, reward, energy homeostasis and arousal state; and impairments to these areas often result in cognitive deficits[1-3]. These nuclei are deep within the brain (*Figure 1*) and contain a diversity of cell types. Imaging tools to access these structures in a cell type-specific manner will greatly empower studies designed to elucidate their neural circuitry and behavioral function(s).

In vivo Ca^{2+} imaging during freely moving behavior

In vivo Ca^{2+} imaging allows scientists to capture neural activity at single cell resolution within specified cell types. nVista microscopy is a practical way to conduct Ca^{2+} imaging during active behavior, and capture Ca^{2+} dynamics repeatedly over time from the same neural populations during various behaviors. Several *in vivo* Ca^{2+} imaging studies[5-8] of deep brain regions (e.g. LH, BF and LDT) have been published using the Inscopix nVista system. nVista imaging provides new ways to study spatial coding, ensemble dynamics during active behavior as well as assess drug effects on brain function and behavior.

nVista materials and supplies

Figure 2. nVista miniaturized microscope and data cable with data acquisition (DAQ) box.

The nVista imaging system (*Figure 2*) is composed of miniaturized fluorescence microscope, data acquisition box, Inscopix data acquisition and data processing softwares, and hardware accessories. *In vivo* imaging in deep brain regions in mouse can now be achieved when the nVista system is paired with our implantable lens probes[4-8]. Other materials needed:

- Stereotax with manipulator
- Microinjection pump
- Approved rodent survival surgery tools and anesthetics
- Dental cement or cyanoacrylate adhesive
- GCaMP virus
- Wildtype mice

In this application highlight, we will outline a brief synopsis of published methods and results showcasing nVista calcium imaging of the BF of freely behaving mice[6].

General experimental workflow*

A. Viral injection: target deep brain region of interest with Ca^{2+} indicator

1. Obtain the optimal virus, brain coordinates, volume and injection rate to label your 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.
3. Use the stereotax manipulator arm to securely hold the lens probe, with the imaging side facing the target brain region.
4. Slowly lower the lens probe through the craniotomy into the brain until the proper depth is reached. Aspiration may be required.
5. Use cyanoacrylate adhesive to fix the lens probe securely to the skull. Use dental cement to create a cranial cap to protect the lens probe and implant site. Ensure that the imaging face of the lens probe is clean and protected.
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.
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 mouse to its homecage for recovery from anesthesia.

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

1. Plug in the nVista system to the computer. Ensure there is plenty of memory and data storage space.
2. Briefly anesthetize or awake restrain the animal, remove the baseplate cover, and attach the nVista microscope.
3. Place animal with attached nVista microscope in the behavioral arena of choice. Allow the animal to recover briefly from anesthesia or handling, and begin experiment!

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

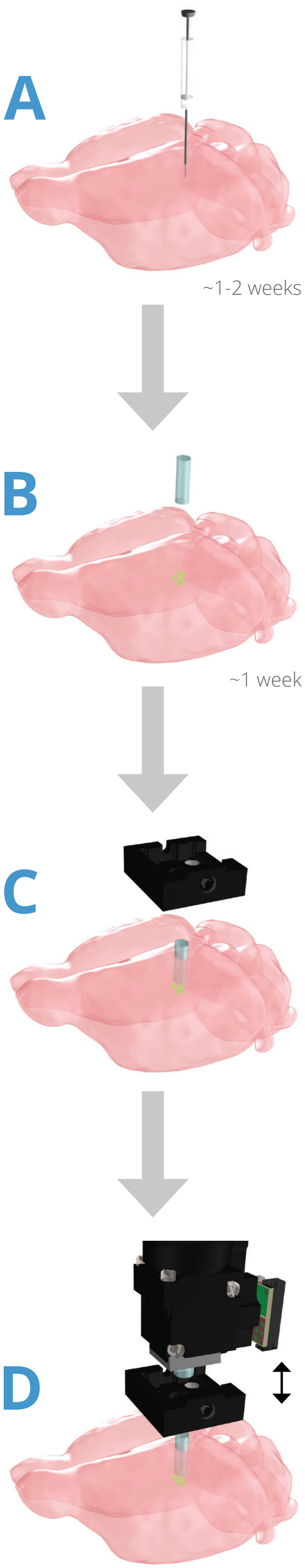

Figure 3. Schematic illustrating abbreviated workflow methods for implanting a lens probe and installing nVista.

Select deep brain publications

Author (year)	Ca^{2+} indicators	Indicator source	Cell type	Animal model	Research application	Theme
Betley (2015)[5]	GCaMP6s/6f	Janelia	LH / AGRP neurons	Agrp-IRES-Cre mouse	Functional cell type mapping	Appetitive / consummatory
Jennings (2015)[6]	GCaMP6m	UNC Core	LH / GABAergic neurons	Vgat-IRES-Cre mouse	Functional cell type mapping	Appetitive / consummatory
Cox (2016)[7]	GCaMP6s	Penn Core	LDT / GABAergic and glutamatergic neurons	GAD2-IRES-Cre / VGLUT2-IRES-Cre mouse	Functional cell type mapping	Arousal state
Harrison (2016)[8]	GCaMP6f	Penn Core	BF / GABAergic, glutamatergic and cholinergic neurons	ChAT-IRES-Cre / GAD2-IRES-Cre / VGLUT2-IRES-Cre mouse	Functional cell type mapping	Cognitive processing
Resendez (2016)[4]	GCaMP6s/m	UNC Core	LH / VTA / BNST	Wild type / TH-Cre mouse	*In vivo* imaging protocol	Imaging & behavioral methods
Douglass (2017)[9]	GCaMP6s	Penn Core	CeA / GABAergic serotonin receptor 2a (Htr2a) neurons	EHtr2a-Cre mouse	Functional cell type mapping	Appetitive / consummatory
Li (2017)[10]	GCaMP6m	Penn Core	MeA neurons	C57BL/6, Vgat-ires-Cre/+, Vglut2-ires-Cre/+ mouse	Functional cell type mapping	Social behavior / sex differences
Remedios (2017)[11]	GCaMP6s	Penn Core	VMHvl / Esr1+ neurons	Esr1Cre/+ mouse	Functional cell type mapping	Social memory
Ryan (2017)[12]	GCaMP6m	In house	PBN / $Oxtr^{PBN}$ neurons	OxtrCre/+ mouse	Functional cell type mapping	Appetitive / consummatory
Chen (2018)[13]	GCaMP6f	Penn Core	DMH / galaninergic & GABAergic neurons	GAL-Cre; GAD2-Cre mouse	Functional cell type mapping	Arousal state
Alves da Silva (2018)[14]	GCaMP6f	Penn Core	SNc / dopaminergic neurons	TH-Cre mouse	Neural coding	Action / Initiation movement
Campos (2018)[15]	GCaMP6m	In house	PBN / $CGRP^{PBN}$ neurons	Calca-Cre/+ & Oxtr-Cre/+ mouse	Functional cell type mapping	Appetitive / con-summatory
Markowitz (2018)[16]	GCaMP6f	Penn Core	DLS / dopamine 1a receptor & adenosine A2a receptor neurons	Drd1a-Cre & Adora2a-Cre mouse	Temporal encoding	Behavior encoding

Data analysis and results

Imaging neuronal activity during spontaneous behaviors[8]

Harrison et al. (2016) examined the activity patterns of BF neurons during spontaneous behaviors in mice fitted with the integrated fluorescence microscope (nVista). Spontaneous behaviors (e.g. sitting, eating/grooming, moving or running) were recorded for 25-50 min using a webcam and scored manually (*Figure 4, next page*).

Processing Ca^{2+} imaging data

After image acquisition, Harrison and colleagues[8] processed the imaging data using Inscopix Data Processing Software. This software is designed to preprocess the raw imaging data and extract Ca^{2+} dynamics of individual cells and allows researchers to correct for brain motion, calculate ΔF/F, and denoise images, among other manipulations. In addition, this software performs PCA/ICA analysis that allows for the detection of cellular events followed by the extraction of independent Ca^{2+} signals and sorts them into a relevant context (*Figure 5, next page*).

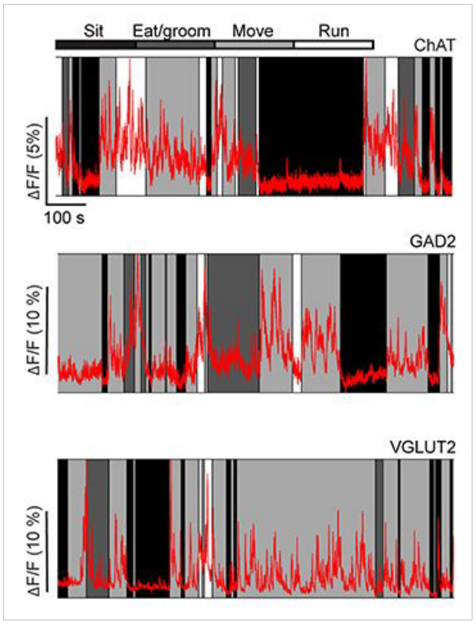

Figure 4. Imaging neuronal activity in BF ChAT, GAD2, and VGLUT2 neurons during spontaneous behaviors in mice[8]. Traces were extracted from nVista imaging data and various behaviors are indicated in the shaded horizontal bar above. Video recording of Ca^{2+} responses can be accessed here.

Discussion

The ability to study the dynamics of neural circuitry and behavioral states in freely behaving animals is vital to gaining a better understanding of the function of deep brain regions in health and disease.

High-throughput *in vivo* calcium imaging at single cell resolution with the nVista system paired with the lens probes opens new possibilities in the mouse brain and allows researchers to ask new questions about how these various deep brain regions modulate essential functions such as arousal state, feeding behavior, reward and cognition, in order to address how these neural circuits become perturbed in CNS disease.

References

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2. Ballinger et al. Basal forebrain cholinergic circuits and signaling in cognition and cognitive decline. Neuron (2016)
3. Stuber and Wise. Lateral hypothalamic circuits for feeding and reward. Nat Neurosci (2016)
4. Resendez et al. Visualization of cortical, subcortical and deep brain neural circuit dynamics during naturalistic mammalian behavior with head-mounted microscopes and chronically implanted lenses. Nat Prot (2016)

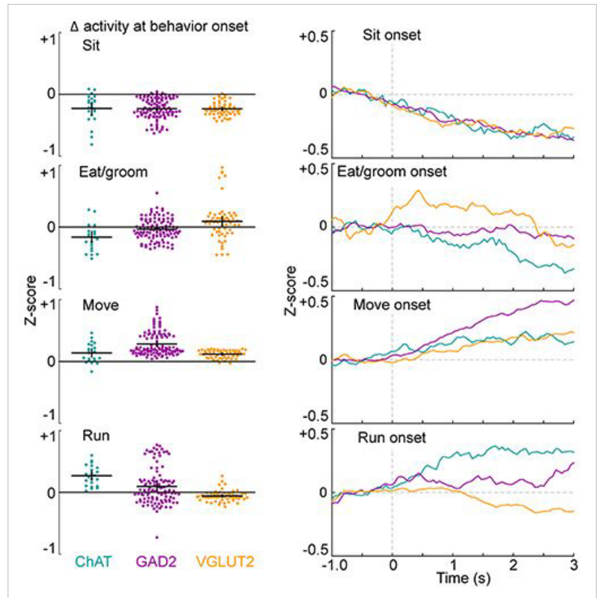

Figure 5. Changes in activity patterns in individual regions of interest (ROIs) of BF neurons at the onset of each behavior (left panel). Mean activity of all ROIs from each cell type, aligned by behavior onset (right panel)[8].

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9. Douglass et al. Central amygdala circuits modulate food consumption through a positive-valence mechanism. Nat Neurosci (2017)
10. Li et al. Neuronal representation of social information in the medial amygdala of awake behaving mice. Cell (2017)
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16. Markowitz et al. The striatum organizes 3D behavior via moment-to-moment action selection. Cell (2018)

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