Video Article

November 2011: This Month in JoVE

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Abstract

The November issue of the Journal of Visualized Experiments begins with a series of behavioral experiments for measuring prehension ability in primates. Investigators from the University of Fribourg demonstrate four grasping and reaching tasks, which all follow common steps for comfortably restraining the animal and correctly positioning its head. The Brinkman Board test is used to assess reaching for static objects, while the Rotating Brinkman Board presents a condition where animals need to anticipate object position. The Brinkman Box test allows dexterity to be investigated under conditions where the monkey has fewer degrees of freedom to perform grasping task and cannot use vision to guide movement. While the Brinkman board and box tests solely rely on analysis of video obtained during experiments, in order to generate data, the drawer reaching task apparatus has detectors that monitor grip and load forces as well as drawer position, making this test appropriate for measuring strength as well as prehension ability. In combination, these four tests provide a powerful approach to assess motor control in the distal forelimb, and the Fribourg group presents data from both lesion studies and experiments aimed to test potential therapies for spinal cord injury.

In Clinical and Translational Medicine, collaborators from the Division of Surgical Research at the University Hospital Zurich and the Institute of Laboratory Animal Science at the University of Zurich, work together to implant a radiotelemetry device in mice, which can be used to take physiological measurements in freely moving animals. The devices consist of two leads, which are capable of measuring biopotential activity (ECG, EEG, or EMG) as well as temperature, and a lightweight radio transmitter (1-200 Hz in bandwidth) that can be implanted in the abdominal cavity. The stress of animal handling as well as anesthesia are known confounding elements, which impact physiological measurements, so radiotelemetry devices are a convenient way to acquire physiological data from animals in an unperturbed, natural state, as well as take measurements of multiple animals, simultaneously.

JoVE also adds to its host of electroporation protocols with a method for introducing macromolecules into the craniofacial mesenchyme of developing *Xenopus laevis* tadpoles, from the Liu Lab at King's College London. *Xenopus* tadpoles are transferred to an electroporation chamber, where they can be injected with nucleic acid, and then subjected to electroporation, which will deliver expression vectors or morpholinos into the developing craniofacial tissue. Gene delivery into differentiated cartilage is inherently problematic, and the Liu lab shows that, through electroporation, morpholinos can be introduced into procartilaginous and cartilaginous tissues of the *Xenopus* tadpole throughout a range of developmental stages.

In Bioengineering, the Zuckerman group from the Lawrence Berkeley National Laboratory illustrates their method for synthesizing peptoids, a class of biologically-active organic molecules that resemble peptides, but have their side chains attached to the nitrogen of the peptide bond, rather than the alpha carbon, which makes them resistant to proteolysis. The Zuckerman group demonstrates submonomer synthesis, where peptide monomers are added sequentially in bromoacetylation and bromide displacement reactions, allowing peptoid chain length and composition to be precisely controlled. Furthermore, the fact that synthesis is done on amide resin, and is hence, solid phase synthesis, greatly simplifies purification of the synthesized peptoids. These authors also show how the newly-synthesized peptoids can be induced to self-assemble into highly-ordered structures, termed nanosheets, which can be visualized by fluorescence and scanning electron microscopy. These nanosheets could serve as a potential platform for protein and membrane mimetics.

Later in the month, JoVE features an assay for measuring phagocytic activity in cells exposed to clinical antibody samples for our Immunology and Infection section. Collaborators from Massachusetts General Hospital and Dartmouth College show that THP-1 cells will engulf antibody-bound antigen coated beads in timelapse video microscopy and present a plate-based flow cytometry method for quantifying the extent of phagocytosis. Our authors use this method to determine the ability of different antibody samples to induce phagocytosis, such as those from HIV patients both treated and untreated with anti-retroviral therapies.

This summary of JoVE's content in November provides a brief overview of JoVE's highlights for the month. Other noteworthy articles include methods for live imaging of cytoskeletal dynamics in endothelial cells, measuring calcium flux in cardiomyocytes, and slice electrophysiology of adult born neurons in the olfactory bulb.

Video Link

The video component of this article can be found at <https://www.jove.com/video/4025/>

Protocol

Measuring Fast Calcium Fluxes in Cardiomyocytes

Urszula Golebiewska¹, Suzanne Scarlata²

¹Department of Biological Sciences and Geology, Queensborough Community College, ²Department of Physiology & Biophysics, Stony Brook University

We present a method to isolate rapid (microsecond) calcium events from slower fluxes in living cells using laser scanning confocal microscopy. The method measures fluorescence intensity fluctuations of calcium indicators by recording line scans of several hundred pixels in a cell. Histogram analysis allows us to isolate the time scales of different calcium fluxes.

Solid-phase Submonomer Synthesis of Peptoid Polymers and their Self-Assembly into Highly-Ordered Nanosheets

Helen Tran, Sarah L. Gael, Michael D. Connolly, Ronald N. Zuckermann
Molecular Foundry, Lawrence Berkeley National Laboratory

A simple and general manual peptoid synthesis method involving basic equipment and commercially available reagents is outlined, enabling peptoids to be easily synthesized in most laboratories. The synthesis, purification and characterization of an amphiphilic peptoid 36mer is described, as well as its self-assembly into highly-ordered nanosheets.

Behavioral Assessment of Manual Dexterity in Non-Human Primates

Eric Schmidlin*, Mélanie Kaeser*, Anne- Dominique Gindrat, Julie Savidan, Pauline Chatagny, Simon Badoud, Adjia Hamadjida, Marie-Laure Beaud, Thierry Wannier, Abderraouf Belhaj-Saif, Eric M. Rouiller

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As manual dexterity is a prerogative mainly of primates, behavioral tasks have been developed in macaque monkeys. Four reach and grasp prehension tasks, measuring hand manipulation ability and force, allow to establish functional recovery after a lesion of the central nervous system and to test the effect of a treatment.

Determining the Phagocytic Activity of Clinical Antibody Samples

Elizabeth G. McAndrew¹, Anne-Sophie Dugast¹, Anna F. Licht¹, Justin R. Eusebio¹, Galit Alter¹, Margaret E. Ackerman²

¹Massachusetts General Hospital, Ragon Institute of MGH, MIT, and Harvard, ²Thayer School of Engineering, Dartmouth College

We present a high-throughput flow cytometric assay to determine the phagocytic activity of antigen-specific antibodies from clinical samples, utilizing fluorescent antigen-coated beads and a monocytic cell line expressing multiple Fc receptors-providing receptor usage and phagocytic activity determinations in a standardized and reproducible fashion for any antigen of interest.

Study of the Actin Cytoskeleton in Live Endothelial Cells Expressing GFP-Actin

Travis M. Doggett, Jerome W. Breslin

Department of Physiology, Louisiana State University Health Sciences Center Microscopic imaging of live endothelial cells expressing GFP-actin allows characterization of dynamic changes in cytoskeletal structures. Unlike techniques that use fixed specimens, this method provides a detailed assessment of temporal changes in the actin cytoskeleton in the same cells before, during, and after various physical, pharmacological, or inflammatory stimuli.

Implantation of Radiotelemetry Transmitters Yielding Data on ECG, Heart Rate, Core Body Temperature and Activity in Free-Moving Laboratory Mice

Nikola Cesarovic¹, Paulin Jirkof², Andreas Rettich², Margarete Arras¹

¹Division of Surgical Research, University Hospital Zurich, ²Institute of Laboratory Animal Science, University of Zurich

A surgical technique for implantation of commercially available telemetry transmitters used for continuous measurement of biopotential (one-lead ECG), heart rate, core body temperature and locomotor activity in freely moving mice is shown. Recommendations and protocols for post-operative care and pain relief, improving recovery, wellbeing and survival rate are also presented.

Electroporation of Craniofacial Mesenchyme

Jacqueline M. Tabler, Karen J. Liu

Department of Craniofacial Development, King's College London

Craniofacial cartilages develop in close contact with other tissues and are difficult to manipulate in live animals. We are using electroporation to deliver molecular tools during growth of the craniofacial skeleton while bypassing early embryonic effects. This approach will allow us to efficiently test candidate molecules *in vivo*.

Slice Electrophysiological Recording and Optogenetic Stimulation of Adult-Born Neurons in the Olfactory Bulb

Matthew Valley, Sebastian Wagner, Benjamin W. Gallarda, Pierre-Marie Lledo

Laboratory for Perception and Memory, Institut Pasteur and Centre National de la Recherche Scientifique (CNRS)

Adult-born neurons expressing ChR2 can be manipulated in slice electrophysiological preparations in order to examine their contribution towards the function of olfactory neural circuits.