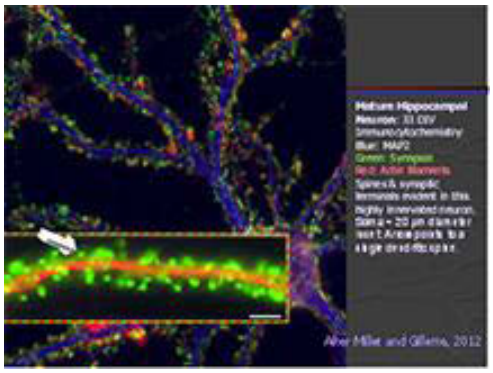
Appendix 1
Descriptions of the Workshop Talks

In what follows, the workshop talks are described including in many cases, the questions asked by the speakers, as well as one or two exemplary references and a figure.

Session 1: *The Grand Challenges in Understanding the Brain: Integrating models, technologies, and scale, from fundamental to clinical*

Presenters: *Martha Gillette*, Leonid Moroz, Anne Andrews

Martha Gillette. ***Grand Challenges in Understanding the Brain: Integrating chemistry, models, technology, & scale.*** Gillette highlighted the tremendous challenges in understanding the brain—a highly complex organ capable of sensation, integration, and actuation as well as multi-modal and highly plastic changes in response to experience. The brain is dynamic at multiple scales in both structure and function. Cellular units are heterogeneous and unusual with small cell bodies bearing highly elaborated, intertwining extensions that form circuits. What new technologies are needed to understand the brain chemically? For example, how do the chemicals released at the tip of a neuronal extension change as spines form and memories are stored or as they are lost? How chemically heterogeneous are brain cells? How does the chemical micro-environment shape construction of 3D neural circuits? How can we measure brain chemicals non-invasively in real time?



Millet, L.J., and Gillette, M.U. New perspectives on neuronal development via microfluidic environments. *Trends in Neuroscience.* 2012 Dec; 35 *(12):*752-761. doi.org/10.1016/j.tins.2012.09.001.

Froeter, P., Huang, Y., Cangellaris, O.V., Dent, E., Gillette, M.U., Williams, J.C., Li, X. Toward intelligent synthetic neural circuits: Directing and accelerating neuron cell growth by self-rolled-up silicon nitride microtube array. *ACS Nano* 2014 Nov 25; 8 *(11):*11108-11117. doi:10.1021/nn504876y.

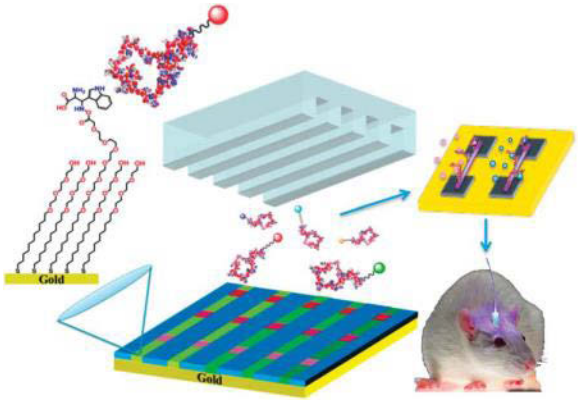
Leonid Moroz. ***Understanding the brain from the evolutionary perspective.*** Moroz highlighted the origins of neurons and the nervous system, and asked a range of grand questions including: why are neurons so different than other cells? Why are there so many transmitters? He demonstrated the extensive convergent evolution of neural systems across the metazoan. Neurons have different functions and different genealogies. Moroz studies the diversity of life using a range of genetic approaches combined with some direct measurement

of transmitters. His overarching goals include creating a census of cell types from neurons from several creatures—*Aplysia* to mammals to establish a "NeuroSystematics" atlas, or, as Moroz calls it, a periodic table of neurons across the diversity of life.

Moroz, L., Koco, K., Citarella, M., Dosung, S., Norekian, T., Povolotskaya, I.S., Grigorenko, A.P., *et. al*. The ctenophore genome and the evolutionary origins of neural systems. *Nature* June 2014; 510:109–114. doi: 10.1093/jhered/est084.

Moroz, L.L., Kohn, A.B. Independent origins of neurons and synapses: insights from ctenophores. *Philos Trans R Soc Lond B Biol Sci*. 2016 Jan 5; 371 *(1685)*:20150041. doi: 10.1098/rstb.2015.0041.

Anne M. Andrews. ***In vivo Electronic Neurotransmitter Sensing.*** Andrews gave an overview of her collaborative and interdisciplinary research efforts to develop and to fabricate aptamer-functionalized field-effect transistor (FET) biosensors.[1] Aptamers are short nucleic acid sequences that can recognize small-molecule targets. Aptamer binding (and unbinding) are transduced into electronic signals *via* aptamer conformational changes and their modulation of local field potentials at the surfaces of nanoscale semiconductor gate electrodes (channels) in FETs. The FETs can be lithographically fabricated using facile chemical lift-off lithography[2,3] on silicon microprobes for acute neurochemical recordings or flexible substrates[4] for chronic measurements. Goals are to identify aptamer-sensors for a wide range of neurotransmitters and to fabricate sensing elements at high densities and nanoscale dimensions to enable measurements of extracellular neurotransmitter signaling in behaving animals with the chemical, spatial, and temporal resolutions needed to decode information processing and to construct a chemical connectome.[5]



Schematic illustrating functional nanomaterials to screen for small-molecule aptamers. Field-effect transistors are functionalized with aptamers to produce electronic sensors for recording neurotransmitter transients in vivo.

[1] Nakatsuka, N. and Andrews, A.M. Neurochips enable nanoscale devices for high-resolution *in vivo* neurotransmitter sensing. *Neuropsychopharmacology* 2016 *41:*378-379.

[2] Liao, W.S., Cheunkar, S., Cao, H.H., Bednar, H.R., Weiss, P.S., Andrews, A.M. Subtractive patterning *via* chemical lift-off lithography. *Science* 2012 (*337):*1517-1521.

[3] Kim, J., Rim, Y.S., Chen, H., Cao, H.H., Nakatsuka, N., Hinton, H.L., Zhao, C., Andrews, A.M., Yang, Y., Weiss, P.S. Fabrication of high-performance ultrathin In_2O_3 film field-effect transistors and biosensors using chemical lift-off lithography. *ACS Nano* 2015 (*9):*4572-4582.

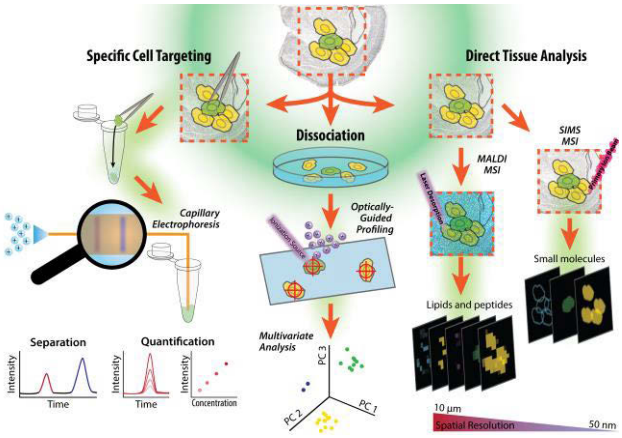
[4] Rim, Y.S., Bae, S.H., Chen, H., Yang, J.L., Kim, J., Andrews, A.M., Weiss, P.S., Yang, Y., Tseng, H.R. Printable ultrathin metal oxide semiconductor-based conformal biosensors. *ACS Nano* 2015 (*9):*12174-12181.

[5] Andrews, A.M. The BRAIN initiative: Toward a chemical connectome. *ACS Chem. Neurosci.* 2013 *(4):*645.

Session 2: *Characterizing the cells making up the brain: a cell census*

Presenters: Jonathan Sweedler, Jim Eberwine, Yong Yao

Jonathan Sweedler. ***Characterizing the cells in the nervous system with mass spectrometry.*** Sweedler's talk focused on understanding cellular heterogeneity from a chemical perspective. How can one measure the chemical contents of thousands (to hundreds of thousands) of cells in the brain in an unbiased way? He focused on high-throughput single-cell mass spectrometry, and presented a brief history and status update on single-cell chemical measurement approaches. Single-cell characterization will enable a chemical cell census and an understanding of cell types and cell content, and how this changes during learning, memory, and behavior. Technological needs include the ability to characterize the chemicals present in larger populations of cells, integrating mass spectrometry data with spectroscopic and transcriptomic measurements.



Outline of the approaches for single-cell mass spectrometry, ranging from isolating the specific cell of interest to a high throughput cell profiling approach.

Chen, X., Love, J.C., Navin, N.E., Pachter, L., Stubbington, M.J., Svensson, V., Sweedler, J.V., Teichmann, S.A. Single-cell analysis at the threshold. *Nat Biotechnol.* 2016 Nov 8; 34 (*11)*: 1111-1118. doi:10.1038/nbt.3721.

Jansson, E.T., Comi, T.J., Rubakhin, S.S., Sweedler, J.V. Single Cell Peptide Heterogeneity of Rat Islets of Langerhans. *ACS Chem Biol.* 2016 Sep 16; 11 (*9)*: 2588-95. doi:10.1021/acschembio.6b00602.

Comi, T.J., Do, T.D., Rubakhin, S.S., Sweedler, J.V. Categorizing Cells on the Basis of their Chemical Profiles: Progress in Single-Cell Mass Spectrometry. *J Am Chem Soc*. 2017 Mar 22; 139 *(11):*3920-3929. doi: 10.1021/jacs.6b12822.

James Eberwine. ***Characterizing the cells making up the brain—how important is distinctness?*** Eberwine described a range of single cell transcriptomics protocols that are used by for studying DNA variation, chromatin dynamics, epigenomics, transcriptomics, mRNA translation, and indirectly aid other –omics measurements such as peptidomics, proteinomics, enzymology, metabolomics, functional genomics. He described the goal of understanding cellular phenotype and pointed out that different groups define this in different ways. Tools to understand the individual cell are not enough; we need tools to measure RNA in a soma versus a dendrite. Eberwine highlighted TIVA (transcriptome *in vivo* analysis) of live hippocampal cells, and discussed the influence of cellular microenvironment upon expression, variance range, and bimodality. The next steps are to combine live cell transcriptomics with chemical assays, physiological measures, and a range of microscopies so that the spatial, temporal, and chemical relationships can be reconstructed.

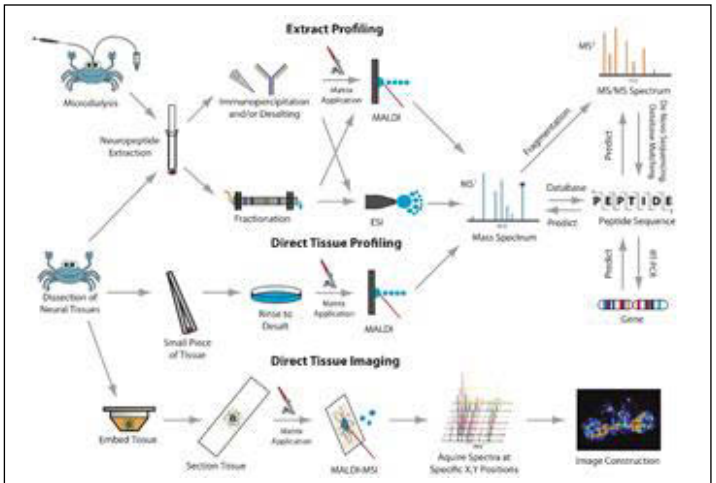
Dueck, H., Eberwine, J., Kim, J. Variation is function: Are single cell differences functionally important?: Testing the hypothesis that single cell variation is required for aggregate function. *Bioessays*. 2016 Feb; 38 *(2)*:172-80. doi: 10.1002/bies.201500124.

Eberwine J. Down the Rabbit Hole of Single-Cell Genome Analysis. *Mol Cell*. 2017 May 4; 66 *(3):*304-305. doi: 10.1016/j.molcel.2017.04.015.

Yong Yao. ***NIH BRAIN Cell Census program.*** Yao highlighted and described the ongoing National Institutes of Health initiative, ***BRAIN 2025: A Scientific Vision***, which is the guiding document for multiple scientific areas to study. For more details, see: http://www.braininitiative.nih.gov/

Session 3: ***Creating a parts list of the brain***
Presenters: Lingjun Li, Jeff Agar, Tom Neubert, Sean Bendall

Lingjun Li. ***Creating a parts list of the brain: Towards generating a molecular atlas of the brain.*** Li's talk focused on challenges, current approaches, and the status of generating a full description of chemical messengers of a nervous system. Using crustacean model organism as an example, Li's presentation highlighted a multifaceted approach employing chemical labeling, tissue imaging and *in vivo* microdialysis coupled with mass spectrometry toward an improved molecular understanding of functional consequences of neuropeptide diversity and multiplicity. Advancements in mass spectrometric instrumentation such as ion mobility and high-resolution Fourier transform mass analyzer enabled more in-depth characterization of neuropeptides with unique post-translational modifications (e.g., D-amino acid containing peptide) and high-throughput quantitative peptidomic studies with new multiplexed mass tagging reagents. Future technological needs include molecular imaging of peptides and neurotransmitters at the subcellular level and integrating multiple anatomical scales and multiple imaging modalities. Improved bioinformatics tools tailored to the unique features of signaling peptides are in demand to accelerate our pace of neuropeptide discovery.



Overview of sample preparation strategies for neuropeptide discovery by a multi-faceted mass spectrometry platform.

Buchberger, A., Yu, Q., Li, L. Advances in mass spectrometric tools for probing neuropeptides. *Annual Review of Analytical Chemistry.* 2015 (*8):*485-509. doi:10.1146/annurev-anchem-071114-040210.

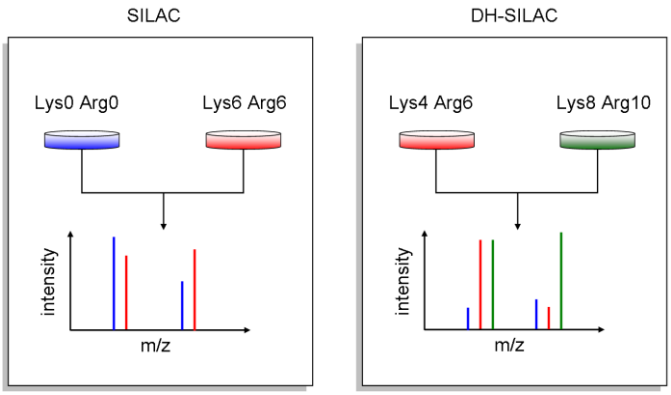
Ouyang, C., Liang, Z., Li, L. Mass spectrometric analysis of spatio-temporal dynamics of crustacean neuropeptides. *Biochim Biophys ACTA (BBA)-Proteins and Proteomics*. Invited contribution to *Special Issue on Neuroproteomics*. 2014 1854(*7):*798-811.

Jeff Agar. ***#Brain 2 Complex 4 2 Day.*** Agar described downscaling proteomics measurements to work with small volume samples such as a milligram of human brain tissue. He highlighted the importance of complete protein characterization with the example that a methyl group addition to a single protein can kill an individual within six months. He also highlighted the use of new technologies such as ambient ionization mass spectrometry in neurosurgery.

Schmitt, N.D., Agar, J.N. Parsing Disease-relevant Protein Modifications from Epiphenomena: Perspective on the Structural Basis of SOD1-Mediated ALS. *J Mass Spectrom*. 2017 May 30. doi: 10.1002/jms.3953.

Calligaris, D., Feldman, D.R., Norton, I., Olubiyi, O., Changelian, A.N., Machaidze, R., Vestal, M.L., Laws, E.R., Dunn, I.F., Santagata, S., Agar, N.Y. MALDI mass spectrometry imaging analysis of pituitary adenomas for near-real-time tumor delineation. *Proc Natl Acad Sci U S A*. 2015 Aug 11; 112 *(32)*:9978-83. doi: 10.1073/pnas.1423101112

Thomas A. Neubert. ***Using mass spectrometry to study signaling in neurons.*** Mass spectrometry can be used to generate fairly comprehensive views of cellular signaling in cultured primary neurons or in some cases homogenized brain tissue. From about a million cells, 8-9,000 proteins can be quantified relative to the proteins from a related or control sample, and changes in tens of thousands of phosphorylation sites in response to growth factors or other stimuli can be monitored using commonly available instruments and workflows. Good affinity tools are available to enrich for several post-translational modifications of interest such as phosphorylation, ubiquitination, acetylation, and glycosylation. Metabolic or chemical stable isotope labeling methods can make relative quantitation more accurate compared to label-free methods and can enable multiplex analysis of ten or more samples. The molecular parts list of key functional molecules identified and characterized in these comprehensive studies of populations of many neurons can be used to inform more ideal, and, likely targeted, future studies of signaling in single neurons in the context of an intact nervous system.



Traditional stable isotope labeling by amino acids in cell culture (SILAC) uses natural or heavy stable isotope-containing amino acids to differentiate proteins from two types of cells or experimental conditions for accurate relative quantitation (left) in mass spectrometric studies of cell signaling. Accurate quantitation can be achieved even when proteins are partially labeled, as can happen in non-dividing primary neurons, if the cell cultures are labeled with medium or heavy stable isotopes (right). In this case the medium and heavy labeled peptides are compared, and the unlabeled peptides are ignored.

Sean Bendall. ***Massively Multiplexed Subcellular Imaging.*** Bendall described several high-speed single-cell mass spectrometry approaches such as the mass cytometer, as well as multiplexed ion beam imaging (MIBI). These innovative approaches combine single cell measures with antibody-based reporters. Instrumental improvements have greater sped and allow measurement of more reporters simultaneously. The researchers section a tissue and

raster a primary ion beam over the sample, liberating the reporter elements one pixel/cell at a time. They have up to 50 simultaneous reporters validated with a spatial resolution of 100 nm - 10 microns. Bendal discussed the need for a subcellular parts list for the aging human brain and asked about the normal distribution of cellular, regulatory proteins, disease related gene products as we age. How does this landscape change with cognition? Improved data repositories somehow indexed to locations are needed.

Porpiglia, E., Samusik, N., Van Ho, A.T., Cosgrove, B.D., Mai, T., Davis, K.L., Jager, A., Nolan, G.P., Bendall, S.C., Fantl, W.J., Blau, H.M. High-resolution myogenic lineage mapping by single-cell mass cytometry. *Nat Cell Biol*. 2017 May; 19 *(5):*558-567. doi: 10.1038/ncb3507. Epub 2017 Apr 17.

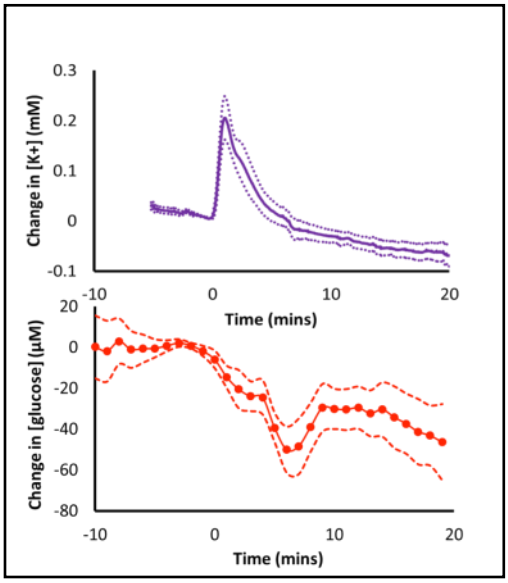
Krishnaswamy, S., Spitzer, M.H., Mingueneau, M., Bendall, S.C., Litvin, O., Stone, E,, Pe'er, D., Nolan, G.P. Systems biology. Conditional density-based analysis of T cell signaling in single-cell data. *Science*. 2014 Nov 28; 346 *(6213):*1250689. doi: 10.1126/science.1250689.

Session 4: *The dynamic brain: sampling and measuring brain chemistry* in vitro *and* in vivo

Presenters: Michael Heien, Adrian Michael, Leslie Sombers, Jill Venton, Ryan White

Michael Heien. ***New tools for measuring the brain.*** Heien started with discussing how to compare the figures of merit of various measurement approaches and their dimensionality across the time/space/chemical dimensions. His examples included methods to implant probes in the brain (amperometry, FSCAV, etc.) where one obtains good spatiotemporal resolution but nothing on chemical, versus micro-dialysis to see lots of distinctions in chemicals, but sacrificing temporal resolution. Techniques chosen will enable and constrain the questions asked. He then described approaches in order to do voltammetry and electrochemistry at the same time using arrays of electrodes, to measure dopamine and neuronal recordings to relate the two processes. Electrophysiologists are ahead of chemists on this as they can have dozens or hundreds of electrodes implanted in animals.

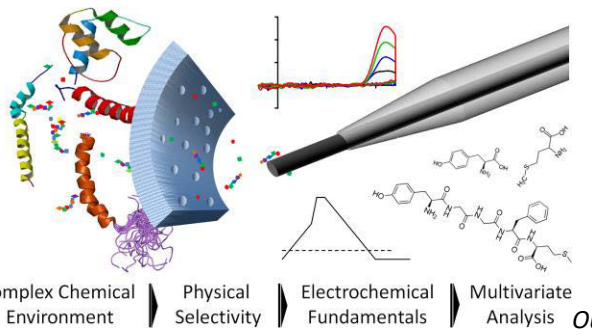
Adrian C. Michael. ***Enhancing capabilities for real time neurochemical monitoring of brain dynamics.*** The central nervous system operates on multiple time scales, from rapid synaptic transmission, to paracrine and hormonal modes of chemical communication. Thus, access to chemical dynamics is vital. Microdialysis is an important tool in this respect, but inserting microdialysis probes into living tissues leads to a traumatic penetration injury that disrupts neurochemistry and, due to scarring, limiting the duration of microdialysis measurements. Using the probe to deliver an anti-inflammatory agent, dexamethasone, directly to the site of the penetration injury is proving to be a very simple, yet highly effective means of mitigating the penetration injury, and the ensuing host tissue response. The figure here shows potassium ion and glucose transients induced by spreading depolarization recorded with microdialysis probes 10 days after they were inserted into the rat brain. Histological studies confirm that dexamethasone abolishes glial scarring at the probe. Mitigation of the traumatic penetration injury normally associated with microdialysis is showing signs of opening a route to new capabilities for monitoring chemical dynamics in the mammalian brain.



Nesbitt, K.M., Varner, E.L., Jaquins-Gerstl, A., Michael, A.C. Microdialysis in the rat striatum: effects of 24-h dexamethasone retrodialysis on evoked dopamine release and penetration injury. *ACS Chem. Neurosci.* 2015 *6*:163-173.

Varner, E.L., Jaquins-Gerstl, A., Michael, A.C. Enhanced intracranial microdialysis by reduction of traumatic penetration injury at the probe track. *ACS Chem. Neurosci.* 2016 *7*:728-736.

Leslie Sombers. ***Characterizing neurochemical dynamics in the living brain with electrochemistry.*** Sombers described the development of new electro-analytical methods to reveal chemical mechanisms of brain function. She focused on monitoring opioid neuropeptide dynamics with sub-second temporal resolution using ultramicroelectrodes. These molecules are



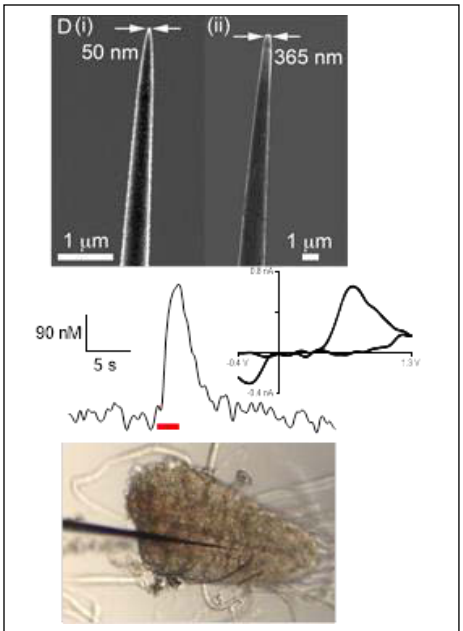
Outline of the approaches used for monitoring endogenous opioid neuropeptide dynamics in situ*, ranging from novel carbon nanomaterials to bolster sensitivity, chemically selective membranes and exploitation of fundamental electrochemical principles to garner selectivity, and multivariate analysis to resolve individual chemical contributors to the complex signal. Figure created by James Roberts of the Sombers group, NC State University.*

critically involved in a variety of physiological functions necessary for adaptation and survival; however, the precise role of members of this class of molecules remains ambiguous. Full optimization and characterization of the novel electrochemical approach to *in situ* quantification will enable clarification of outstanding questions regarding the fundamental nature of endogenous opioid peptide neurotransmission, including determination of the physiological parameters required to elicit endogenous opioid peptide release, the time scale and diffusion distance of this chemical signaling, and an evaluation of peptidergic processing in the extracellular space.

Schmidt, A.C., Dunaway, L.E., Roberts, J.G., McCarty, G.S., Sombers, L.A. Multiple Scan Rate Voltammetry for Selective Quantification of Real-Time Enkephalin Dynamics. *Anal. Chem.* 2014 86 *(15)*:7806-12. doi:10.1021/ac501725u.

Carbon Nanotube Yarn Electrodes for Enhanced Detection of Neurotransmitter Dynamics in Live Brain Tissue. *ACS Nano.* 2013 *7*:7864-7873. doi:10.1021/nn402857u.

Jill Venton. ***The dynamic brain***. The question of how brain chemistry changes during behavior and disease is complex. One popular measurement technique is to use carbon fiber microelectrodes to detect molecules *in vivo* using fast-scan cyclic voltammetry. The time-scale of these measurements is 100 ms, nearly real time. However, this technique mostly looks at changes in extracellular dopamine, and probing synaptic changes is just becoming possible. By far, dopamine has been the most studied molecule using this approach, and experiments over the past 30 years have shown that it is an important signal for reward and motivation. However, there has been a recent push in the field to expand the use of dynamic, real-time measurements beyond dopamine. For example, the Venton lab has recently discovered fast release of adenosine, which can be identified using electrochemistry. The spontaneous releases of adenosine occur every 30 s in the prefrontal cortex. In addition, oxygen changes can be measured as well to correlate changes in neurotransmitters. One lesson from these forays into making dynamic measurements in the brain is that every time we measure faster, we find faster changes in the brain! The field is also pushing for smaller model systems and smaller probes. For example, a new model system for neurochemistry is *Drosophila*, in which carbon fiber probe and optogenetic stimulations have been used to make dynamic measurements of the dopamine and serotonin changes. However, carbon-fiber microelectrodes (7 um in diameter) are "big" compared to the size of the fly brain or a single synapse. The Venton lab has developed carbon



Carbon nanopipettes are submicron electrodes used to measure dopamine in the brain of a fruit fly larva.

nano-pipette electrodes that have a diameter of less than 1 um. Christian Amatour's group in France recently etched electrodes to make them small enough to place in a single synapse to monitor exocytosis. Challenges for the future include making even smaller electrodes, including functional nanoelectrodes that actually work for us. Batch fabrication is needed and electrodes that are nano in size but sturdy enough for implantation. In electrochemistry current scales with area, so smaller means also less current and that's an issue. Another challenge for the future is multiplexing to make arrays that would either measure different neurochemicals or the spatial distribution of neurotransmitter release.

Rees, H.R., Anderson, S.E., Privman, E., Bau, H.H., Venton, B.J. Carbon nanopipette electrodes for dopamine detection in *Drosophila*. *Analytical Chemistry*. 2015 *87*:3849-55.

Ngyuen, M., Lee, S.L., Ross, A.E., Ryals, M., Choudhry, V.I., Venton, B.J. Characterization of spontaneous, transient adenosine release in the caudate-putamen and prefrontal cortex. *PLoS One*. 2014 9 *(1):*e87165.

Li Y.-T., Zhang S.H., Wang X.-Y., Zhang X.-W., Oleinick A.I., Svir I., Amatore C., Huang W.-H. Nanoelectrode for Amperometric Monitoring of Individual Vesicular Exocytosis Inside Single Synapses. *Angew Chem Int Ed Engl*. 2014 53 *(46):*12456–12460.

Ryan White. *Developing Electroanalytical Tools for the Extracellular Space.*

White's talk focused on leveraging the specific recognition abilities of nucleic acid aptamers and protein channels with the sensitive and high spatiotemporal resolution afforded by electrochemical detection. By combing these attributes, electrochemical sensors can expand to a wide dynamic range of peptide and molecular dynamics in the brain. Folding-based aptamer sensors enable a wide range of molecular target detection from single cells to *in vivo*.

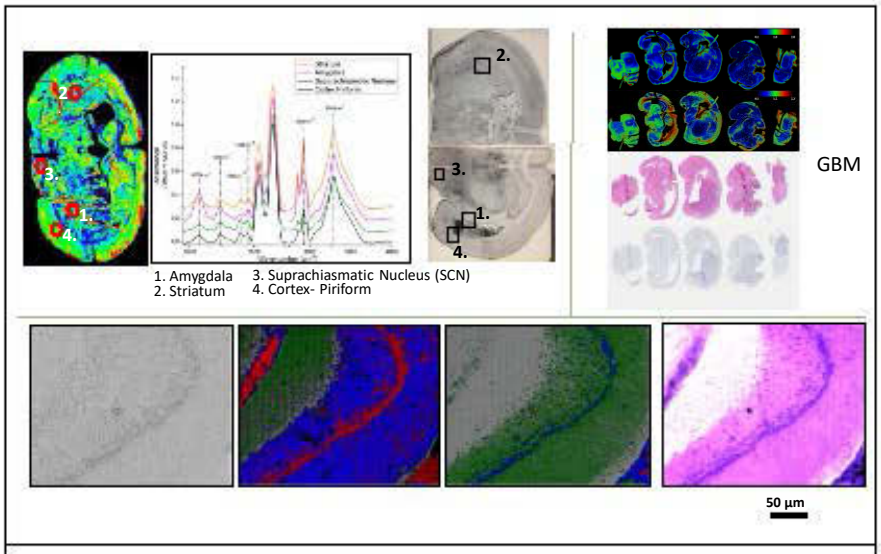
Liu, J., Wagan ,S., Dávila Morris, M., Taylo,r J., White, R. J., Achieving Reproducible Performance of Electrochemical, Folding Aptamer-Based Sensors on Microelectrodes: Challenges and Prospects. *Anal. Chem.* 2014 *86*:11417-11424.

Macazo, F. C., and White, R. J. Bio-Inspired Protein Channel-Based Scanning Ion Conductance Microscopy (Bio-SICM) for Simultaneous Conductance and Specific Molecular Imaging. *J. Am. Chem. Soc.* 2016 *138:*2793-2801.

Session 5: *Advances in molecular imaging*

Presenters: Rohit Bhargava, Conor Evans, Fahmeed Hyder, Partha Basu

Rohit Bhargava. *Advances in Molecular Imaging.* Vibrational spectroscopies (infrared and Raman) offer powerful new tools for brain characterization with sub-cellular resolution and large scale imaging capability. The presentation focused on describing recent developments in IR imaging technology that include high-resolution and high-signal-to-noise ratio images at rapid speed using quantum cascade lasers. The talk suggested the capability of building a "Google maps"-like applications for brain research where each region could be coded with chemical information while allow a full spatial view of various molecular data. The talk also emphasized that multimodal technique, multiscale techniques and hyphenated techniques are likely to prove useful and should be developed. Data handling from imaging, with computational analysis and merging of multiple data types was emphasized. Finally, the importance of educational programs that merge sensing, with computational analysis and basic science was emphasized.



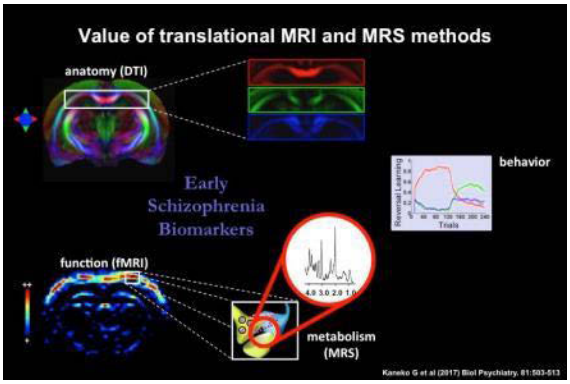
Chemical imaging of brain sections using infrared (top, left) vibrational spectroscopic imaging reveals chemical differences among structural units. (Top, right) Glioblastoma multiforme (GBM) affected animal brain samples. (Bottom, from left to right) White light images of brain sections do not show contrast but chemical contrast can be obtained at various frequencies using stimulated Raman scattering. With advanced numerical techniques, images can be colored to resemble those used clinically or for research that are accessible via chemical stains such as H&E (far right).

Conor Evans. ***Optical Toolkits for Molecular Imaging***. Coherent Raman Scattering (CRS) imaging is a powerful toolkit for molecular imaging in biomedicine. CRS imaging enables the visualization of specific molecules of interest *via* their unique chemical vibrations, which are derived from molecular structure. The presentation included examples of this toolkit as well as specific research carried out by leaders in the field. Of note, Daniel Cote's (Laval University) work in visualizing myelin in the brain was discussed, as well as ongoing efforts by Wei Min (Columbia University) to develop Raman labels that can be used to visualize drugs and metabolites. Also included in the talk was work related to the Evans lab's efforts to create oxygen imaging systems, specifically phosphorescent molecules that can report tissue oxygen concentration.

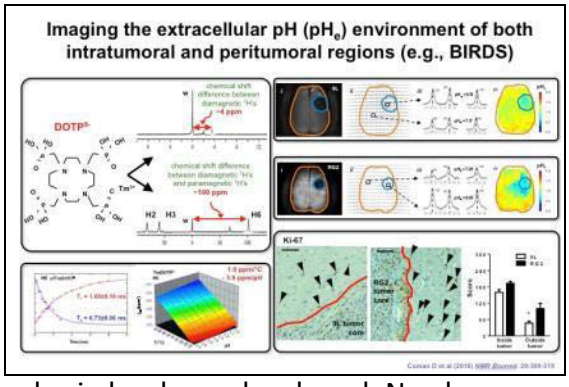
Evans, C.L., and Xie, X.S. Coherent anti-stokes Raman scattering microscopy: chemical imaging for biology and medicine. *Annu. Rev. Anal. Chem.* 2008 *1*:883–909

Roussakis, E., Li, Z., Nichols, A.J., Evans, C.L. Oxygen-Sensing Methods in Biomedicine from the Macroscale to the Microscale. *Angew. Chem. Int. Ed Engl.* 2015 *54*:8340–8362.

Fahmeed Hyder. ***Measuring the Brain: From Synapse to Thought.*** Hyder uses magnetic resonance (MR) methods to map physiology and chemistry that underlies brain function, for early disease detection but also for targeted drug delivery and monitoring treatments. MR imaging (MRI) is attractive because of its non-invasive and 3D nature to capture anatomical and functional information. The brain has high-energy demands to support its neural infrastructure and functional connections, but how much energy supports anatomical *vs*. functional needs is unknown. Pathways by which neural cells use nutrients to fuel their growth *vs*. function are measured by MR spectroscopy (MRS). Among our contributions are high-resolution functional MRI (fMRI) developments, but with calibrated fMRI for imaging energy demanded by activities at the neuropil. Another area is a new class of exogenous paramagnetic MRS agents with a method called Biosensor Imaging Deviation in Shifts (BIRDS) that can map pH and other physicochemical parameters important in cancer. These breakthroughs provide insights into the brain at work, from synapse to networks, in health and disease.



Partha Basu. ***Metals in Brain***. Basu described the importance of metal ions in neuroscience; after all, they are ubiquitous in tissues including brain, and are present as a part of biomolecules present in the brain tissue. These biomolecules often carry out important chemical transformation for normal functioning. Exposure to toxic metal ions can lead to accumulation of those ions in the brain tissues, impairing normal physiological functions. Detection and quantification of metal ions in the brain remain challenging, and consequently no atlas of metals in the brain has been developed. Newly developed fluorescent probes provide a means to take the inventory of select metals in the brain.



Sparacino-Watkins, C.E., Tejero, J., Sun, B., Gauthier, M.C., Thomas, J., Ragireddy, V., Merchant, B.A., Wang, J., Azarov, I., Basu, P., Gladwin, M.T. Nitrite reductase and NO synthase activity of

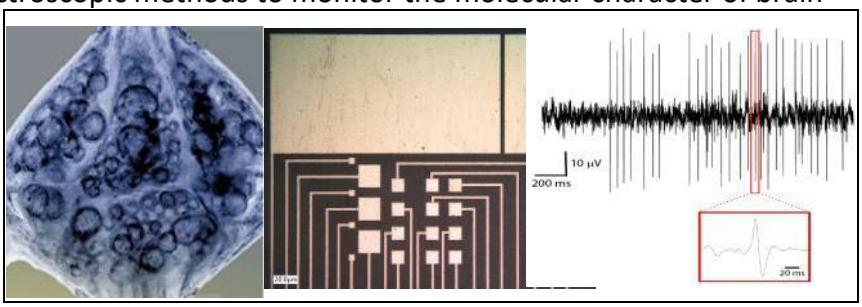
the mitochondrial molybdopterin enzymes mARC1 and mARC2C. *J. Biol. Chem.* 2014 *289*:10345-10358.

Deibler, K. and Basu, P. Continuing issues with Lead: Recent Advances in Detection. *Eur. J. Inorg. Chem*. 2013:1086-1096.

Session 6: *Sensors around neurons and in the brain*

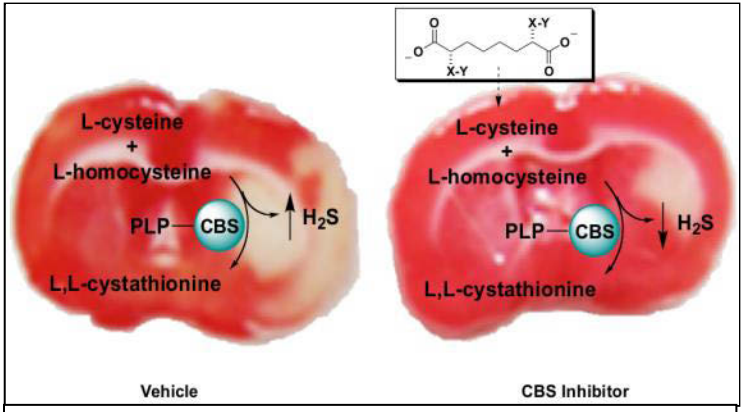
Presenters: Christy Haynes, David Berkowitz, Tim Glass, Lin Tian, Mande Holford

Christy Haynes. *Sensors around neurons and in the brain.* Haynes' talk focused on both electrochemical and spectroscopic methods to monitor the molecular character of brain cells or tissue either *ex vivo* or *in vivo*. She discussed the strength of electrochemical methods for monitoring secreted electroactive species with otherwise unavailable temporal resolution using both fast-scan cyclic voltammetry and amperometry. In addition, implantable chips are in development that include multiple electrodes to monitor both the firing of neuronal cells and the chemical messenger secretion from neuronal cells in a confined region of the brain. Haynes also suggested the potential utility of the vibrational spectroscopic method of spatially offset surface-enhanced Raman scattering (SESORS), a method that can be used to detect Raman active species through containers, to measure small molecules through the skull. Haynes concluded by identifying several materials and analytical chemistry challenges related to measurements in the brain, including challenges related to (1) electrode or SERS substrate fouling; (2) avoiding tissue damage during device implantation; (3) eliminate tethers between sensors and signal transduction equipment; and (4) measuring high value small molecule species that are not electroactive in water or don't present a useful chromophore.



David B. Berkowitz. *Understanding and Modulating the Control of Neurotransmitter Biogenesis via PLP Enzymes: Focus on H_2S & D-Serine.* Berkowitz spoke about the importance of pyridoxal phosphate (PLP)-dependent enzymes in neurochemistry; PLP enzymes generate key neurotransmitters including dopamine, GABA, serotonin, D-serine and hydrogen sulfide. The focus of the talk was on the mechanistic study and development of small molecule modulators for the enzymes that biosynthesize *D*-Ser (serine racemase) and H_2S (CBS; cystathionine β-synthase), respectively. *D*-Serine is to the first *bonafide D*-amino acid in human biology and H_2S is one of three known gaseous hormones.

Both neuromodulators display complex biology and are currently the subjects of intense study, the former being masked in a sea of *L*-serine and the latter being stored in a varied of polysulfide and protein hydrosulfation forms. Technological needs include the development of potent, specific chemical biological tools for the study of such neuronal signaling and communication mechanisms, as well as the improvement of non-intrusive analytical methods for selectively measuring neurotransmitter levels and for assessing activity levels of key neuronal enzymes.



Schematic view of small molecule modulators of CBS and their effect upon neuronal biology. Here, the bis*-hydrazine compound is assayed with the purified enzyme, in neuronal cell culture and* in vivo*. This mechanism-based inhibitor attenuates CBS activity in all three systems and decreases H_2S levels in living systems (rat brain model – collaborative with Peter T.H. Wong –NUS-Singapore). Figure designed by Matthew L. Beio, Christopher D. McCune, and Su Jing Chan.*

McCune, C.D., Chan, S.J., Beio, M., Shen, W., Chung, W.J., Szczesniak, L., Chai, C., Koh, S.Q, Wong, P.T-H., Berkowitz, D. "Zipped Synthesis" by Cross-Metathesis Provides a CBS Inhibitor that Attenuates Cellular H_2S Levels and Reduces Neuronal Infarction in a Rat Ischemic Stroke Model. *ACS Central Science* 2016 2 *(4):*242-252; doi:10.1021/acscentsci.6b00019

Nelson, D.L., Applegate, G.A., Berkowitz, D.B. A Useful Platform for Human Serine Racemase Studies: Variation of the Position-84 Base Reveals a Hotspot for β-Elimination Function and Provides Mechanistic Insight. 2017, submitted.

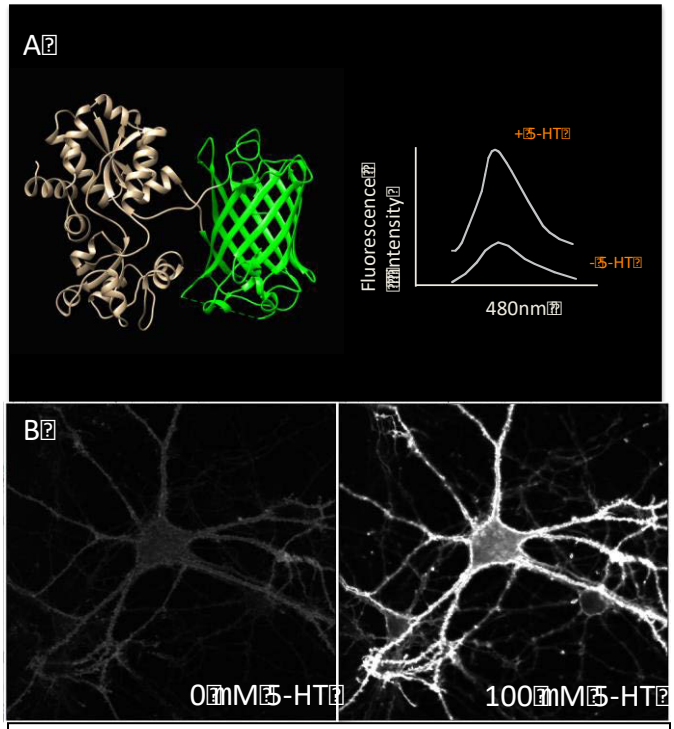


Figure A. Structure of genetically encoded indicator of neuromodulators. Ligand binding induced conformational changes leads to modification of chromophore environment of cpGFP from dark protonated state to bright deprotonated state. B. The fluorescent intensity increases in response to 100uM 5-HT in rat hippocampal neurons.

Lin Tian. ***Light up the brain with genetically encoded indicators of neural activity.*** To study the neural circuitry, the action of one cells under the context of others, one would precisely measure specific neuronal populations and molecules in behaving animals that are specifically engaged in performing the computation or function of interest. A key challenge has been difficulty of assessing a

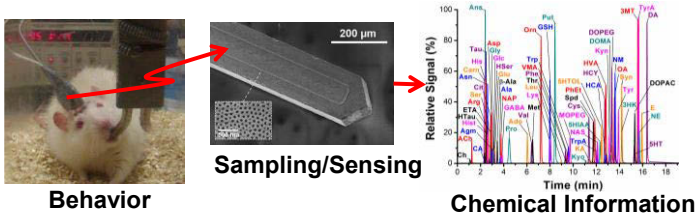
large invisible network of neuromodulators and neuropeptides with single-cell resolution. Tian discussed recent development and applications of genetically encoded indicators in measuring the spatiotemporal profiles of monoamine neuromodulators, such as 5-HT and norepinephrine, with sub-cellular resolution. We expect that a broad application of these tools will ultimately be important for uncovering emergent properties of neural circuitry.

Mande Holford. ***An Integrated approach to identify new tools for characterizing the brain.*** Holford described the characterization and discovery of novel peptide toxins to probe brain physiology. They use a variety of snails venoms, and she described the venom as a "cluster bomb" of hundreds of peptides and other small molecules. Venom peptides act as levers to turn on and off the ion channels—thus also relevant to pain and cancer. She is currently focusing on terebrids snails. She described the approach of "Venomics" to look at combine phylogenetics, transciptomics and proteomics to parse out the venom arsenal. Teretoxins are distinct from conotoxins (from another genus of marine snails).

Session 7: Engineered structures

Presenters: Robert Kennedy, Albert Folch, Han Xue, Hang Lu, Steve Weber

Robert Kennedy. *Monitoring neurotransmitters in the living brain*. Kennedy's goal is to identify chemical signals associated with behavior, learning, pharmacology, and pathophysiology. Understanding intercellular chemical signaling of the brain is critical for a complete view of neurotransmission. Reaching this goal requires meeting the "Grand Challenge" of measuring release and dynamics for all neurochemicals *in vivo* at high spatial and temporal resolution. Current techniques are electrochemical sensors, PET/MRI, genetically encoded fluorescent sensors, and microdialysis. Each has trade-offs that affect their utility. Emerging technologies based on microfabrication, novel assays, and flexible probes have potential for significant breakthroughs.



Probes in the brain of behaving animals allows in-depth analysis of the chemical changes underlying the behavior.

Kennedy, R.T. Emerging trends in *in vivo* neurochemical monitoring by microdialysis. *Curr Opin Chem Biol*. 2013 Oct; 17 *(5):*860-7.

Hamid, A.A., Pettibone, J.R., Mabrouk, O.S., Hetrick, V.L., Schmidt, R., Vander Weele, C.M., Kennedy, R.T., Aragona, B.J., Berke, J.D. Mesolimbic dopamine signals the value of work. *Nat Neurosci*. 2016 Jan; 19 *(1):*117-26.

Albert Folch. ***Microfluidic Chips for Measuring the Brain.*** Folch's talk focused on microfluidic approaches to observe and measure axon guidance growth *in vitro*. Compared to traditional, pipette-based or gel-based gradient generation approaches, microfluidic chips produce reliable, stable, and quantitative gradients. The Folch group has presented a gradient generator that is ideally suited for neurons because it is shear-free, its equilibration time is independent of MW, and it seeds/keeps cells in a convenient, accessible open chamber configuration. The approach can be parallelized and a device with >1,000 gradients in parallel has been used to show that the axons of E18 primary mouse hippocampal neurons respond differently to the same gradient of netrin when they are exposed to different concentrations of netrin: axons exposed to low (0-50 μg/mL) netrin concentrations were chemorepelled whereas axons exposed to high (150-200 μg/mL) netrin concentrations were chemoattracted.

Bhattacharjee, N., Li, N., Keenan, T.M., Folch, A. A neuron-benign microfluidic gradient generator for studying the response of mammalian neurons towards axon guidance factors, *Integrative Biology* 2010 *2*:669.

Bhattacharjee, N., and Folch, A. Large-scale Microfluidic Gradient Arrays Reveal Axon Guidance Behaviors in Hippocampal Neurons, accepted for publication by *Microsystems and Nanoengineering* (2017).

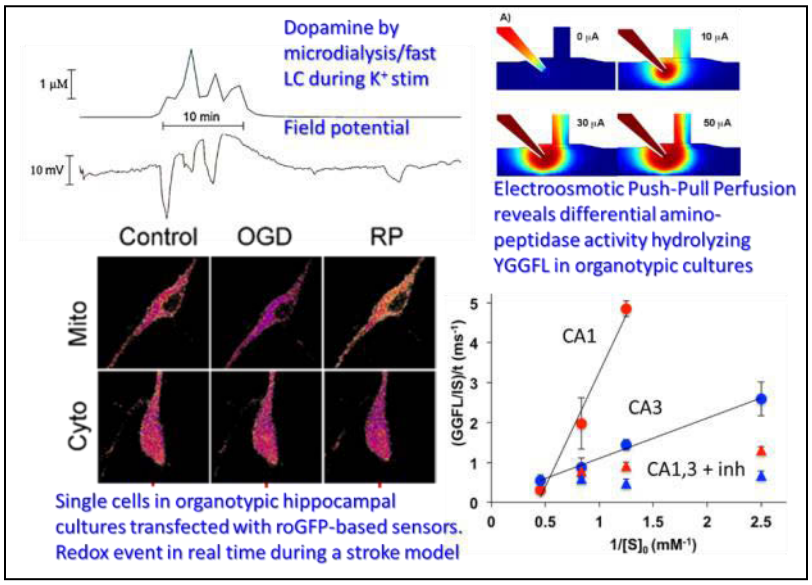
Xue Han. ***Photo uncaging of bioactive molecules for time resolved analysis of neurotransmitters and neuropeptides in the brain.*** Han's talk focused on the development of a new strategy that uses light to liberate encapsulated bioactive molecules from DNA nanostructures. This strategy works by encapsulating cargoes within DNA nanocages through a photo-labile cross-linker, and then release the cargoes upon brief exposure to light that cleaves the photo-labile linkers. Given the large size of the DNA nanoparticles, this strategy has been used to successfully uncage molecules ranging in size from small molecules to full-sized proteins. This novel molecular uncaging technique offers a general approach for precisely releasing a large variety of neurotransimitters and neuropeptides. Integrating this uncaging technology with high-resolution *in vivo* real-time imaging techniques, Han highlighted the potential of probing the function of specific neurotransmitters and peptide hormones in the brain with high spatiotemporal resolution.

Kohman, R.E., Cha, S.S., Man, H.Y., Han, X. Light-Triggered Release of Bioactive Molecules from DNA Nanostructures. *Nano Lett*. 2016 Apr 13; 16 *(4):*2781-5. doi:10.1021/acs.nanolett.6b00530. Epub 2016 Mar 3. PubMed PMID: 26935839.

Mohammed, A.I., Gritton, H.J., Tseng, H., Bucklin, M.E., Yao, Z., Han, X. An integrative approach for analyzing hundreds of neurons in task performing mice using wide-field calcium imaging. *Sci Rep*. 2016 Feb 8; (*6*):20986. doi:10.1038/srep20986. PubMed PMID: 26854041; PubMed Central PMCID: PMC4745097.

Hang Lu. ***Quantitative Neurobiology via Microfluidic, Automation, Genetic/Genomic, and Computational Approaches*****.** Lu's talk described quantitative neurobiology *via* microfluidic, automation, genetic/genomic and computational approaches. She emphasized the use of well-defined simpler models; in her case, she uses *C. elegans* as a model organism and studies the development and function of the nervous system, as an example, the genes affecting synaptogenesis. She highlighted her worm inside a chip device. She uses microfluidics and computers to streamline experiments and can handle thousands of worms per hour with this automated set up. Quantification is automated, image recognition software etc. With her approach, she has found classes of mutants and addresses the discovery of subtle alleles—phenotypic heterogeneity. Lots of future opportunities in genetic/genomics, including knock-ins, knock-outs, knock-downs, measurement tools, data management all moving towards understanding.

Stephen Weber. ***Better measurement for improved understanding of the brain.*** Weber emphasized improving the information content of selective measurements: redox behavior in stressed hippocampal pyramidal neurons, the fate of neuropeptides in the extracellular space, and measuring natural changes in extracellular neurotransmitter concentrations with improved time resolution by microdialysis[1-3]. Key objectives are making better measurements on the most relevant model (awake *in vivo* better than anesthetized *in vivo* better than slide better than cell culture …). Key future objectives are the facilitation of making measurements in more relevant models by minimizing the perturbation caused by the measurement and making multiple measurements simultaneously.



(1) Ngo, K.T., Varner, E.L., Michael, A.C., Weber, S.G. *ACS Chemical Neuroscience* 2017 (*8)*: 329-338.

(2) Yin, B., Barrionuevo, G., Weber, S.G. *ACS Chem. Neurosci.* 2015 (*6)*: 1838-1848.

(3) Ou, Y., Wu, J., Sandberg, M., Weber, S.G**.** *Anal. Bioanal. Chem.* 2014 (*406*):6455-6468.

Session 8: ***Opportunities in data analysis, informatics and integration.***
Presenters: George Komatsoulis and Bill Miller.

George Komatsoulis. ***The Commons Credit Model.*** As the associate director for the NIH Office of the Data Science, the presentation focused on data management issues. Big data is expensive to store, move, compute, and this is a growing problem as the amount of data increases. They are working on cloud computing to assist to deal with mobility, storage, on demand capabilities. He introduced a 'pay as you go' model; NIH is developing "The Commons" – a shared virtual environment to work on data sets, workflow, etc. Commons infrastructure will sit on public/private clouds. NIH will provide Commons credits to investigators and they spend these credits in the Commons. Market forces to set up a marketplace for useful resources. The eventual outcome is that the individual researchers can then decide what data to store and how accessible to make their data. This is a pilot project of NIH and may be expanded in the future.

Bill Miller. ***NSF investments to enable data-intensive brain research.*** Miller described NSF's investments in cyberinfrastructure for research; understanding the Brain (UtB) is NSF's umbrella program across NSF directorates. He emphasized a number of NSF programs that impact brain research including neurotechnology hubs, the NeuroNex proposals, and other NSF efforts, including XSEDE, available to all researchers, and more resources.

Appendix 2:
Contributions by Workshop Attendees Who Did Not Present

Peter Nemes. ***Single-cell mass spectrometry for advancing metabolomics and proteomics of neural fate specification in the vertebrate embryo.*** The Nemes Laboratory focuses on developing next-generation mass spectrometry approaches to characterize metabolites and proteins as single embryonic cells differentiate into neural tissues in the frog (*Xenopus laevis*) embryo and as neuron heterogeneity is maintained in the mammalian (mouse) nervous system. Obtaining these molecular insights will elevate our systems cell biology understanding of molecular mechanisms that are necessary for early patterning and long-term maintenance of the healthy nervous system. Technological needs range from development of specialized approaches to sample single identified cells, advancement of single-cell mass spectrometry sensitivity, and obtaining and integrating multi-omics (proteomics, metabolomics, and transcriptomics) data in developing systems.

Onjiko, R.M., Moody, S.A., Nemes, P. Single-cell mass spectrometry reveals small molecules that affect cell fates in the 16-cell embryo. *Proc. Nat. Acad. Sci.* 2015 *112*:6545–6550, doi:10.1073/pnas.1423682112, PMID: 25941375

Lombard-Banek, C., Moody, S.A., and Nemes P. Single-cell mass spectrometry for discovery proteomics: quantifying translational cell heterogeneity in the 16-cell frog (Xenopus) embryo. *Angew. Chem. Int. Ed.* 2016 *55*:2454–2458, doi:10.1002/anie.201510411, PMID 26756663

Lombard-Banek, C., Portero, E.P., Onjiko, R.M., Nemes, P. New-generation mass spectrometry expands the toolbox of cell and developmental biology. *genesis* 2016 *55*: e23012, doi:10.1002/dvg.23012.

Appendix 3:
Editorial Describing the Workshop

The Chemistry of Thought: The Role of the Measurement Sciences in Brain Research

Anne Milasincic Andrews, Rohit Bhargava, Robert Kennedy, Lingjun Li, and Jonathan Sweedler

Anal. Chem., **2017**, *89* (9), pp 4757–4757. **DOI:** 10.1021/acs.analchem.7b01364 Publication Date (Web): April 14, 2017

On April 2, 2013, the White House announced the BRAIN Initiative, a federal agency-spanning effort to stimulate the creation of new neurotechnologies for advancing our understanding of the brain. While the long-term goal is to elucidate brain function, there is an initial focus on tool development, with many analytical chemists involved in multiple efforts.

We were the organizing committee of an NSF-sponsored workshop held in October 2016 in Arlington, VA, on the role of the measurement sciences in moving this grand challenge forward, with a symposium held on this topic at Pittcon in March 2017. The workshop had 48 attendees representing a range of disciplines, government agencies, and programs.

As exemplified in the article: "*Why recruit more chemists? Neuroscientists don't know all of the chemicals that are active in the brain*," in ***Chemical & Engineering News***,(1) there are ample opportunities for chemists and measurement scientists to contribute uniquely by identifying and investigating the functions of important molecules involved in intra- and intercellular signaling. There are also many opportunities for chemists to create new tools (2) and therapeutics that will enable deeper understanding and control of brain function.

Workshop participants articulated a path toward creating novel chemistry-centric tools to enable new understanding of brain organization, activity, and function across the metazoan. Measuring the spatially and temporally dynamic chemical content of the brain is itself a grand challenge. As outlined in the prior decade's 2006 NSF Brain workshop on measurement challenges, a neuron can respond to an external signal by releasing the small gaseous cell-to-cell signaling molecule nitric oxide or by opening a mega-dalton ion channel complex; these molecules vary in mass by a hundred thousand-fold. A nanoscale synapse can be located at the end of an axon that is tens of centimeters away from the cell soma to which it transmits information. And, of course, synaptic connections, which function largely through chemical transmission, can vary their efficacy over milliseconds, yet memories persist for a lifetime.

These widely varying chemical, temporal, and spatial scales are difficult to bridge using existing measurement modalities, leaving many critical measurements unobtainable.

The technological challenges associated with *in vivo* imaging, molecular characterization, and speed and spatial resolution of measurements strike at the heart of chemical measurements

and, broadly, the fundamentals of chemistry. While enormous progress toward these goals has been made, much more remains to be accomplished. It is almost inconceivable that more than a decade into the -omics era, we still do not know the full "parts list" of the brain nor do we have a complete census of the cell types within this most complex organ in the body.

Addressing these and other challenges will be at the heart of chemical measurement efforts that will rely on experts in spectroscopy, spectrometry, separations, electrochemistry, electronics, optics, genetics, and nanotechnology as well as informatics.

A grand challenge for the integration of measurement and brain science remains: to map the full extent of neurochemical signaling in terms of its ***chemical***, ***spatial***, and ***temporal*** information and organization within the brain. We envision that new tools will enable fundamental questions related to the nature of memory and thought to be addressed. For more details on the workshop and these measurement needs, see the draft workshop report.

As measurement scientists, we are ready to embrace these challenges.

1. Widener, A. Why recruit more chemists? Neuroscientists don't know all of the chemicals that are active in the brain. *Chem. Eng. News* 2016 94 *(22)*:22– 23.
2. Andrews, A. M. Why monitor molecules in neuroscience? *ACS Chem. Neurosci.* 2017 *8*:211– 212, DOI: 10.1021/acschemneuro.7b00052