THE KAVLI FOUNDATION

Key Events in the Formation of the BRAIN Initiative
September 2011 – April 2013

Date	Event	Outcome	Documents
Sept 2011	Allen/Gatsby/Kavli Workshop: "Opportunities at the Interface of Neuroscience and Nanoscience"	Birth of BAM: White paper is written and sent to OSTP. Technological Foundations White paper written	Agenda Participants Group Photo OSTP White Paper Technological Foundations Paper

AGENDA

Saturday, 10th September 2011

From early afternoon Arrivals, registration and check-in at Chicheley Hall

18.30 Opening reception (Sarah Caddick, Miyoung Chun, Allan Jones; Winton Suite 2)

19.00 Dinner (Great Hall)

Sunday, 11th September 2011

07.00 – 08.15 Breakfast (Winton Suite 2/Great Hall)

08.30 Scene setting (Wolfson Lecture Theatre Hall 1)
Neuroscience by Cori Bargmann
Nanoscience by Paul Alivisatos

09.00 Perspective 'blast' session (three minutes each; Wolfson Lecture Theatre Hall 1)

- Each attendee will have a three-minute slot to present.
- Please talk about your work; your interests; ideas for promoting interaction within the fields; and/or current opportunities; etc.
- You can use PowerPoint slides; a flipchart with pens; or just talk.
- Given the brevity of the presentations, each attendee should plan on having not more than 3 or 4 slides if using Powerpoint.

10.15 Coffee break (Wolfson Suite Glazed Enclosure)

10.45 Blasts continue (Wolfson Lecture Theatre Hall 1)

12.00 Lunch (Great Hall)

13.30 Sessions 1 and 2 (Wolfson Lecture Theatre Hall 1)
Measurement and Control
Co-chairs: Paul Weiss and Rafa Yuste
10-minute summary talk on imaging -Elizabeth Hillman
10-min summary talk on electrophysiology - Massimo Scanziani
10-min summary talk on optogenetics - Karl Deisseroth
10-min summary talk on spectroscopy - Martin Hegner
Discussion

15.40 Coffee break (Wolfson Suite Glazed Enclosure)

16.10 Session 3 (Wolfson Lecture Theatre Hall 1)
Delivery and Transport
Co-chairs: Ed Callaway and Michael Roukes
5-min summary talk on current vectors for delivery into the brain/neurones - Ed Callaway
5-min summary talk on microfluidics and possible applications - Hongkun Park
5-min summary talk on low dimensional, nano-structures and possible applications - Philip Kim
5-min summary talk on compartments of neurones and parts of the brain that could be novel targets and associated issues - Matteo Carandini
Discussion

17.15 Session 4 (Wolfson Lecture Theatre Hall 1)

Materials and Functional Limitations

Co-chairs: Joanna Aizenberg and George Church

10-min summary talk on nano-fabrication (what is possible at moment, process, timescales, costs, etc) - Michael Roukes

5-min talk on the quantum domain - David Awschalom

5-min talk on power sources for nano-devices - Gabriel Aeppli

Discussion

18.20 End of Sessions

19.00 Pre-dinner drinks (Bar/Great Hall area)

19.30 Dinner (Great Hall)

Monday, 12th September 2011

07.30 – 08.45 Breakfast (Winton Suite 2/Great Hall)

Team breakout sessions – you will be assigned to a group.

09.00 First break-out (Main Hall Meeting Rooms 1, 2 & 3: groups 1+4; 2+5; 3+6 respectively)

10.00 Report back (Wolfson Lecture Theatre Hall 1)

10.30 Coffee break (Wolfson Suite Glazed Enclosure)

11.00 Second break-out (Main Hall Meeting Rooms 1, 2, & 3; groups 1+5; 2+6; 3+4 respectively)

12.00 Report back (Wolfson Lecture Theatre Hall 1)

12.30 Lunch (Great Hall)

14.00 Condensation of ideas (Wolfson Lecture Theatre Hall 1)

15.30 Coffee break (Wolfson Suite Glazed Enclosure)

16.00 Discussion: key points and priorities (Wolfson Lecture Theatre Hall 1)

Closing comments

18.00 End of sessions

19.00 Pre-dinner drinks (Bar/Great Hall area)

19.30 Dinner (Great Hall)

Tuesday, 13th September 2011

From 07.00 Breakfast (Winton Suite 2/Great Hall)

Check out/transfers to airport or train station

List of attendees

CONVENORS

1.	Allan Jones	Allen Institute	http://www.alleninstitute.org/about_us/staff/allan_jones.html
2.	Amy Bernard	Allen Institute	http://www.alleninstitute.org/about_us/staff/amy_bernard.html
3.	David Sainsbury	Gatsby	http://www.gatsby.org.uk/index.html
4.	Gary Wilson	Gatsby	http://www.gatsby.org.uk/index.html
5.	Jessica Roberts	Gatsby	http://www.gatsby.org.uk/index.html
6.	Max Liu	Kavli	http://www.kavlifoundation.org/officers-and-staff
7.	Miyoung Chun	Kavli	http://www.kavlifoundation.org/miyoung-chun
8.	Robert Conn	Kavli	http://www.kavlifoundation.org/conn-robert-w
9.	Sarah Caddick	Gatsby	http://www.gatsby.org.uk/index.html

NANOSCIENTISTS

1.	Dustin Tyler	Case Western	http://bme.case.edu/FacultyStaff/PrimaryFaculty/Tyler/
2.	David Awschalom	UCSB	http://awsch-web.physics.ucsb.edu/research/
3.	Elizabeth Hillman	Columbia	http://www.bme.columbia.edu/~hillman/Hillman.html
4.	Gabriel Aeppli	UCL	http://www.london-nano.com/our-people/academics/gabriel-aeppli
5.	Hongkun Park	Harvard	http://www.people.fas.harvard.edu/~hpark/
6.	Jeremy Baumberg	Cambridge	http://www.phy.cam.ac.uk/people/baumbergj.php
7.	Joanna Aizenberg	Harvard	http://www.seas.harvard.edu/aizenberg_lab/
8.	Martin Hegner	Trinity, Dublin	http://www.crann.tcd.ie/index/Research/PIResearch/HegnerPIPage
9.	Michael Roukes	Caltech	http://www.its.caltech.edu/~nano/
10.	Molly Stevens	Imperial	http://www3.imperial.ac.uk/people/m.stevens
11.	Paul Alivisatos	Berkeley	http://www.cchem.berkeley.edu/pagrp/overview.html
12.	Paul Weiss	UCLA	http://www.nano.psu.edu/~psw/Research.html
13.	Philip Kim	Columbia	http://www.columbia.edu/~pk2015/
14.	Viola Vogel	ETH	http://www.nanomat.mat.ethz.ch/

NEUROSCIENTISTS

1.	Catherine Dulac	Harvard	http://www.hhmi.org/research/investigators/dulac_bio.html
2.	Cori Bargmann	Rockefeller	http://lab.rockefeller.edu/bargmann
3.	David Anderson	Caltech	http://biology.caltech.edu/Members/anderson
4.	Ed Callaway	Salk	http://snl-c.salk.edu/SNL-Callaway/Callaway%20Lab.html
5.	George Church	Harvard	http://arep.med.harvard.edu/gmc
6.	John O'Keefe	UCL	http://www.ucl.ac.uk/cdb/research/okeefe
7.	Karl Deisseroth	Stanford	http://www.stanford.edu/group/dlab
8.	Massimo Scanziani	UCSD	http://biology.ucsd.edu/faculty/scanziani.html
9.	Matteo Carandini	UCL	http://www.carandinilab.net
10.	Rafa Yuste	Columbia	http://www.hhmi.org/research/investigators/yuste_bio.html
11.	Ralph Greenspan	UCSD	http://kibm.ucsd.edu/profile/2006-10/index.php
12.	Thanos Siapas	Caltech	http://biology.caltech.edu/Members/Siapas
13.	Tobias Bonhoeffer	MPI	http://www.neuro.mpg.de/english/people/bonhoeffer_t.shtml

OTHERS

1.	Brian Cox	Manchester	http://www.manchester.ac.uk/research/brian.cox/personaldetails
2.	Heather Mayfield	Science Museum	www.sciencemuseum.org.uk

THE BRAIN ACTIVITY MAP

Merging Nanoscience and Neuroscience for Science, Technology, and Health

Nanoscience and Neuroscience are at a crossroads. Each field is currently thriving in its own right, and the two are now on the threshold of an alliance that will mutually and significantly enhance one another. This alliance will provide a wide range of practical benefits.

The Ultimate Goal

The ultimate goal of this project is to construct the **functional connectome map of the human brain**, by assembling a coordinated network of researchers deploying next-generation nanotechnological tools with unprecedented capabilities. Mapping the functional connectome will unravel the fundamental, long-sought paradigms of how the brain computes. Together with these new technologies, this will enable accurate diagnosing, and restoring, of normal patterns of activity to injured or diseased brains; will foster the development of broader biomedical and environmental applications; and will produce a host of associated economic benefits.

What Is the Functional Connectome?

The connectome is a scientific effort currently under way that will give us a detailed anatomical highway map of the brain. The functional connectome, that we are proposing, will make that map comprehensible and useful by telling us about the traffic on those highways. It will map the patterns and sequences of nerve cell firing activity, and in so doing, reveal the brain's code.

Why We Need It

How the brain makes sense of the world is still basically unknown. One major reason for this is the distinct possibility that the neural code is a property that emerges from widespread patterns of nerve cell activity in many parts of the brain at the same time. Current imaging techniques are too local, and either too slow (fMRI) or too blurred (electro- and magneto-encephalography) to record these patterns with enough coverage, detail, and accuracy. If we can obtain higher resolution data over the entire brain, and then correlate the recorded activities with their anatomical circuits and behavioral consequences, we would be in a position to decipher the neuronal code, and to understand how it relates to behavior.

Why Now

Mapping of the functional connectome requires the development of a nanoscale analytical system of unprecedented complexity. Current technology for detecting, storing, and analyzing data of this sort exists to some extent for small brains, but a system of this type that can work at the scale of large animal brains, or even human brains, will require methods and technologies that are now emerging from the first decade of the National Nanotechnology Initiative. Nanosystems offer the only feasible way forward; they will allow for comprehensive coverage,

sufficient sensitivity, and minimal invasiveness in neurophysiological research. Nanosystems comprise large coherently engineered ensembles of nanodevices and nanoparticles, assembled in a fashion yielding capabilities that are immensely greater than the sum of the parts. The technological capabilities for producing such nanosystems *en masse*, can originate by leveraging the immense worldwide resources for producing microchips, and integrating these with bottom up fabricated nanostructures that can report on local neural phenomena with electrical and chemical specificity. The new innovation and capabilities developed in the course of this project will provide utility far beyond the bounds of this project and specific research application. They will open up new industrial avenues for our nation.

Anticipated Benefits

Easily imagined benefits from the project include devices and techniques for diagnosing brain disorders with much greater accuracy and sensitivity than are currently feasible, thus allowing a diagnosis to be made earlier in a disease's progress. Because the project will rely on stimulating nerve cells as well as monitoring their activities, the outcome will point the way towards workable strategies for interventions more refined and longer lasting than those currently used in deep-brain stimulation for Parkinson's disease and chronic depression. Even subtler manipulations can be foreseen for rebalancing circuits that have become imbalanced, as treatments for schizophrenia and autism.

On the engineering side, the nanosystems developed and deployed for this enterprise will have potential uses in a broad range of engineering and environmental applications, where sensitive, miniature, and intelligent systems can fulfill functions that are currently impossible with existing devices. This project realizes the potential for merging of nano- and bio-technology outlined several years ago in a "Nanotechnology White Paper"[1] published by the U.S. Environmental Protection Agency, which stated:

The convergence of nanotechnology and biotechnology with information technology and cognitive science is expected to rapidly accelerate in the coming decades. The increased understanding of biological systems will provide valuable information towards the development of efficient and versatile biomimetic tools, systems, and architecture.

Non-Obvious Economic Benefits

The economic activities emanating from this project are likely to reach far beyond those related to the outcomes outlined above. We anticipate profound attendant benefits such as occurred in the aftermath of the Human Genome Project. In fact, there are numerous similarities between what is proposed here and the Human Genome Project: it is a comprehensive approach to issues that had previously been treated piecemeal; it requires concerted team effort (unusual for basic researchers); it is a project that will be based on large-scale deployment of new technologies and, as such, requires formidable strategic thinking and the assembly of substantial technological resources; it is an initiative that capitalizes on emerging technologies to open up entirely new realms of scientific inquiry and economic activity; and it falls outside of the current funding programs because of its bridging of distant fields, and its ambitious scale. And like the Human Genome Project, the resulting economic benefits are

[1] http://www.epa.gov/nanoscience/files/epa_nano_wp_2007.pdf

likely to be much broader and greater than anyone imagined, and perhaps will be realized much sooner than anticipated.

A recent report from the Battelle Technology Partnership Practice found that the $3.8 billion investment in the Human Genome Project has generated $796 billion in economic impact.[2] The economic modeling study showed that every dollar invested in the U.S. elements of the project generated $141 in the economy. They estimated that in 2010 alone, academic and commercial genomic sequencing and research supported 310,000 jobs and generated $67 billion in economic output.

This exceptional return on investment is probably an underestimate, since the major anticipated impacts of the Human Genome Project on health care are still merely on the verge of realization. The study's authors conclude that the project is "arguably the single most influential investment to have been made in modern science."

The State of the Art

Although neuroanatomy is a very old science, connectome biology is relatively new. The first complete nervous system wiring diagram was accomplished with the 300 cell nervous system of a model organism, the nematode in the 1980's.[3] The next example is only now coming online for another model organism, the 150,000 neuron brain of the fruit-fly *Drosophila*.[4] A connectome project for the mouse (4 million neurons) is just being launched[5] with an anticipated completion time of at least 5 years, and the prospects for having a human connectome map (100 billion neurons) is decades away.[6] All of these efforts have proceeded slowly because of the painstaking nature of determining the shape of each individual nerve cell. Perhaps surprisingly, the technical barriers to obtaining the functional connectome map are not as daunting, and are much closer to being solved, than for the anatomical connectome map.

Functional mapping can currently be done on the smallest of these brains, the nematode *C. elegans* and the fruit fly *Drosophila*, and on the superficial layers of the mouse brain, but the sensitivity and speed of the recordings is not yet adequate. The tools are proteins or chemical dyes that fluoresce when calcium levels in the cell change, a technology originally applied to

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[2] http://www.battelle.org/publications/humangenomeproject.pdf; http://online.wsj.com/article/SB10001424052748704681904576315253143162630.html

[3] White JG, Southgate E, Thomson JN, Brenner S. (1986) The structure of the nervous system of the nematode *Caenorhabditis elegans.* Philos Trans R Soc Lond, SerB, Biol Sci. 314: 1-340.

[4] Chiang AS, et al.. (2011) Three-dimensional reconstruction of brain-wide wiring networks in *Drosophila* at single-cell resolution. Curr Biol. 21: 1-11.

[5] Bohland JW, et al..(2009) A proposal for a coordinated effort for the determination of brainwide neuroanatomical connectivity in model organisms at a mesoscopic scale. PLoS Comput Biol. 5: e1000334.

[6] Sporns, O., Tononi, G., Kötter, R. (2005). "The Human Connectome: A Structural Description of the Human Brain". PLoS Computational Biology 1 (4): e42

nerve cell recording[7] and more recently combined with advances in microscopy to enable recording from up to 1,000 nerve cells at once in brain tissue up to half a millimeter thick.[8]

The technology for targeted stimulation of nerve cells has existed for many years in the crude form of implanting electrodes into the brain. More recently, a finer grain technique has been developed that uses light-activated proteins to stimulate nerve cells.[9]

Technology has been demonstrated for producing nanoscale probes capable of sensitive and fast responses to changes in voltage.[10,11] This is just starting to be exploited for use in nerve cells, but capabilities for its large-scale integration and production not been assembled, hence its subsequent wide deployment to the neuroscience community has not yet become possible.

Next Steps

By analogy to the role played by *C. elegans* and *Drosophila* in troubleshooting and laying the foundations for the Human Genome Project, these model systems will play a similar role in part of our progression towards the human functional connectome. On this front, we will start with existing technology (calcium imaging and state-of-the-art microscopy) to produce initial functional connectome maps. The same technology will be used on a piece of mouse brain cortex (10,000 cells) that is thin enough to be imaged. A key element in our strategy, which will permit us to determine the input and output for each nerve cell, is the ability to stimulate in a targeted and controlled manner. This will be done with the existing optogenetic technology, which is available for all three model organisms.

In parallel, on a second front, we will develop and deploy a new generation of integrated nanoscale probes that combine integrated electrophysiological and photonic technologies. We will also pursue development of new classes of nanoparticles permitting optically based neurophysiological stimulation and recording from local, very selectively targeted regions of neuronal tissue. Together these nanotechnologies will enable simultaneous observation and stimulation across deep, vast, and previously-inaccessible regions in the brain, and thus enable the construction of a full functional connectome map. We will strategically assemble resources to enable robust mass-production of systems that utilize these tools in a highly reproducible manner, multiplexing them at unprecedented scale, and deploying them to newly-assembled networks of researchers who will become capable of mapping the functional connectome.

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[7]Smetters D, Majewska A, Yuste R. (1999) Detecting action potentials in neuronal populations with calcium imaging. Methods. 18: 215-221.

[8]Nikolenko V, Watson BO, Araya R, Woodruff A, Peterka DS, Yuste R. (2008) SLM Microscopy: Scanless Two-Photon Imaging and Photostimulation with Spatial Light Modulators. Frontiers in Neural Circuits. 2: 5.

[9]Fenno L, Yizhar O, Deisseroth K. (2011) The development and application of optogenetics. Annual Rev Neurosci. 34: 389-412.

[10] See, for example, Du J, Riedel-Kruse IH, Nawroth JC, Roukes ML, Laurent G, Masmanidis S. (2010) High-Resolution Three-Dimensional Extracellular Recording of Neuronal Activity With Microfabricated Electrode Arrays. J. Neurophysiology 101(3): 1671, and references contained therein.

[11]Du J, Roukes ML, Masmanidis S (2009) Dual-side and three-dimensional microelectrode arrays fabricated from ultra-thin silicon substrates. J. Micromechanics Microengineering19(7): 075008

The Way Forward

Perfecting the technological capabilities, mass-producing the requisite nanosystems, and assembling the collaborative research networks that will use them constitutes the core vision for the primary organizing phase of this effort. The initial research phase consists of producing the preliminary functional maps in model systems, outlined above. Beyond these immediate next steps, we envision working towards the ultimate development of subsequent generations of untethered, nanoscale neural probes that can locally acquire, process, and store accumulated data, and that would ultimately be configurable into a communications network within the tissue. Such device networks could potentially address the long-standing problem of how to obtain sufficient coverage in deep tissue layers. These networks of "intelligent" nanosystems would also be capable of providing specific responses to externally applied signals, or to their own readings of brain activity. Their responses could be used to trigger nerve cell activity in a measured manner, and could comprise the first steps in controlled restoration of normal patterns of activity in damaged brains. This would initially be done in animal models for brain injuries, such as TBI and PTSD, or for psychiatric disease.

The Authors

A group of scientists, whose research spans neuroscience, nanoscience, genomics, and systems biology have collaborated to produce this document. This group – Paul Alivisatos (LBNL and UC Berkeley), George Church (Harvard), Ralph Greenspan (UCSD), Michael Roukes (Caltech), and Rafael Yuste (Columbia) – formed at a recent workshop exploring the juncture of nanoscience and neuroscience that was organized by The Kavli Foundation, the Gatsby Charitable Foundation, and the Allen Institute for Brain Science. The workshop was held in September, 2011 at Chicheley Hall, home of the Kavli Royal Society International Centre, UK.

Date: **31 Oct 2011**
Version: **8**

The Brain Activity Map: **Technological Foundations**

Overview

This document is intended to provide an overview of the technical underpinnings that must be assembled to map the "functional connectome" of the brain. We start by listing the overarching technical goals of this national program, delineating the underlying assumptions made in defining these goals. We then formulate a list of requisite technological thrusts, with specific milestones, that we see as necessary to achieve these program goals. We conclude by drawing parallels of the project's scope with that of the Human Genome Project.

A) Technical Goals

We identify four principal goals:

- Ultra-large-scale, extremely high-density **measurement** (recording) of neural activity across widely distributed brain areas.
- Ultra-large-scale, distributed **stimulation** of neural circuits with arbitrarily complex spatiotemporal patterns and extremely high spatial resolution;
- Realization of large-scale **system integration and production *en masse*** to enable creation of powerful and robust "tools" permitting this work to be undertaken
- **Validation** of the technology, its wide-scale **deployment** to the academic and corporate neuroscience community, and **user training** will be required.

These are each outlined below.

Measurement (Imaging/Recording):

To elucidate the functional connectome, the type of measurements we envisage will involve the following:

a) Measurement with single-neuron resolution of both the **extracellular potential** (spiking) and **intracellular potential** (that is, spiking and, ultimately, subthreshold behavior) are of interest -- at the characteristic magnitudes and time scales on which they occur. (For both intracellular and extracellular potentials, sensitivity at least at the ~20uV level with 10KHz bandwidth is necessary.)

b) **Multiparametric physical/chemical measurements**, that is, recording the temporal dynamics of neurophysiological parameters beyond potentiometric measurement alone, are of interest. Such measurements may include: local chemical concentrations (*i.e.* the chemical analog of a local field potential), dynamical local forces involved in neural association, *etc*.

c) **Local interactions** with a "probe" or "reporter" located <100um from neuronal bodies will be required for measurements with single-neuron resolution and <1nm from the plasma membrane for optical voltage measurements. The methods to be developed will always be carried out through some form of remote readout (see below), however we anticipate that a **local reporter** will be often involved. This will be placed no further than the length scale (exponential

decrement) characterizing the spatial decay of the "fields" (electrical, chemical, force) of interest. Such probes/reporters may be arrays of narrow neural probes (shanks) inserted deep into the brain tissue, functional nanoparticles placed in the membrane either by direct intervention or developmental transport, or, ultimately, complex functional nanosystems capable of both local recording and local storing of the time records of the "fields" they measure.

d) **Deep interactions** (measurements) with neural tissue are required. As an example, for the rat, requisite interaction depths will typically be 0-10 mm. For primates, even deeper interaction lengths will be required.

e) **Non-local readout** of the neuronal fields[1] is essential to transport the acquired data to external measurement and analysis systems. In first phase efforts this will be achieved through direct connection involving electrical leads, optical fibers/waveguides, etc. Later generations may involve non-local interrogation fields, such as radio-frequency waves, of types that are capable of deep penetration of neural tissue. For the case of autonomous (untethered) probes, capable of local sensing and local data storage, an entirely different class of readout could involve tissue dissection and recovery of the individual probes -- in which case their internal data would be subsequently downloaded.

f) Highly **biocompatible interactions** with neural tissue are required, to prevent gliosis and attendant loss of sensitivity -- and, thereby, to enable chronic recording.

f) **Dense distributed recording with single-neuron resolution** to decipher a complete picture of the functional connectome.

g) Definitive identification of the **spatial location** within the brain of *each* of the local probes/reporters is essential, in order to correlate the measured functional connectome with the morphological connectome. For non-isotropic neuronal fields, **spatial orientation** of direction-sensitive probes/reporters may also be required.

Stimulation:

To elucidate the functional connectome, we anticipate that direct **stimulation** of specific neurons (followed by *measurement* of the response to such stimuli) will be essential. We envisage such stimulation will involve the following:

a) **Electrical stimulation** at the single-neuron and, possibly, at the single-synapse level. This could be carried out by direct electrical stimulation from the extracellular environment via electrophysiological probes, or, indirectly, through optical stimulation of membrane-bound, genetically inserted light-sensitive ion channels, or through photo-uncaging of active neurotransmitters (such as glutamate, GABA, Ach or glycine).

[1]Here we use "neuronal field" to mean the time-varying fields (electrical, chemical, mechanical, …) driven by the behavior of a *single* neuron. This is to avoid confusion with "local field", used in neurophysiology to convey an average field generated by multiple neurons within some characteristic volume.

b) **Multiparametric physical/chemical neurostimulation**, that is, stimulation by alteration of neurophysiological parameters beyond direct neuronal depolarization. Such stimulating interactions may include: local delivery of neurochemical stimuli through microfluidic devices, mechanostimulation by imposition of dynamical local forces through, e.g. nanoelectromechanical actuators, *etc*.

c) **Stimulation of artificial actuators** through signals applied, presumably, <<100um from neuronal bodies, so as to achieve selective, single-neuron effects. Similar to the case for local recording, the stimulation methods to be developed will always be controlled through some form of remote input (see below), however we anticipate that **local trigger** might be involved. This local entity, although controlled remotely, would deliver the stimulus only to a restricted, specifically-targeted region. Such probes/triggers may be arrays of narrow neural probes (shanks) inserted deep into the brain tissue, or functional nanoparticles placed either by direct intervention or developmental transport.

d) **Deep interactions** (stimulation) with neural tissue are required. The previous examples for recording also hold here -- for the rat, requisite interaction depths are ~10mm. For primates, even deeper interaction lengths will be required (several centimeters).

e) **Non-local control** of the stimulating fields is essential to permit automated, spatially and temporally complex stimulation protocols under computer control. As in the case for recording, in first phase efforts this will be achieved through direct connection involving electrical leads, optical fibers/waveguides, etc. Later generations of technologies may involve non-local interrogation fields, such as radio-frequency waves, that permit deep penetration of neural tissue (unlike optical fields).

f) Highly **biocompatible interactions** between the probes/triggers and neural tissue are required, to prevent gliosis and attendant loss of effectiveness.

f) **Dense, distributed stimulation with single-neuron resolution, using natural sensory stimuli (e.g., visual, auditory, olfactory, tactile, and proprioceptive)** will ultimately be necessary, in order to decipher a complete picture of the functional connectome.

g) Deterministic programming of **spatial location** within the brain for *each* of the local probes/triggers is essential, in order to correlate the functional connectome with the morphological connectome. For non-isotropic stimulation fields, **spatial orientation** of direction-sensitive probes/triggers may also be required.

System Integration and Production

Academic research is, and will continue, driving specific innovations in nanodevices, nanoparticles, and synthetic & molecular biology to create new technology for this program. The complex technology we will assemble for this project requires the coherent concatenation of many such individual innovations. Systems-level engineering and **system integration** and of new powerful and robust “tools” (instruments) is required. Further, these tools need to be widely deployed to address the monumental task of deciphering how brains work; accordingly, **mass**

production of these new tools, with fidelity and uniformity, will be essential. Similarly, there will be a need for developing mathematical and computational strategies capable of amalgamating and analyzing the massively parallel time series data that will be generated from the measurements described above.

The integrative nature of this work and the capabilities required to achieve them places these technical goals well outside the scope of what can be accomplished by single-investigator-driven university research. Their pursuit must involve highly coordinated efforts between state-of-the-art microelectronic foundries, experts in instrument development and assembly, directed characterization and validation (discussed below), and centers devoted to computational innovation. This is unlikely to be jump-started in the corporate sector in the near term. No viable business model can be currently formulated to finance a program for prototype instrument development and manufacturing. Hence, we believe this essential work must be initiated through a sharply focused national initiative that would create a center, or several centers, that will amass the requisite technological competencies.

Validation, Technology Deployment, and User Training

In parallel with technological development, wide-scale **technology deployment** to the academic and corporate neuroscience community will be required, as well as the requisite **user training** in its use by a cadre of technological experts from the principal technology center(s). Early stage adopters of the technology will provide the absolutely essential role of **validation** of the technology by, for example, pilot neurophysiological studies at progressively increasing scales. These beta-testing projects will pave the way to wide-scale technology deployment of robust, well-characterized tools to the academic and corporate neuroscience community. Examples of locations that could serve as teaching sites for these new technologies are the neuroscience techniques summer courses held at the Marine Biological Laboratory in Woods Hole and in Cold Spring Harbor Laboratories.

B) Technical Milestones

To achieve the goals listed above, we anticipate that pursuit of the following technological thrusts will be necessary:

1. Large-Scale Measurement/Recording

- Our first goal is to measure activity from very large numbers of neurons in a model nervous system (for example: *C.elegans*, Drosophila and mouse neocortical brain slices. This will begin with a parallel efforts using optical, electrical and genomic techniques i) optical imaging (e.g., by two-photon methods) of neural circuits loaded with either calcium indicators or voltage-sensitive dyes, and ii) electrophysiological interrogation of neural tissue via neural probe arrays enabling highly multiplexed electrical recording and

(iii) indirect reading of neuronal activity using genomic signatures of it, such as DNA sequence errors that correlate with the spiking patterns generated by calcium-dependent polymerases

- Innovation to develop new classes of nanoparticle reporters of voltage appear as a crucial aspect of this work. For example, the creation of highly-sensitive potentiometric reporters at small enough scale to embed within the neuronal cell membrane would permit a new era of large-scale recording of the intracellular potential, simultaneously, of large numbers of neurons.
- Critical to the success of large-scale efforts to permit deep tissue imaging/recording will be the packaging developed to interface the multichannel technology with the subject (e.g. animal) under test. This should permit minimally invasive, free movement of the awake animal with minimal-to-no discomfort.
- Subsequent technological innovation will permit deep electrophysiological or optical measurements using dense arrays of electrical or optical probes based on integrated photonics coupled with next-generation nanoparticle or genetically-introduced voltage-sensitive reporters. Local introduction of calcium or voltage-sensitive reporters may require probe technology comprising co-integrated microfluidics to permit controlled local delivery of fluorophores or viral vectors.
- Among possible long-range technological goals might be the development of complex, untethered, nanoscale probes – which could be based on semiconductor nanotechnology or synthetic biology – that permit local sensing, local data storage, and, potentially, remote interrogation/activation by externally-imposed electromagnetic fields (e.g. at radio frequencies).

2. Large-Scale Stimulation

- Our second goal it to be able to stimulate individual neurons in the nervous system independently at first, then combinatorially and also by means of natural sensory stimuli. This will first begin with: i) optical stimulation (e.g. by two-photon uncaging or photoactivation) of neural tissue perfused with optogenetic constructs or caged compounds, and ii) electrophysiological interrogation of neural tissue via neural probe arrays enabling highly multiplexed electrical stimulation. Subsequent technological innovation will permit deep electrophysiological measurements using dense arrays of probes based on integrated photonics coupled with next-generation nanoparticle or genetically-introduced optogenetic reporters.
- For optogenetic stimulation, new classes of genetically introducible, light-sensitive ion channels providing higher sensitivity and more varied spectral coverage will need to be developed, particularly ones that can be excited with two-photon light, to permit single-cell resolution in vivo.

3. Local Reporters, Passive (Genomic and Nanoparticle Reporters and Stimulators)

- Our third goal is to enable patterned stimulation, in principle in any arbitrary spatio-temporal pattern. In a way, this will resemble “playing the piano” with the neural circuit.

- To achieve any arbitrary stimulation patterns, optical efforts will harness holographic methods based on phase-only spatial light modulators (SLMs), using two-photon excitation.
- For electrical stimulation, dense arrays of neural probes can be used for highly-multiplexed, complex patterns of neural stimulation. Work to optimize the stimulation electrode/tissue interface to permit stable, long-term stimulation is critical; use of nanotechnology-based approaches to deterministically engineer optimal electrode interfaces is a unexplored area that will be tackled.
- Zero-, one-, and two-dimensional artificial nanostructures and nanoparticles will play critical roles in this project.
  - Zero-dimensional nanostructures can be manipulated to produce a new generation of local optical reporters for neuroscience. These reporters will need to be capable of being embedded into neural membranes (thickness ~5nm) and of being sensitive to local electric fields as well as local chemical environments. The design of these nanostructures will draw from the newly established ability to control plasmonic behavior in metallic nanoparticles, quantum size effects in semiconductor heterostructures with designed asymmetries, and nanoparticles with embedded dopants possessing sharp emission spectra. These inorganic nanoparticle optical probes can be tuned for to match the photon energy requirements of the various excitation and detection systems. Further, compared to organic optical probes, they will be photochemically robust during extended interrogation. They will need to be combined with organic nanostructures, that is, biofunctionalized, to direct/embed them within neural membranes or synapses. They may be combined with selective molecular binding moieties to confer sensitivity to changes in local neurotransmitter concentrations.
  - One-dimensional structures such as nanotubes and nanowires may be used for highly local electrical measurements, for the delivery of photons to specific locations, and for the local release or collection of chemicals.
  - Two-dimensional nanostructures such as graphene may be engineered into artificial membrane patches, providing new interfaces of our electrical systems to biological membranes.
- Among possible long-range technological goals might be the development of complex, untethered, nanoscale probes – which could be based on semiconductor nanotechnology or synthetic biology – that permit local sensing, local data storage, and, potentially, remote interrogation/activation by externally-imposed electromagnetic fields (e.g. at radio frequencies).

4. Long-Term Brain/Probe Interaction

- The goal of the project is to develop long-term approaches that can observe and manipulate the response of whole brains to complex stimuli. This requires non-perturbative interactions between the technological interfaces that are introduced into brain tissue.

- The response of neural tissue to foreign entities, that is, to each individual probe/reporter/stimulator introduced for this work must be understood and controlled. This will require coordinated work between the technology developers and researchers in **anatomical neuropathology**.
- The **biocompatibility** of materials used in the development of the nanodevices and nanoparticles must be assessed. This will involve coordinated, iterative research in close collaborations between technology developers and neurophysiological researchers.

5. Database Assembly and Computational Analysis

- This project will generate immense volumes of data in the form of multichannel time records of neuronal activity. These channels will be indexed by position within the brain, and time-correlated with various complex stimuli (which also could be complex, multichannel time records of directly-applied neural stimuli).
- Data mining of this immense body of digital information will require new paradigms in multidimensional correlative analysis of massively parallel time series data.
- New models for brain processing must aid and inspire this numerical analysis. These models are likely to originate by new collaborations involving engineers with expertise in the theory of networks, time series analysis, and physicists with expertise in synchronization phenomena and the nonequilibrium thermodynamics of pattern formation.
- Centralized supercomputer facilities to enable the handling of the massive data sets and their numerical analysis and physical modeling will be essential for this work.

6. Large-Scale Systems Integration

- Large-scale integration of the micro- and nano-scale devices used as the “front ends” of these systems must be fabricated en masse in state-of-the-art semiconductor foundries. Sufficient resources to permit research and development to arrive at stabilized processes for the wafer-scale production of device arrays will be essential.
- Large-scale production techniques for nanoparticle probes will be required. Once the prototype reporters or stimulation particles are perfected, their mass production will likely involve engaging a commercial factory that adheres to “best practice” standards.
- Creation of systems-level instrumentation will require assembly of a cadre of engineering experts. Small-scale production of such complex instrumentation is feasible within a center with a dedicated professional staff. (An analogy here could be made to the building of space probes, which is undertaken in similar fashion at, for example, Caltech’s Jet Propulsion Laboratory. Such a paradigm can provide prototype instrument systems to a community of beta-testers selected and engaged to help drive the program forward.
- Mass production will ultimately be required to deliver the technology in large scale to the neurophysiology community who will actually map the functional connectome. This is probably best done by commercializing the beta-tested prototypes; this will permit infusion of commercial capital to optimally engineer the systems for production.

7. Local Reporters, Active (Smart Nanoprobes)

- Long term possibilities may include the creation of "smart nanoprobes" achieved through either synthetic biology or advanced nanotechnology.
- The synthetic biology approach would involve developing voltage sensitive reporters that would record time records of neural response embedded through protein or nucleic acid synthesis into artificial constructs (for example using an ion-sensitive processive polymerase). Post-experiment dissection and analysis would permit subsequent readout of these local time records of neural response (for example by in situ DNA sequencing).
- The nanotechnological approach would involve engineering local potentiometric sensors within, say, ten-micron-scale capsules. These capsules would also include sufficient memory to store time records of local neuronal signaling and/or a means of real-time readout of the data. Such entities could be powered by immersion of the brain into radio-frequency fields or red light. Post-experiment data acquisition could be carried out either through dissection and downloading (as discussed for the case of the synthetic biology approach) or by remote interrogation.
- Remote interrogation of implanted smart-nanoparticle probes will require both assigning a position to each nanoparticle probe, and sequential download of their information in a coherent manner.

C) The Brain Activity Map: **Overarching Perspective**

We briefly address the scope and complexity of the undertaking we envisage, and compare it to known, present benchmarks.

In 1990, the possibility of sequencing a viral genome of 100,000 base pairs (at an error rate of 0.001) was considered feasible, but sequencing one human genome of 3×10^9 bp was considered unrealistic. Today, one group with 20 machines is able to sequence 10,000 human genomes per year; in other words a single group can sequence **6×10^{13} bp** = $10^4 \times (6\times10^9$ bp) at an error rate of 1×10^{-5}. This generates about 3×10^{15} bytes (3PB) of data. It is noteworthy that the fast pace of continuing technological advances are, at present, multiplying this already-impressive capacity by roughly 5-fold per year.

At the outset of the Human Genome Project, even the most rudimentary task of genomic combinatorics – that is, comparing each short region to each other region – was predicted by some to require an unapproachable number, $>1\times10^{19}$ $=(3\times10^9)^2$, of computer operations. Today, many clever linear algorithms (such as BLAST) have displaced the naïve N-squared algorithms (like Needleman-Wunsch) invoked at the outset of genomics analysis. These advances have allowed combinatorics analysis using 3×10^9, and not $(3\times10^9)^2$, separate calculations. Also, it has since been discovered that genomic data are much more

compressible than some originally thought; in general, 5×10^6 bytes (5MB) is sufficient per each 6×10^9 bp genome. This compressibility has translated into reduced requirements, from those originally projected, for genomic data.

We now draw analogies to the structural complexity of the brain. The human cortex comprises 1.6×10^{10} neurons (80% glia), the whole human nervous system 1×10^{11}; the mouse cortex 4×10^6 neurons (65% glia), the whole mouse nervous system 4×10^7.

To estimate **data storage capacities** required for a brain activity map we consider the anatomic connectome. Bock *et al*. (Nature 2011) have covered 1500 cell bodies with 1×10^{13} raw pixels. By analogy we can estimate that 7×10^6 mouse cortical cells would require something of order 5×10^{16} bytes. We note that this is less data than the current genome image data worldwide. Further, astronomy and astrophysics are already awash with data; currently 1 PB (1 petabyte = 10^{15} bytes) of public data is electronically accessible, and this volume is growing at 0.5 PB per year. The availability of this data has transformed astronomy research. Projections indicate that by 2020, more than 60 PB of archived data should be accessible to astronomers.

We turn to estimates of **data bandwidth** required for real-time imaging of brain activity. For an activity map of the brain, a bandwidth of 10^4 bytes per second per neuron may be required. For 3×10^9 actively firing neurons in the brain this would correspond to a raw data acquisition rate of 3×10^{13} bytes (30TB) per second. As a benchmark, in 2012 the Australian Square-Kilometer Array Pathfinder (ASKAP) radio telescope is on track to handle a data stream of 10TB/second from the telescopes to its digital correlators. This bandwidth will be aggressively pushed for the next generation radio telescopes, estimated to require 100X the 2012 bandwidth.

In more close consideration of the **complexity** of the grand challenge of mapping brain activity, we acknowledge that direct analogies to genomic bioinformatics and data handling are, most likely, somewhat limited. For example, the informatics associated with brain activity mappings are of much higher dimensionality than are linear genomics sequences. Brains are dynamical systems with operations on a very wide range of time scales. Their component neurons are complex dynamical systems in their own right, and the synapses between them are plastic over a vast hierarchy of time scales (from milliseconds to, presumably, years). The spectrum of behavior of even the simplest neural circuits (for example, two reciprocally connected inhibitory neurons) has many solutions – comprising a few stable and many unstable ones. These solutions are all dependent on dynamic parameters describing the neurons and the connections between them.

Hence, in addition to the sheer numbers, brain activity maps will differ from genomics in other ways. Prominent among these are: (i) resulting combinatorics, (ii) the state dependence of interactions between neurons (from short-term facilitation to more complex nonlinear interactions) and (iii) neuronal biophysics, which are extremely varied, adapted and complex. Further, to make headway, it is most likely that some foreknowledge will be required of the

function of neural circuits. To date, it has not been possible to predict function (as in: to compute invariance to size or contrast) from connectivity.

Given these considerations, we anticipate that connectivity studies will likely need to be embedded in functional studies within a "traditional" framework (e.g. hippocampal function and spatial coding, or memory formation and retrieval, etc.). In other words, brain activity mapping will likely have to focus first on near term studies of brain subsystems: networks, circuits, and areas. Acquired data will become truly valuable when both connectivity and functional studies are carried out in the same brain. For this reason, we will initially espouse focusing on technologies that enable ultra-dense neuronal sampling of small brains, or in restricted areas in behaving animals, to studies that focus on being exhaustive over the entire brain, at the expense of compatibility with simultaneous functional studies.

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This working group, with members whose research spans neuroscience, nanoscience, genomics, and systems biology, formed at a recent workshop exploring the interface of neuroscience and nanoscience. The workshop was organized by The Kavli Foundation, The Gatsby Charitable Foundation, and the Allen Institute for Brain Science and was held in September 2011, at Chicheley Hall, home of the Kavli Royal Society International Centre, UK.