The Brain Activity Map and Functional Connectomics

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Abstract

The function of complex neural circuits in the brain is an emergent property based on the coordinated activity of immense numbers of neurons. We propose launching a large-scale, international public effort, that we are calling the *Brain Activity Map Project*, to reconstruct the full record of neural activity across complete neural circuits. We are convinced this will prove to be an invaluable step toward understanding both fundamental and pathological brain processes.

Emergent Properties of Brain Function

Understanding the brain is arguably one of the greatest scientific challenges of our time. Although there have been many piecemeal efforts to explain how different brain regions operate, no general theory of brain function is universally accepted. A fundamental underlying limitation is our persistent ignorance of the topology of the brain's micro-circuitry – the minute and multitudinous connections contained within. This heavily interconnected, intermixed, and dynamical network of different cell types results in a daunting complexity of *"impenetrable jungles where many investigators have lost themselves"* (Ramón y Cajal, 1923). Moreover, another equally fundamental shortcoming is our inability to monitor network interactions and coordinated brain activities densely, and to do so simultaneously across extended regions of the brain, and with sufficient temporal and spatial resolution.

To navigate these jungles, neuroscientists have traditionally relied on electrodes that sample brain activity only very sparsely – from just a few neurons of the whole or, at best, from groups of cells within a few regions. However, since neural circuits can comprise up to billions of neurons, it is probable that *functional circuits* are to be found at hierarchical levels involving very large, coherently-operating, neuronal ensembles. This level of organization will certainly be invisible from single neuron recordings – just as it would be pointless to understand the architecture of a building by studying the atomic structure of its bricks, or to view an HDTV program by looking at a just a few pixels on a screen. Coherent multi-neuronal activity, spanning extended hierarchical regions of brain tissue is an *emergent* property. In this context, by emergent we allude to computational or cognitive functions that arise from complex interactions among the constituents; one

that is, by definition, irreducible and not explainable from the individual elements in isolation. The expression *"*more is *different"* coined by physics Nobel Laureate P.W. Anderson succinctly expresses this basic notion (Anderson, 1972)

The hypothesis that most brain regions operate at emergent functional levels is supported by the well-known recurrent and widespread architecture of their connections (Lorente de No, 1938). Indeed, most neural circuits are distributed, with individual neurons making and receiving synaptic contacts from hundreds or thousands of other neurons. In such distributed circuits, the role of any specific neuron is deemphasized: the larger the connectivity matrix, the greater redundancy within the network and the less important is each neuron. Moreover, given their distributed connections, neurons are likely to be subject to continuous, dynamical rearrangement, participating at different times in different ensembles. Nonetheless, neuroscience has gravitated toward detailed descriptions of the feature selectivity of *individual* neurons. This is, of course, the natural consequence of neuroscience having long being armed with methods to record from individual cells, a toolset that is limited to a very restricted domain. On the other hand, concepts such as emergent codes, functional states, and dynamical attractors (Hopfield, 1982; Rabinovich, 2001), are major departures from the neuroscience tradition of using receptive field responses from individual cells to characterize the functional properties of a circuit (Hartline, 1938). Indeed, in the few instances where more comprehensive population monitoring of neuronal ensembles has been possible, results confirm the premise that emergent properties are not predictable from individual cell phenotypes or local response (Briggman et al., 2005; Lubenov et al, 2009).

This emergent-level problem is not unique to neuroscience. There are many historical precedents in science where understanding complex systems has been stymied by insular and persistent adherence to single-element measurements and analyses, and where breakthroughs have come only from shifting the focus to the emergent level. Examples include complex systems, and their emergent properties involving statistical mechanics, non-equilibrium thermodynamics, many-body and quantum physics (Feynman, 1965; Anderson, 1972). The outcome from taking this path has led to a very rich new science describing novel states of matter involving correlated particles – magnetism, superconductivity, superfluidity, the quantum Hall effects, and macroscopic quantum coherence. In the biological sciences, the sequencing of entire genomes and the ability to simultaneously measure the expression level of many genes have enabled the generation and testing of novel emergent models of gene regulation, developmental control, and disease states. These advances have fostered explanations of these processes with an increasing degree of prediction accuracy (Pe'er and Hacohen, 2011).

We believe similar richness is in store with the neuroscience of emergent systems. An emergent level of analysis appears to be crucial for understanding the most compelling questions of how brain functions create sentience. Likewise, the pathophysiology of illnesses like schizophrenia and autism, which have been resistant to traditional, single-cell level analyses, could potentially be transformed by an infusion of work from emergent levels.

The Functional Connectome: Measuring Every Spike from Every Neuron

We propose that elucidating emergent levels of neural circuit function requires, ultimately and unequivocally, recording every action potential from every neuron in a circuit. This must be carried out over a time scale on which the coherent behavior emerges. Similarly for the spatial scale, observation must subtend the spatial extent of a *circuit* – which can range from the smallest to the largest assemblage of neurons exhibiting coherent behavior. Using such measurements will enable a complete functional description of the system: a ***Brain Activity Map*** (BAM). This activity will transcend mapping the "structural connectome", which aims at a detailed but *static* anatomical map of a circuit by reconstructing its synaptic "highways", that is, its junction and connection matrix. Instead, what we propose is mapping the "functional connectome", which will complement and augment maps of the structural connectome, by revealing the *dynamical* traffic on its synaptic highways. In other words, the functional connectome will map the patterns and sequences of nerve cell firing activity. If it proves possible to correlate this firing activity with the connectivity of the circuit and with the functional or behavioral output of this activity, it will be possible to formulate cogent hypotheses, decipher the neuronal code, and understand how that code regulates behavior. We believe this is feasible.

Together with the new technologies needed to achieve this vision, this new level of understanding will enable the accurate diagnosis and restoration 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.

Imaging Every Spike From Every Neuron

To date, it has not been possible to reconstruct the circuit activity patterns of even a single complex region of the brain. While conventional technologies like fMRI or MEG can be used to partially capture activity patterns, these whole brain imaging techniques lack both single-cell acuity and sufficient temporal resolution; moreover, they cannot detect the detailed firing patterns of a multiplicity of neurons. To preserve single-cell information while recording the activity of complete circuits, we propose that vigorous efforts be launched to massively upscale the capabilities of both *imaging* and *nanoprobe sensing*. We shall elaborate upon these below. First we must point out that very significant investment must be made today – as was the case at the start of the Human Genome Project – to insure these next-generation technologies are in place for the BAM Project. We are convinced the efforts we propose will not evolve sufficiently quickly without this concerted financial impetus. Strategically placed funding will insure the requisite acceleration of technology development.

Over the last two decades, neuroscientists have made transformational advances in optical techniques both to monitor and manipulate the activity of neuronal ensembles, *in vitro* and *in vivo*. Optical techniques now nicely complements electrical measurements, since light is essentially non-invasive and can provide great spatial and temporal flexibility (Helmchen et al., 2011). Optical techniques have single-cell resolution, which is an essential feature for the BAM Project because neural circuits are built with many different cell types that are spatially intermixed. Particularly with two-photon excitation (Denk et al., 1990), optical methods can enable imaging and manipulation of neurons in the middle of living brains (Denk et al., 1994).

Measuring the multi-neuronal activity of a circuit can be carried out with calcium imaging (Yuste and Katz, 1991), something made possible by the design of fluorescent calcium indicators with high-affinities (Tsien, 1980). Measuring the associated calcium influxes in the soma of a neuron can be used to monitor its spiking, since every neuron has voltage-sensitive calcium channels and these channels are opened by action potentials (Smetters et al., 1999). Moreover, calcium imaging is very sensitive because resting concentrations of intracellular free calcium ($[Ca^{2+}]_i$) are close to nil. Under ideal conditions, even a brief, millisecond-long opening of calcium channels by an action potential causes a significant increase in $[Ca^{2+}]_i$, and this can enable the detection of individual action potentials (Figure 1). Finally, acetoxymethyl ester forms of organic calcium indicators (Tsien, 1981), or genetically-encoded calcium indicators (Miyawaki et al., 1997), can be loaded into populations of neurons *in vitro* (Yuste et al., 2011) or *in vivo* (Garaschuk and Konnerth, 2010), allowing one to simultaneously image thousands of neurons, and capture the increase in fluorescence associated with their spiking (Cossart et al., 2003). A drawback of present-day calcium imaging, however, is its poor time resolution. Calcium indicators are cytoplasmic, whereas action potentials trigger calcium entry at the plasma membrane, so calcium must diffuse into the cytoplasm before it binds the calcium indicators (Tank et al., 1995). In addition, calcium is heavily buffered in neurons, and further buffered by high–affinity calcium indicators (Neher and Augustine, 1992). Therefore, a significant time delay occurs until spike-associated fluorescence signals are detectable over background. In practical terms calcium imaging cannot be used at present to pinpoint the spike timing with a resolution better than 40 Hz (Smetters et al., 1999). Therefore, trains of action potentials are difficult to detect with high affinity

calcium indicators, which become saturated in high ($[Ca^{2+}]_i$. Despite these limitations on time resolution and detection of spike trains, calcium imaging has been used effectively to reconstruct the firing patterns of large (>1,000) populations of neurons (Cossart et al., 2003).

Calcium imaging, while useful, can only approximate the real functional signals of neurons, which communicate by altering their membrane potentials. It is clearly preferable to capture the complete activity of a circuit by voltage-based functional imaging (Wu et al., 1994). However, current methods for voltage imaging in vertebrate preparations do not enable imaging of action potentials in neuronal populations with single-cell resolution (Peterka et al., 2011). This is partly due to the significant technical difficulties that hamper voltage imaging. For example, the plasma membrane is very thin and can only accommodate a few sensing chromophores, and these must be precisely positioned within the Debye length of the electric field to be effective. The electric field is attenuated within only a few nanometers outside the membrane. Another deterrent is that the plasma membrane is the cellular defense barrier; any damage caused to it, such as photodamage triggered by reactive oxygen species, can have catastrophic consequences for the cell. Positioning extra charged molecules in the membrane, such as voltage-sensitive chromophores, can also measurably perturb the electrical properties of the plasma membrane (Blunck et al., 2005). Further, efforts to stain plasma membranes with voltage indicators selectively are also complicated: the plasma membrane makes up a small proportion of all cellular membranes. Voltage imaging thus still remains an experimentally challenging technique, and does not provide single-cell resolution when

applied to imaging mammalian circuits. The current state-of-the-art is not adequate for large-scale reconstruction of neuronal activity.

Technological Challenges: Speed and Depth of Imaging

To realize the goal of imaging every spike from every neuron, many technological hurdles must be overcome. As mentioned, the BAM Project will require significant investment in new technology to succeed. It is obviously necessary to improve voltage imaging to the point where it can supplant calcium imaging. Novel voltage sensors with better signal-to-noise, less photodamage, and faster time resolution are needed. Examples of the new sensor designs include opsin-based genetic indicators of voltage (Kralj et al., 2011), second harmonic and electrochromic chromophores (Millard et al., 2003). Particularly promising are new entries from the realm of nanoscience – nanoscale reporters such as nanowires (Miller et al., 2012) or color centers of diamonds (Mochalin et al., 2012). This is discussed further below.

Even if choosing calcium imaging as the technological platform with which to image neuronal activity, one needs to make it high-throughput in order to capture all the spikes from all the neurons. A major drawback of current forms of calcium imaging is their limited temporal resolution in reconstruction of the action potential. Mixing calcium indicators of different affinities and targeting them to plasma membranes could partly alleviate these limitations. In addition to increases in the temporal accuracy of the action potential reconstruction, major improvements are also needed to increase the number of imaged neurons and the depth of the imaged tissue. Another avenue of improvement is the design of lower magnification objectives with high numerical apertures. In this

respect, a recently designed "Megalens" enables the fluorescence imaging of an entire fish embryo, while maintaining single-cell resolution (Amos, 2011). But there are two fundamental limitations to optical imaging techniques to these scanned, high-intensity, laser-light-based methods. The first is the slow temporal resolution that scanning laser microscopes provide (Pawley, 1995). The second is focal planes for traditional optical methods are essentially two-dimensional (2D) surfaces, while neural circuits have three-dimensional (3D) structure. Simply put, one can only "see" a small subset of the neurons within the slice. Recent 3D methods employ fast moving piezo-mounted objectives combined with galvanometer scanners (Helmchen et al., 2011), fast random-access serial scanning using acousto-optic deflectors (Kremer et al., 2008), or electrically-tunable lenses (Grewe et al. 2011). However, these advanced methods have a fundamental limitation to their speed: it is still necessary to wait at each imaging position to collect enough photons to generate a usable image. An interesting solution to the speed and thickness problems is to parallelize the imaging so that multiple sites can be simultaneously illuminated and detected (Hell and Andresen, 2001); this can be done with fixed diffractive optical elements (Watson et al., 2009) or, with great flexibility, using spatial light modulators (SLMs) (Lutz et al., 2008; Nikolenko et al., 2008). SLMs are holographic optical devices that modulate the phase of a coherent light source, generating any arbitrary set of beamlets. Thus, one can deliver light to any location and simultaneous excite multiple 3D sites.

Two-photon (2P) excitation can significantly alleviate the depth limitation of light microscopy (Denk et al., 1990). Infrared light can penetrate deep into neural tissue (Denk et al., 1994; Svaaland and Ellingsen, 1983), whereas with visible light, imaging is

effectively restricted to 50 μm from the surface. High quality two-photon imaging can be routinely done down to 500 μm from the surface of mouse brains using living preparations (Denk et al., 1994). With increased laser power, one can image fluorescence calcium transients effectively at depths of 1.5 mm *in vivo* (Rueckel et al., 2006; Theer and Denk, 2006). These current limits may be surpassed with novel approaches, such as adaptive optics (Rueckel et al., 2006), PSF design (Quirin et al., 2012), wavefront correction (Conkey et al., 2012), or computational reconstruction of the signals. Also, the recent development of light field cameras (Levoy et al., 2009), and, more generally, the field of computational optics, may allow the "optical triangulation" of signals emerging at different focal points. Finally, the use of GRIN fibers and endoscopic approaches can allow for imaging deeper brain structures, such as the hippocampus, although at the expense of some invasiveness (Reed et al., 2002).

Nanoparticles as Next-Gen Reporters

An exciting area of improvement for optical methods is the development of nanoparticles, small inorganic – metal or semiconductor – compounds with well-defined electronic structure and precise quantum states. Composed of many atoms or molecules, they can have very strong interactions with the light field, leading to very large absorption and highly efficient emission. The design of these nanostructures draws 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 are photochemically robust during extended interrogation. For their use in neuroscience, nanoparticles need to be combined with organic nanostructures, that is, biofunctionalized, to direct/embed them within neural membranes or synapses. The specialized structure of semiconducting nanoparticles enables the generation of excitons, which can, in turn, be very sensitive to the external electric field. This sensitivity can turn these nanoparticles into local reporters with externally modulated fluorescence intensity, spectra, or lifetime. They may be combined with selective molecular binding moieties to confer sensitivity to changes in local neurotransmitter concentrations.

More specifically, zero-dimensional nanostructures – also known as quantum dots (Hallock et al., 2005) – can be manipulated to produce a new generation of local optical reporters for neuroscience. One of the exciting prospects, given the innate voltage-sensitivity of nanoparticle reporters, is that they could be used directly as optical readouts of membrane potential. These reporters need to be capable of being embedded into neural membranes (thickness ~2nm) and of being sensitive to local electric fields as well as local chemical environments. If nanoparticles could be properly targeted to the membrane, their optical properties and voltage sensitivity could make them ideal voltage sensors (Fan and Forsythe, 2008). In fact, recent work using tools from atomic physics has shown that optically manipulated color centers in diamond provide exceptionally sensitive magnetic and electric field probes at sub-100 nm distances (Mochalin et al., 2012). Moreover, diamond is uniquely suited for studies of biological systems because it is chemically inert, cytocompatible, and ideal for coupling to biological molecules.

Besides nanoparticles, other nanomaterials could be of great use in neuroscience applications. For example, 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.

These types of nanoparticles could be used alone, or combined with a conventional organic chromophores, as under certain conditions, they have been shown to greatly enhance optical signals, acting as an "antenna" for the light (Stiles et al., 2008; Tam et al., 2007). Indeed, membrane-bound, antibody-linked gold nanoparticles have been already used to increase SHG from single dye molecules allowing site specific measurements of membrane potential (Peleg et al., 1999). Hence, one of their uses for the BAM Project could be to harness this plasmonic enhancement to increase the signals emitted by organic voltage reporters in fluorescence, Raman, or SHG modalities.

Concerted development of new classes of nanoparticle reporters could be key for the BAM Project. Traditional organic chromophores suffer from several drawbacks – they are large and can geometrically or chemically perturb the cellular environment. Also important is that they can bleach, that is, become ineffectual after exposure to light. Nanoparticles, by contrast, can be coated with a passivation layer or specialized shell that limits direct interaction with the surrounding media, this greatly minimizes bleaching, and in the cell, the generation of reactive oxygen species. In the near term, work must proceed to address key limitations exist in the palette of reporters available to the BAM Project for functional optical imaging. Such near-term challenges include developing

inorganic nanoparticles with enhanced voltage sensitivity, and orchestrating plasmonic enhancement of existing optical reporters. It should also be feasible to develop inorganic nanoparticles with voltage sensitivity; verify their biocompatibility; and to identify and validate routes to targeted delivery. We also see it as possible to develop of new organic nonlinear voltage probes. A third very important area we target for development is development of multifunctional nanoprobes that can locally report not only voltage, but strain, chemical species (e.g. calcium, neurotransmitters, etc.), and local temperature or ionic environment. The ability to perform multifunctional optical imaging of complementary physical fields in neural tissue will open many new frontiers.

Nanoscience for Neural Probes

Aside from optical techniques, for the BAM Project we propose to harness the new developments of nanofabrication, which are generating a veritable revolution in or ability to electrically record neuronal activity. As the reader knows, electrophysiology has been the mainstay for functional observation from neurons ever since the first *extracellular* recordings of Galvani (Galvani, 1791), and the early *intracellular* recordings of Renshaw, Eccles and Hodgkin and Huxley (Hodgkin, 1939). Although hand-assembled tetrode technology (O'Keefe, 1993; Gray, 1995) arguably still represents the state-of-the-art in terms of signal quality and robustness for chronic extracellular recording, beginning in the mid-1960's microelectronics technology was already beginning to be explored for creation of regularized, batch-fabricated structures with multiple electrodes (Wise, 1970; Wise 1975). The state-of-the-art has progressed very significantly in terms of both probe and system complexity over the intervening forty

years (Hetke, 2002); indeed, silicon based neural probes with several dozen electrodes are now available commercially and are used in many laboratories worldwide. Recently, with the infusion of nanoscale features onto microscale probes – features fabricated by nanolithographic processes and microelectromechanical systems (MEMS) technology, respectively – a huge leap forward in electrode density has become possible. It is now feasible to realize dozens of recording sites per silicon neural probe (that is, per shank), densely, at a pitch commensurate with the scale on which extracellular fields evolve (~a few x 10μm) (Du et al. 2009a; Du et al. 2009b). Previously, the limitation was not the size of the recording sites (electrodes) themselves – electrode physics dictates a minimum size of order $(10\mu m)^2$ for optimal signal-to-noise. Rather, it is the need to run wires from these sites up the length of the shanks to external connections that becomes the problem. With nanoscale traces, narrow shank widths can be preserved; this is important to minimize tissue damage as mentioned below. These narrow silicon probes can be fabricated in two-dimensional multi-shank arrays, then stacked to create three-dimensional probe arrays, in future with thousands of sites. This technology is now in place to orchestrate, ultimately, a massive scale-up to systems with hundreds of thousands of recording sites, but significant system integration and engineering issues must first be surmounted.

With so-called spike sorting algorithms, multiple electrode ensembles can enable monitoring of many more individual neurons than the actual number of recording sites (electrodes). Spike sorting allows, in effect, each individual electrode within a tetrode or multisite silicon probe to distinguish between the spiking of many nearby neurons. In this vein, discussions of increasing the density of neural recording technology are, in fact,

often largely focused upon improved computational approaches (Einevoll, 2011), but the aforementioned methods from nanoscience for building new generations of highly-multiplexed probes, *in concert with* advanced spike-sorting algorithms, now make it feasible to ultimately envisage recording from hundreds of thousands, if not millions of neurons.

Neural probes are singular in that they can be fabricated with centimeter-scale lengths (Fomani, 2011; NeuroNexus, 2012) to facilitate penetration into the deepest recesses of brain tissue –well beyond the realm where electromagnetic waves and light imposed from the outside will have long since decayed. Their implantation does indeed come at the risk of disturbing the proximal tissue involved; recent work has begun to explore how to ameliorate tissue trauma so as to circumvent the gliotic response that can ultimately render a probe ineffectual (Prasad, 2012). Such work will ultimately enable chronic recording from implanted probes (Kipke, 2008), although we note that high-quality long-term (6-12 month) recordings are already possible today (Siapas, 2012).

Nanoprobe-based, Massively-Multiplexed Stimulation and Recording

As mentioned, traditional electrodes are being superseded by nanoprobes, shank-like structures that are inserted into neural tissue and comprise arrays of functional elements – for example, recording electrodes – along their length. Examples are depicted in Figure 2. Looking ahead, it is possible to envision a realistic scenario where the microfabrication of nanoprobes could be used to record neuronal activity. Specifically, one goal could be to measure activity from very large numbers of neurons in a model nervous system by

electrophysiological interrogation of neural tissue via nanoprobe arrays enabling massively-multiplexed electrical recording and stimulation.

With today's state-of-the-art technology it should be feasible to use microelectronic foundry (factory) production to create probes that enable electrical recording from 1,000 electrodes channels, each at a 25KHz sampling rate sufficient to record the full bandwidth of a spike (approximately 10kHz). The technology can easily enable creation of centimeter-length probes to access deep tissues, for example the rodent cortex. The sampling density would be of order 50 or 100 μm pitch. The holy grail will be to record from millions of electrodes, keeping the same bandwidth, reducing the electrode pitch down to distances of order ~15μm, and increasing the probe length to cortical dimensions of several centimeters. This will require significant innovation in systems engineering, especially to handle the data throughput. This is discussed in a section to follow.

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 subject with minimal-to-no discomfort.

Recent technological innovation is extending the concept of a silicon neural probe to include those based on integrated photonic circuits. Shanks containing optical waveguides can route light deep into tissue to permit optical stimulation of deep brain tissue, as a technology to complement existing electrically-based methods for such stimulation.

Hence, our second goal is 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 naturally begin with pursuit of optical stimulation, for example, by two-photon uncaging or photoactivation of neural tissue perfused with optogenetic constructs or caged compounds and, in parallel by 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, for example, 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.

Nanosystems comprise large coherently engineered ensembles of nanoprobes, nanodevices and optical nanoparticles, assembled in a fashion yielding capabilities that are greater than the sum of the parts. The technological capabilities for producing such nanosystems *en masse*, can originate by leveraging the large-scale worldwide resources for producing microchips if the funding resources are secured for this enterprise. Integrating these with bottom up fabricated nanostructures that can report on local neural phenomena with electrical and chemical specificity would offer new windows into neural processes.

Untethered Local Recording and Synthetic Biology Approaches

As a third technological platform, perhaps distant but no less important to pursue than optical or nanoprobes sensing, we envision the future application of approaches that

could enable a wireless, non-invasive readout of the activity of neuronal populations. These might include wireless electronic circuits based on silicon very large scale integration (VLSI), synthetic biological components, or their hybrids.

It is easy to underestimate the potential of today's electronic technology; it could be feasible to deploy small microcircuits in living brains for local monitoring of neuronal activity in the near term. The miniaturization of circuits has followed Moore's law (of 1.5-fold size reduction per year) to the point that radiofrequency identifiers (RFID) with 128 bit encoding were as small as 50×50×5 microns with 90 nm IC feature size (Nozawa, 2007). In 2012 a feature size of 22 nm is feasible, while in 2013 and 2015 production sizes are expected to be 14 and 10 nm (Bohr and Mistry 2011). The analog components may not scale as easily as the digital ones. Another challenge to RFID implementation is scaling down the antennae. This might be done by exploring beyond 2.45 GHz RF to THz or optical frequencies. For RFIDs, the electromagnetic waves typically handle both the digital signal and the energy transmission (Figure 3).

There are significant technological challenges to make wireless measuring of neuronal activity possible. Optical or electrical wires have the advantage of knowledge of the position of each datum in space and time. So these would have to be provided in other ways if wireless options are pursued for reasons of scaling or lower perturbation. This can include the use of synchronization signals, interferometry, and self-analyzing local area networks. Delivery of the wireless devices or synthetic cells ideally would be minimally invasive, possibly exploiting the ability of natural cells (e.g. T-cells) to move both ways across the so-called "blood brain barrier" (Engelhardt, 2006).

Synthetic biology could also provide an interesting set of novel techniques to enable non-invasive recording of activity (Figure 4). Technically, this could be considered a wireless option, but radically different from approaches based on microelectronics. For example, polymerases can have error rates that are responsive to cation type and concentration (Frank and Woodgate, 2007) and this property can be genetically enhanced and inserted into neurons or synthetic cells in electrical contact with neurons. Pre-chosen DNA molecules would record patterns of errors corresponding to the patterns of spikes in each cell, since polymerase errors would be calcium-sensitive. The cells could then be analyzed using in situ sequencing of fixed tissues or by sequencing from the recorded DNA collected peripherally. The density of DNA storage is quite remarkable. In principle, a 5μm diameter synthetic cell could hold at least 6 billion basepairs of DNA, which could encode 7 days of data at 100 Hz and 100-fold redundancy (coding inefficiency factor).

Roadmap – How the BAM Project Might Unfold: Choice of Species

Once the technology to reconstruct the complete activity patterns of a circuit is developed, which circuit should be worked on, and in which order? We envision a parallel effort with several different species, progressing from reconstructing the activity of small, simpler circuits to more complicated and larger ones. We propose a series of short to long-term goals. We will briefly comment on some potential targets, reminding the reader that this is not an exclusive listing, but only a tentative plan. For any target circuit, one could proceed in two steps. First, initial, lower resolution mapping will be done using calcium imaging of action potentials. Reconstruction of the spiking will be

carried out at 100 Hz with single-cell spatial resolution. This could be performed with existing calcium indicators, either genetically encoded, or organic ones using acetoxymethyl ester loading. In a second step, voltage imaging of the action potentials or subthreshold electrical activity would be carried out using two-photon excitation of voltage-sensitive probes, genetic constructs, organic chromophores or inorganic nanoparticles. Native signals could be increased by plasmonic enhancement to achieve a signal to noise adequate to ideally measure every action potential with a temporal resolution of 1 kHz and spatial resolution of a single cell in 3D.

In a short term (5 year timeframe), we propose to reconstruct the activity of a series of small circuits, all less than 50k neurons. By analogy to the role played by *C. elegans* and *Drosophila* and the mouse in the evolution of the Human Genome Project, these model systems are perfectly suited to play a similar role in the evolution of the mammalian functional connectome, with the important caveat that the BAM Project and the Human Genome Project have very different goals and methods.

The worm is the only complete connectome at present (302 neurons and 7,000 connections at EM resolution) (White et al., 1986), and has the capability of being imaged *in toto* by 2-photon microscopy for calcium imaging with current genetic tools. Whether imaging can be done of freely behaving animals is an issue, but with current techniques (see above), all cells can be imaged simultaneously. In addition to the worm, in the first 5 years we would propose the reconstruction of a discrete region of the *Drosophila* brain, such as an optic lobe (e.g., the medulla, with ~15k neurons). The Drosophila connectome is currently 20% complete at the meso scale (Chiang et al., 2011), and will likely be 100% complete within two or three years. Genetically encoded

calcium imaging is currently available and the brain size permits complete 2-photon imaging, and with current imaging techniques (see above), comprehensive imaging can be done of 10% of the brain, or the whole brain at a 10% sample level. Tethered flies can be imaged during perceptual tasks, learning paradigms, and during tethered walking or flying. Finally, as a short-term goals in vertebrate circuits we would suggest the choice of the mouse as the main experimental preparation. In the first 5 years, one could aim to reconstruct the activity of all the ganglion cells in a mouse retina (~50k neurons), or all the mitral cells in the mouse olfactory bulb (~70k). A mouse neocortical brain slice has roughly 10-40,000 neurons and could be serve a benchmark for future scaling of the project to the entire cortex of the mouse. In fact, 4,000 cells can be already imaged simultaneously in a mouse brain slice (Cossart et al., 2003).

For mid-term goals (10 year timeframe), one could then aim at imaging the entire Drosophila brain (135k neurons), the CNS of the zebrafish (~1M neurons), and an entire mouse retina, or the hippocampus from a mouse, all under a million neurons. As additional mid-term goal, one could reconstruct the activity of an entire cortical area in a wild type mouse or in mutant mice that are models of diseases or have interesting phenotypes. Finally, it would be also interesting to consider the Etruscan shrew, the smallest known mammal, with a cortex of only a million neurons (Roth-Alpermann et al., 2010).

For a long-term goal (15 year timeframe), we would expect that technological developments will enable the reconstruction of the neuronal activity of the entire neocortex of an awake mouse, and proceed towards primates, perhaps starting with the cortex of a marmoset, and leading to human subjects. Indeed, we do not exclude the

extension of the BAM Project to humans, and if this project is to be applicable to clinical research or practice, its special challenges are worth addressing early. Thinking out of the box along these lines may even contribute to ideas applicable in early years to model systems. Potential options for a human BAM Project include safely and transiently introducing engineered cells, the use of wireless electronics or a combination of these approaches. T-cells go back and forth across the blood-brain barrier and it could be explored whether it is feasible to engineer them to make tight (transient) junctions with neurons for recording and possibly programmable stimulation. The miniaturization of electronics and molecular recording will have spin-offs well beyond biology.

It is of the upmost importance that the BAM Project sets its goals clearly and its budget wisely. Furthermore, its scientific accomplishments and costs should be re-evaluated at every stage in order that it is comprehensive and cost effective.

Data Access and Ethical Considerations

We feel strongly that an effort such as the BAM Project should be squarely put in the public domain, with all data becoming accessible as soon as it is captured. The generation of complete records of the activity of a neural circuit will require large-scale coordination between many participants, and the information will benefit mankind in many different ways. Because of this, it makes sense for this project, to be run as a public enterprise with unrestricted access to its resulting data.

One should also discuss the potential ethical ramifications of a project such as the BAM Project. It addition to humane treatment of animals, ethical and policy questions arise if this technology moves as swiftly as genomics has done in the last few years. The

scientific community needs to be proactive well in advance; engaging diverse sets of stakeholders and lay public as early and as thoughtfully as possible. Perceptions and misconceptions can be as crucial as reality. For example, the reaction to a small number of adverse events for gene therapy and GMOs in the 1990s had large chilling effects for a decade thereafter. Issues that may appear during the realization of the BAM Project include mind-control, discrimination, health disparities, unintended short- and long-term toxicities and other consequences.

Computational Analysis and Modeling

Our stated goal of recording every spike from every neuron raises the specter of a data deluge, so it becomes important to discuss strategies for data reduction, management and, as the end result of this effort, data analysis. In this respect it will be helpful to gain perspective by reviewing the Human Genome Project as a comparative benchmark for the BAM Project (Figure 5).

For perspective on a computing scale, in 2011 a single genome center with 20 machines sequenced 3000 human genomes × (6×10^9 bp) at 40X =8×10^{13} bp (or bytes)/year. In 1984, 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 speculative at best. The fast pace of continuing technological advances are multiplying this already impressive capacity by roughly 10-fold per year (Carr and Church, 2009). Similar skepticism of progress can accompany associated computing tasks. At the beginning of the Human Genome Project, even an elementary task of genomic analysis -- comparing each short region to each other region – was

predicted by some to require an unapproachable number of computer operations: $(3\times10^9)^2 \sim 1\times10^{19}$. Today, clever linear algorithms (such as BLAST) have displaced the naïve N-squared algorithms (like Needleman-Wunsch) invoked at the outset of genomics analysis allowing the above analysis with on the order of 3×10^9 calculations. Also, as genomic accuracy improves, it becomes much more compressible than generally imagined: 4×10^6 bytes is sufficient per each 6×10^9 bp genome (Christley et al. 2009). To estimate data storage capacities required for a brain activity map we consider the anatomic connectome. Bock et al. (2011) covered 1500 cell bodies with 1×10^{13} raw pixels (Bock et al., 2011). 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 global genome image data and comparable with 10^{15} bytes of public astrophysics data. These volumes are growing at 1.5-fold per year. The availability of these data has transformed biology and astronomy research. Some might argue that analogies to genomics are limited in that brain activity mappings are of much higher dimensionality than are linear genomics sequences. High dimensionality and dynamics of transcriptomes, immunomes and indeed whole body analyses are increasingly enabled by the plummeting costs. Indeed, integration of transcriptional, anatomic and functional connectomes may be a desirable path (Bota et al, 2012, Kording 2011). 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. Brain activity maps, like the broader omics and systems biology paradigms, will need (i) 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 likely that knowledge of the function of neural circuits will require manipulation of those circuits. It may be unnecessary to insist on predicting function from connectivity alone if we can measure and manipulate both, but still an interesting challenge.

With such data management strategies in place, we envision the creation of large data banks where the complete record of activity of entire neural circuits could be freely downloadable. This could spur a revolution in computational neuroscience, since the analysis and modeling of a neural circuit will be possible, for the first time, with a comprehensive set of data. As in the case of the Human Genome Project, generating a new field of inquiry ("Genomics"), it is likely that the generation of these datasets could enable the creation of novel fields of neuroscience.

Finally, one could mine the spiking raster plots to infer the connectivity present in the sample. Indeed, there is already precedent for successful detection of synaptically-connected neurons from calcium imaging of neuronal populations (Aaron and Yuste, 2006; Kozloski et al., 2001). In the case of *C. elegans* one could compare inferred connectivity with "ground truth" collected from electron microscopic reconstructions of its nervous system. In the case of *Drosophila*, one could also confirm predictions with ground truth, using the new Chiang dataset that reveals connected sets of neurons

(Chiang et al., 2011). In the case of mouse brain slices, one could confirm the predictions using dual whole-cell electrical recordings between putatively connected neurons (Peterlin et al., 2000). Predictions of the connectivity could be carried out by first applying fast deconvolution algorithms to extract estimates of the neuronal spike times from the optical data. Employing generalized linear models to represent these neurons, one can estimate the connectivity of the circuit by quickly fitting an elastic net model path via coordinated descent (Mishchenko et al., 2011). Opportunities exist for integration of anatomical, activity and omic maps at cellular or even subcellular resolutions.

Outcomes and Anticipated Benefits

We expect the BAM Project to result in a host of scientific, medical, technological, educational, and economic benefits to society. Indeed, the widespread effect of this research underscores the need for it to be controlled by the public.

In terms of anticipated scientific benefits, the original motivation behind this project--the reconstruction of every spike in every neuron--will generate a complete functional description of the circuit. This information will be invaluable in addressing an array of outstanding questions in neuroscience for which the emergent functional properties could be key.

Near- and long-term questions that could be directly examined from the BAM Project datasets include the following: what is the functional connectivity diagram of the brain? What detailed computations take place locally in the brain? What are the real-time, multiple, long-range interactions that underlie cognitive functions and behavior? How do

local computations and long-range interactions influence each other? What are the paths of information flow in the brain? What alternative pathways produce similar outputs? When the brain "organizes" itself during development, or "reorganizes" itself after an injury, what is actually happening to activity locally and globally? When pharmacoactive drugs alter behavior, what are the local and global effects on activity? When memories are transferred from one brain region to another over time, how are their activity patterns changing? What design principles can be discerned in how the brain functions? Is there an underlying functional architecture to the brain's networks? What are the true functional underpinnings of perception, recognition, emotion, understanding, consciousness, and subconscious processes? Together, answers to these questions can open the doors to deciphering the neural code, or to disproving the existence of a neural code altogether, as well as unlocking the possibility of reverse engineering neural circuits.

In addition to answering critical basic research questions, we anticipate countless medical benefits from the BAM Project. Among these are novel and sensitive assays for brain diseases and better, more sensitive diagnostic tools that will allow brain disorders to be detected earlier and more accurately; generation and validation of novel biomarkers for mental disease (a biological "DSM5" type classification); elaboration of detailed disease etiology from its subtlest beginnings, thus allowing a diagnosis to be made earlier in a disease's progress. We also expect improvement in animal models of disease that could lead to testable new hypotheses for pathophysiology of brain disease and development of novel devices and strategies for fine control brain stimulation to rebalance diseased circuits. These devices and strategies will be more refined and longer

lasting than those currently used in deep-brain stimulation for Parkinson's disease and chronic depression, as the project will rely on stimulating nerve cells as well as monitoring their activities. Not least, we can expect therapies for diseases such as schizophrenia and autism.

Furthermore, we anticipate many technological breakthroughs to arise from the work on BAM Project. In fact, significant technological investment is critical; the project itself sits crucially at the convergence of biotechnology and nanotechnology and is likely to be substantially driven by development of unique technologies. These new technologies should include new optical techniques to image in 3D, useful both for microscopy and photography; sensitive, miniature, and intelligent nanosystems for engineering and environmental applications; new capabilities for storage and manipulation of massive datasets, and development of novel, biologically-inspired, computational devices.

The clinical devices, technological innovations, and computing capabilities developed in the course of this project will provide economic benefits, far beyond the bounds of this project and its specific research applications, potentially leading to the emergence of entirely new industries and commercial ventures around the world. On the engineering side, for instance, the nanosystems developed and deployed for this enterprise will have potential uses across a broad range of engineering and environmental applications, where sensitive, miniature, and intelligent systems can fulfill functions that are currently impossible with existing devices.

We also anticipate profound attendant benefits similar to those that occurred in the wake of the Human Genome Project. In fact, BAM Project shares a number of

similarities with 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 current funding programs because of its bridging of distant fields, and its ambitious scale. Furthermore, like the Human Genome Project, the resulting economic benefits are likely to be much broader and greater than anyone imagined, and perhaps may be realized much sooner than anticipated. A recent report from the Battelle Technology Partnership Practice found that every dollar invested in the U.S. elements of the Human Genome Project generated $141 in the economy (Battelle, 2011). 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 only on the verge of realization. The study's authors conclude that the project was "arguably the single most influential investment to have been made in modern science". The BAM Project, we believe, will have comparable ramifications.

Finally, we should not underestimate the repercussions that such a project could have for education. The proposed activities are interdisciplinary, cutting across a swath of approaches, so this project will lead to the training of a new generation of

interdisciplinary scientists and the opening up of new strategies for evaluating pedagogical effectiveness.

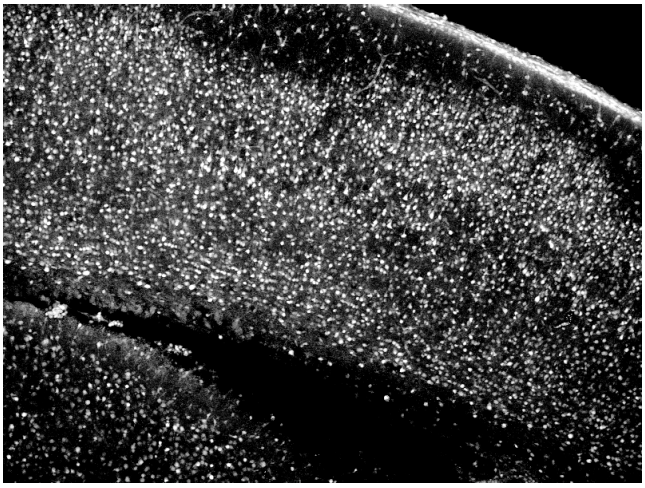
A Call for a Community Effort

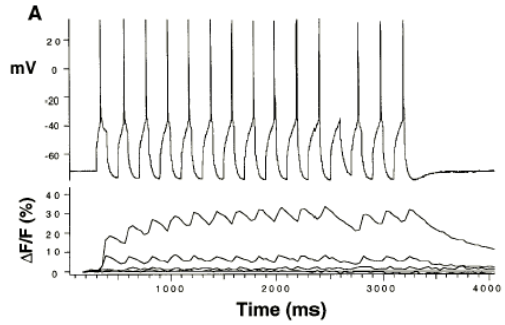
To succeed, the BAM Project needs two critical components to be in place: strong leadership from both funding agencies and scientific administrators, and the recruitment of a large coalition of scientists. Several disciplines appear key, from experimental neuroscientists exploring worm, fish, mouse, rat, turtle, and primates, to computer scientists at the forefront of massive data mining technologies, to computational neuroscientists building models/analyses with increasing complexity. Chemists, biochemists and nanoscientists will need to develop nanoparticle and molecular reporters, using state-of-the-art microchip research foundries to translate "one-off's" into prototypes capable of scale-up and production *en masse*. Finally industrial partners are essential for enabling mass production and system integration and deploying robust, integrated measurement instruments.

To conclude, we believe that neuroscience is ready for a large-scale functional mapping of the entire neural circuits and that such mapping will precisely address the emergent level of their function, providing light in the *"impenetrable jungles"*.

Figures and Legends

Figure 1: Large scale imaging of neuronal activity using calcium imaging.
A. Labeling of a living neural circuit with calcium indicators. A brain slice from primary visual cortex of a mouse was stained with bulk incubation of fura-2 AM. More than a thousand neurons are fluorescently labeled and can be imaged with a two-photon microscope (Yuste et al., 2011). B. The calcium concentration in the soma of one of these neurons faithfully tracks the electrical firing pattern of the cell (Smetters et al., 1999). C. Example of a reconstructed "raster plot" of the spontaneous activity of 754 neurons from a similar experiment (Cossart et al., 2003).





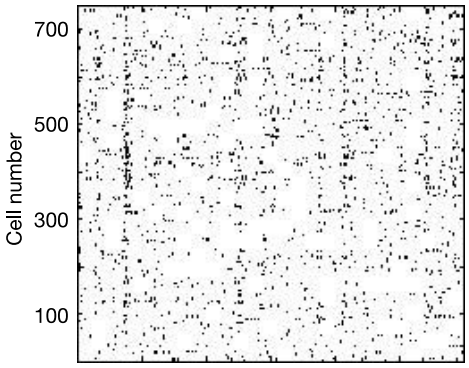


Figure 2: Nanoprobe sensing.

Commercial silicon multisite neural probes. NeuroNexus (2012); Silicon nanoprobe arrays. Du, Roukes, Masmanidis (2009); Integrated waveguides on probe for optogenetic stimulation. Boyden et al. 2009)

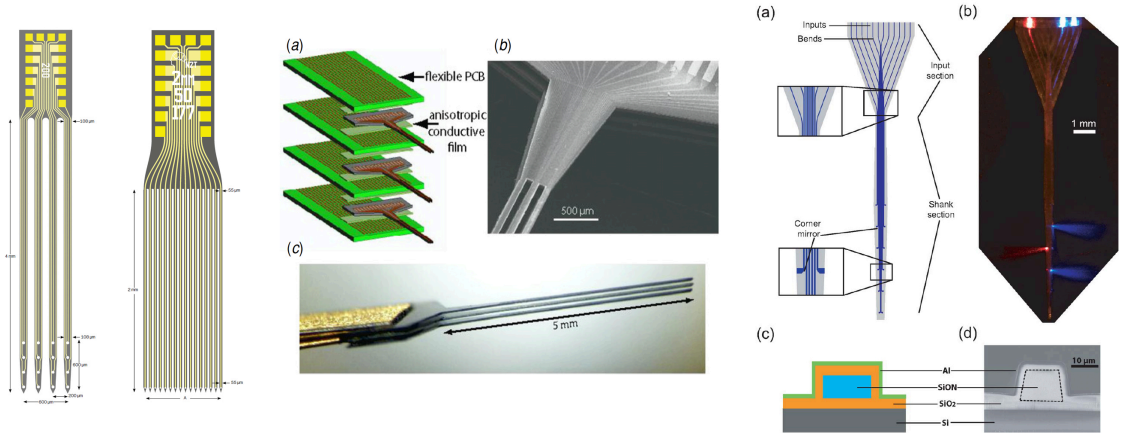


Figure 3: Miniaturization of electronic circuits for wireless recording.

Wireless electronics (RFID).

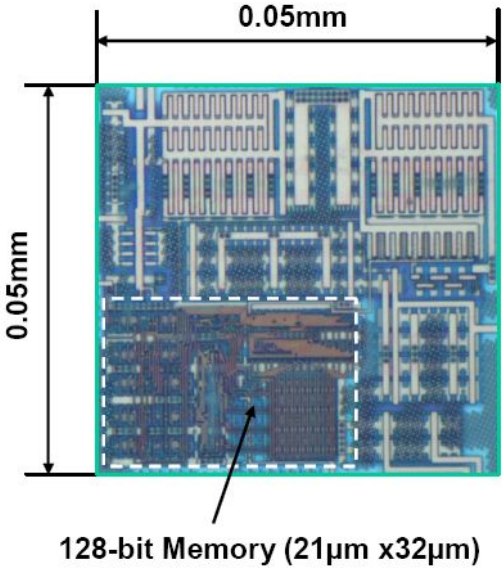


Figure 4: A synthetic biological strategy for measuring neuronal activity.

Polymerase encoding of ion flux spikes. A voltage sensitive calcium channel is shown influencing an engineered DNA polymerase. X marks sites of mismatch between "T" in the template strand (lower) and "G" new copy strand. Note scale of the various devices and cells.

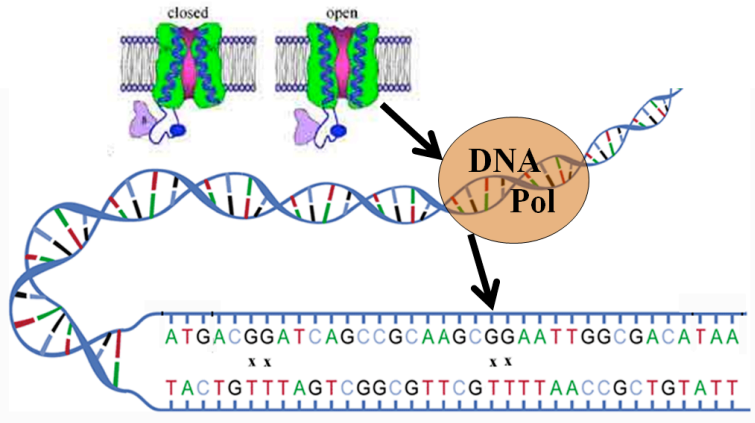
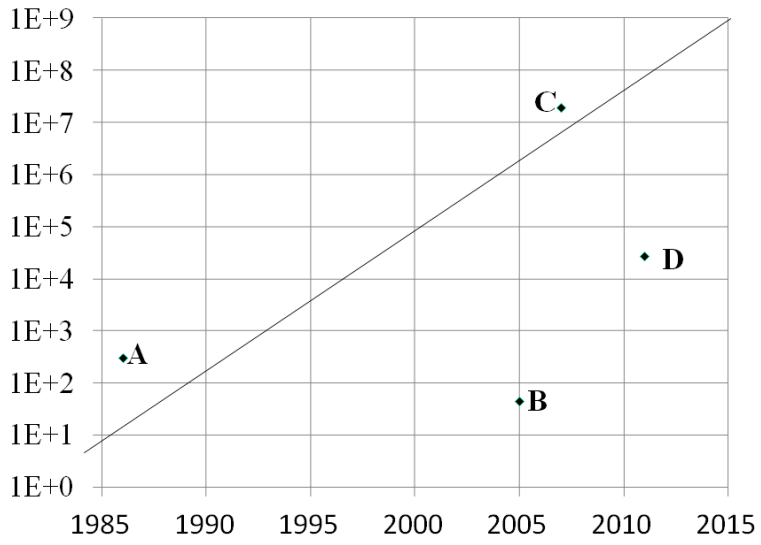


Figure 5: Potential scaling for the BAM Project. The straight (exponential) line is the trend in disk drive storage measured along the vertical axis in Kbytes/$ as a function of year on the horizontal axis. This curve is symbolic of a variety of technological enablers of the BAM Project. Letters represent key brain mapping milestones with number of neurons on the vertical. **A:** Worm 302 neurons by electron microscopy (White et al. 1986). **B:** Extracellular 45 electrodes (Quiroga et al. 2005) **C:** Transcriptome: 2 million neurons by 20,000 RNAs in the Allen Brain Atlas (Lein et al. 2007). **D:** Fly Fluorescent anatomical connectome (Chiang et al. 2011). Note that the 4 points chosen are not monotonically improving with time, as they represent a sparse sampling of experiments with very different goals.



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