

Foresight Cognitive Systems Project
Research Review

Advanced Neuroscience Technologies

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The objective of the project is to examine recent progress in two major areas of research – computer science and neuroscience, and their related fields – and to understand whether progress in understanding cognition in living systems has new insights to offer those researching the construction of artificial cognitive systems.

Further details are available at the Foresight web site: <http://www.foresight.gov.uk/>

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1 Executive summary: Technological developments in cognitive and imaging neuroscience

Richard Morris

Unlike other Research Reviews in the Life Sciences for the Foresight Cognitive Systems Project, this document relates to advanced techniques. The neurosciences are not unusual in science in having periodically been transformed by the advent of new techniques. Striking examples in recent memory include the advent of single-unit recording in awake animals by Hubel and Wiesel in the early 1960s, and the introduction of patch-clamping to observe single-channel ion-currents by Sakmann and Neher in the 1980s. Both developments were justly rewarded with Nobel Prizes to these pioneers.

While a mature theoretical understanding of many issues in the brain sciences must marry understanding at different levels of analysis, research often arrives at obstacles that require new techniques to circumvent them. In this summary, we highlight three examples of new technologies that offer considerable promise for the future.

First, the widespread use of non-invasive human brain imaging has transformed cognitive neuroscience. Instead of relying on techniques from experimental psychology in the analysis of neurological patients, human brain imaging allows us to derive a spatial picture of brain activation in normal subjects as they undertake different tasks. Second, single-cell and multiple single-unit recording technologies are advancing to the point where we can simultaneously record from large ensembles of cells, sometimes in different brain areas. In this way we can obtain data about how the firing of one or more cells may influence or be influenced by that of other cells. Third, optical imaging provides a new window on the brain, enabling microscopic techniques to reveal the molecular dynamics of activity within individual neurons. A wide variety of optical techniques are available, with the most recent using two-photon confocal microscopy.

Each technique can be coupled to others. Indeed, they often have to be. Functional brain imaging is of limited value without ingenious neuropsychological tests, refined to suit the constraints and opportunities afforded by brain scanning. Single-unit recording must likewise be coupled to analytic behavioural tests in primates, rodents or other species before we can draw useful inferences about the significance of different patterns of cell firing. Optical techniques on their own are similarly limited, but can provide remarkable information when used in conjunction with appropriate physiological procedures or molecular-genetic probes such as green fluorescent protein.

In focusing on these three techniques, we do not mean to imply that they alone will transform neuroscience. Many other techniques will also be very important, ranging from novel behavioural techniques through to targeted manipulations of individual genes. Rather, with these three examples we aim to illustrate the importance of advanced technological development in a seemingly mature science such as neuroscience.

2 Imaging the human brain

Karl Friston and Jon Driver

2.1 State of the art

The past decade has seen a profound paradigm shift in cognitive neuroscience. This has been enabled, in large part, by several spectacular technological advances in functional brain imaging that offer entirely new ways of relating cognitive processes to their neural substrates. The ability to map measures of brain activity in response to particular cognitive or sensorimotor challenges now allows cognitive neuroscientists to think about brain function in explicit neurobiological terms. Moreover, the agenda of cognitive neuroscience has been transformed. The focus is no longer merely on the fractionation of mental processes into putative components, a feature of the era when the only obtainable data was from the careful study of neurological patients, but on how neuronal information processing and cognition actu-

ally proceed in the brain, and how different components, and different brain areas, influence each other.

Key developments in neuroimaging began in the 1980s with the application of positron emission tomography (PET) to measure evoked changes in regional cerebral blood flow. In the 1990s, functional magnetic resonance imaging (fMRI) came to the fore, using changes in cerebral hemodynamics as an intrinsic signal for measuring brain activations.

fMRI is increasingly supplanting PET in brain imaging. This is due not only to the wide availability of MRI scanners in medical and academic settings, but also to its higher spatial and temporal resolution. It also permits event-related designs that were previously impossible with PET, which could measure only summed activity over periods of 90 seconds or so.

Parallel developments in the recording of electrical brain signals, with electroencephalography (EEG), and magnetic brain signals, with magnetoencephalography (MEG), now allow us to measure evoked responses, either with a fine temporal resolution on the order of milliseconds (EEG and MEG), or with a spatial resolution of a few millimetres.

State-of-the-art fMRI neuroimaging allows us to acquire an image of the whole brain within a second or so. Thus we can generate several hundred images in a scanning session of half an hour or so. This is sufficient for brain mapping because the hemodynamic sequelae of neuronal responses have time-constants in the order of 4 to 8 seconds, meaning they only have to be sampled every second or so. Typical evoked responses are on the order of 0.1 to 1% of the average MRI signal, although scanners with higher magnetic-field strengths may offer not only higher signal-to-noise ratios but also larger percentage signal changes in some cases. This relatively small absolute magnitude of the changes observed is a serious worry to some critics of the use of fMRI, but robust statistical techniques can ensure that, although small, the reported changes are reliable and potentially replicable.

In a number of specialist units, concurrent EEG recording during fMRI is technically feasible, allowing temporally precise and spatially precise measures to be combined, but this still requires considerable expertise to remove the artefacts induced by MRI acquisition. In addition, several laboratories can now combine fMRI scanning with transcranial magnetic stimulation (TMS) of localized brain regions. This allows an assessment of the effects of disrupting one brain region on activations in remote but connected brain regions.

The more psychological features of the experimental design in neuroimaging have now become quite sophisticated, with some consensus about optimal approaches. Most designs are multi-factorial and are often motivated by well-established paradigms in psychology, psychophysics or psychopharmacology. These designs may also relate to studies of brain function in animals from basic neuroscience.

It is now difficult to think of an area in cognitive or clinical neuroscience that has not been advanced by human functional neuroimaging at many levels. To give just a few examples, functional imaging has already substantially influenced research on perception, cross-modal integration, attention, spatial cognition, plasticity and learning, memory, language, intelligence, individual differences, and motor control in the normal brain. It has also advanced understanding of the effects of brain injury and neurological disease, and of pharmacological (drug) manipulations. We think that functional neuroimaging will play an increasing role in the study of cognitive and social development.

The prospects for non-invasive human brain imaging are exciting and diverse. Many open questions remain to be resolved at the technical, theoretical, empirical and applied levels. We will deal with these under the headings of neurophysiology, multimodal integration, biomathematics, computational neuroanatomy, clinical applications and new technologies. The final section on new technologies draws on some of the open questions established in preceding sections.

2.2 Neurophysiology

Fundamental questions in imaging neuroscience centre on the relationship between neuronal responses, the hemodynamic signals measured by fMRI, and the electrical and magnetic signals measured by EEG and MEG. These issues are important because they link non-invasive brain imaging to neuronal information-processing, both at a theoretical level and in relation to multi-neuron electrode recordings of neuronal responses, as in animal studies. The latter are clearly central to understanding non-invasive imaging results in terms of invasive work in basic neuroscience.

Initial work along these lines has used either conjoint recording of electrical single neuron activity and fMRI signals in monkeys; or related measures of hemodynamics in smaller animals – e.g. optical imaging and laser-flow Dopplerometry. Although vital, these empirical set-ups are technically challenging. Only a handful of research groups around the world can implement them.

A major open question here is: “Which exact aspects of neuronal responses produce the signals measured by non-invasive brain imaging, such as fMRI?”

The possibilities currently range from simple things such as mean synaptic activity, to more complicated relationships that speak to the temporal dynamics of signal generation – e.g. specific fMRI correlates of oscillation at different frequencies in neuronal assemblies. A full understanding of these relationships will be predicated on plausible mathematical models of neuronal populations, and of how their microscopic organisation leads to the emergence of measurable ensemble responses. We return to this theme below.

2.3 Multimodal integration

One way forward is to combine different non-invasive measurements of human brain activity. In doing so, it is self-evident that fMRI and EEG/MEG have complementary strengths and weaknesses in terms of spatial versus temporal resolution. Harnessing the resolution of multiple modalities concurrently, within a single multimodal observation, is a major technical aim in the field.

While there has been some progress in coupling different measurements, a central outstanding issue remains to be addressed. To date, most fusion approaches merely harness the spatial precision of fMRI to provide constraints on the inverse source-reconstruction problem posed by EEG and MEG – i.e. resolving where the temporally well-specified EEG and/or MEG components come from spatially in the brain. In this context, under most current approaches, functional neuroimaging simply provides a prior spatial constraint. While this has certainly finessed the interpretation of EEG and MEG results, it does not represent true integration. A proper integration should allow the estimation of some aspect of functional anatomy that was hitherto inaccessible using either technique alone.

More formally, one would like to use multimodal data to estimate the parameters of a model that would otherwise be inestimable. **This relies upon the construction of forward models of neuronal populations that can generate both electromagnetic and hemodynamic signals, an ambitious but not unthinkable objective for the next few years.**

We have already made much progress on biophysical models, linking dendritic currents and local-field potentials to EEG signals measured at the scalp. There has been similar progress with models of how changes in mean synaptic activity are expressed hemodynamically, and thus detected by fMRI, through changes in blood volume, flow and oxygen content. Combining these two components with a model of interacting neuronal populations could lead to a complete forward model. The biologically meaningful parameters of this model would then be obtained by finding those parameters that produced a best match to observed data in both approaches.

The open questions here pertain to the nature of the underlying neuronal model. It is likely that neural mass and mean-field approximations will be useful. These approaches, based upon statistical physics and thermodynamics, rest upon modelling the dynamics not just of the states of a neuronal system, but rather of the probability densities of those states. Clearly, this vital enabling endeavour will require close collaboration between imaging neuroscientists and biologically invested physicists. Indeed, such interdisciplinary collaboration has been critical in every major breakthrough in functional neuroimaging to date.

2.4 Biomathematics and functional integration

Biomathematics underpins imaging neuroscience at two levels:

- it enables the development of increasingly sophisticated methods of data analysis;
- it drives the interpretation and motivation of brain mapping experiments from the viewpoint of computational neuroscience and theoretical neurobiology.

Open questions in the analysis of brain imaging data now particularly address the integration of anatomically dispersed brain systems — i.e. not just which brain areas are activated in a particular condi-

tion overall, but how does activity in one brain area influence activity in connected brain areas? This specific issue in neuroimaging relates to an increasing interest in neuroscience, and cognitive science more broadly, in how different components of the mind and brain work together. The notion of network operations is supplanting old ideas of strictly modular processes. In the neuroimaging context, the issue, referred to as ‘functional integration’, is usually thought of in terms of the effective connectivity among neuronal populations or cortical regions.

While many of the classical inference problems in analyses of spatially extended data (e.g. functional brain images) have now been resolved (by use of Gaussian field theory, for example), further work is required on the outstanding issue of how best to approach functional integration. There has been a recent move towards Bayesian inference and the incorporation of prior knowledge into estimation and inference procedures for imaging analysis. This is particularly important in relation to the analysis of functional integration because the latter is a fundamental ill-posed problem that requires priors for its solution.

Research over the next few years will investigate such questions as “How do the evoked responses in one brain area depend upon the state of others?”

To answer such questions, we require biomathematical models of interacting brain areas that will likely harness the same mean-field approaches we described in the previous section. Models and estimation schemes of this sort are central to understanding the complex functionality of the brain. Not only will they enable understanding of how remote brain areas can influence each other in the normal brain, in different cognitive states, but they should also shed light on the effects of different forms of localized brain damage, and of pharmacologically induced neurotransmitter-specific manipulations.

At present, the forward or estimation models used for data analysis and characterising empirically observed brain responses are not linked to mathematical models of brain function (e.g. generative models or predictive coding models of brain function). There is a wealth of understanding and expertise in machine-learning and computational neuroscience that may provide important mathematical constraints on possible functional brain architectures and how these might work.

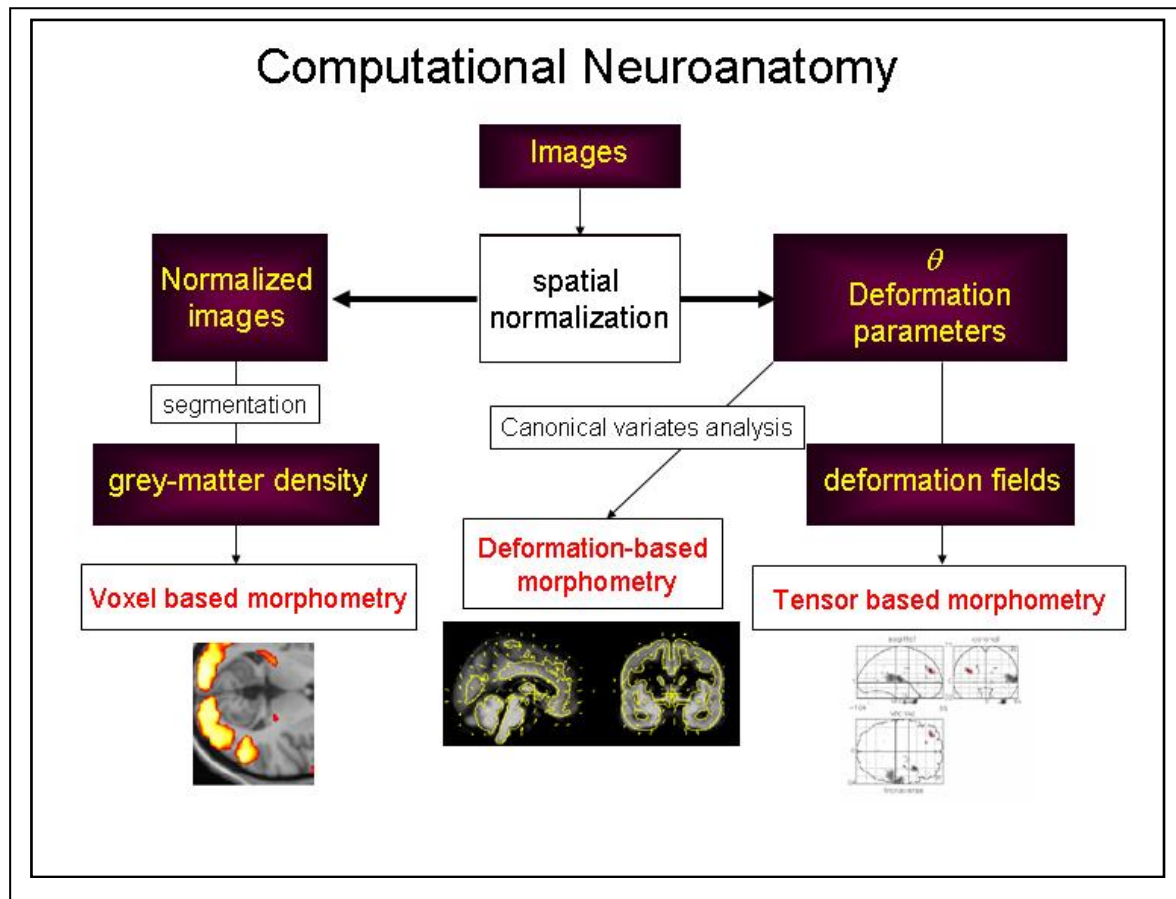
A fundamental development over the next decade will involve attempts to use such formally specified theoretical models as explicit explanations for observed brain responses. To enable this, mathematical models from computational neuroscience of how we learn and perceive things, will have to be reformulated in terms of the kind of estimation models used in data analysis. Again, such an approach will require collaboration between different disciplines; here including theoretical neurobiologists, computational neuroscientists, functional imagers and mathematicians/statisticians.

2.5 Computational neuroanatomy

In addition to functional brain imaging of changes in neural activity under particular conditions, there have been substantial developments in the computational characterization of structural brain images of anatomy. This was the traditional clinical use of MRI prior to the advent of fMRI. Initially, functional imagers saw variations between subjects in neuroanatomy largely as a ‘nuisance’ factor that had to be removed by analysis procedures prior to pooling functional data in a common or normalized brain-space. However, over the past few years, some analysis techniques used to remove such differences in individual anatomy (e.g. through nonlinear warping and other morphological operations) have been applied to longstanding questions about anatomy per se.

The application of these techniques has become known as computational neuroanatomy. From the point of view of clinical neuroscience, this may have been as important as developments in functional brain imaging. For example, we can now detect, objectively, very subtle and previously undetectable changes in the neuroanatomy of various patient cohorts – e.g. schizophrenic, depressive, normal elderly, Alzheimer’s, fragile-X etc. This is important because it provides an unbiased assessment of pathology that can be applied to the entire brain. Moreover, it can assess changes that are distributed in a structured fashion over multiple brain areas.

These techniques of structural anatomy rely on the statistical analysis and characterisation of warping fields that map an individual’s brain on to some reference template. Over the coming years, computational analysis techniques developed independently in machine vision and image processing could have a profound impact on the characterisation of these warps, and should find important applications in



clinical neuroscience. These developments will again rely upon interdisciplinary collaboration – e.g. here between clinical neuroscientists, neuroradiographers and experts in image processing.

Technological advances in diffusion-weighted imaging may enable a substantial further development in this field. Diffusion-weighted imaging allows one to assess the anisotropic diffusion of water in the brain. It can thus be an indirect measure of the integrity and orientation of white-matter tracks beneath the grey-matter formed by neurons – i.e. bundles of nerve processes that connect different brain regions, including areas that are otherwise anatomically remote. This is important because the ensuing data are high dimensional (tensor-fields) that may be sensitive not only to regionally specific changes in brain anatomy, but also to anatomical connections amongst these regions.

It will be impossible to analyse these data sets by eye. We will rely upon the continued development of computational neuroanatomic techniques. This is important for a clinical understanding of many neuropsychiatric disorders that may relate in part to subtle ‘disconnections’ between remote brain areas – as perhaps in dyslexia. It should also furnish critical evidence in the study of functional, rather than purely anatomical, integration between remote brain areas.

2.6 Potential clinical applications of neuroimaging

The potential for applied clinical uses of recent technical advances in functional and structural neuroimaging seems enormous. Notable examples include the study and treatment of deficits following localized brain-injury (e.g. caused by stroke or tumour) or other neural pathology (as in Alzheimer’s etc). It could also be useful for psychiatric disorders (e.g. schizophrenia, depression, anxiety), developmental disorders (ranging from autism to dyslexia), including those of known genetic origin (e.g. fragile-X or Williams’ syndrome), and for studying the functional neural effects of various treatment options, includ-

It is a common cliché in neurology textbooks that an experienced clinician is the most sensitive instrument of measurement for pathology. However, one only has to look at how structural brain imaging has supplanted clinical examination as the method of choice for investigating lesion-site, to see how the textbooks may have to be rewritten to accommodate the new technology.

ing pharmacological interventions.

Some advances we described in the previous section should lead to an increasingly sophisticated assessment of structural anatomical pathology in different clinical populations. Even more exciting is the potential for functional neuroimaging to shed light on how brain processes are altered in such populations, and how this constrains the observed deficit and the possibilities for recovery.

Sophisticated invasive studies of brain function in animals have, for many years, shown that lesions or disruptions to one brain region, or to fibres of passage there, can disrupt or modulate brain function in remote but interconnected areas, thus documenting how a functional network is influenced. Before the advent of functional imaging, such an approach was largely impossible in studies of human brain function. Such work was mainly restricted to correlating the local lesion-site with cognitive or behavioural impairment, as if each brain area operated in isolation. With fMRI we can now study how damage in one area or set of areas affects functional neural activity elsewhere, and how this correlates with the clinical deficit, relates to recovery and the effects of treatment.

With fMRI, in principle we can now relate the success or failure of brain implants, surgery or stimulation to the functional networks found to be differentially activated. Likewise, we can now monitor pharmacological effects in the intact and damaged brain.

Unlike PET, we can apply fMRI repeatedly. We can track changes in relative brain activity under different conditions across days, months or even years. Indeed, such experiments have already documented previously unsuspected plasticity in the adult brain, both in the course of so-called 'spontaneous,' but actually rather slow, recovery, and as a result of prolonged training. While conventional fMRI seems unlikely to be applied to very young babies – although fMRI *in utero* is now established – other imaging methods, such as optical imaging, can be administered at birth to assess brain function in relation to risk-factors such as oxygen deprivation etc.

In addition to providing critical information on how different forms of pathology influence neural networks, and how they respond to treatment interventions, we can foresee that functional imaging might also be routinely used in basic diagnosis of deficit for many clinical populations, and possibly even in legal contexts. For example, it could supplement behavioural tests of whether brain damage has produced, say, visual-field cuts or attentional deficits; memory-retrieval versus memory-consolidation deficits; hysterical or real paralysis; different forms of acquired or developmental dyslexia; even the extent of psychosis or psychopathy, and so on.

In addition to its clinical relevance, functional imaging of clinical populations should also provide information on issues that are fundamental to basic research on cognition. To give just one example, clinical studies of lesions have established that certain areas of frontal cortex seem intimately involved in 'executive processes' concerned with the planning and co-ordination of temporally extended behaviours in daily life. However, at present we know very little about how these frontal areas modulate brain activity elsewhere to produce such coordination. Once again, progress on such major issues is likely to require interdisciplinary collaboration – here between clinicians and basic researchers, involving both neuroscientists and computational modellers.

2.7 New technologies

Pursuing the theme of connections between remote areas in the brain, the use of manganese as an MRI axonal tracer is currently being explored. Manganese can pass through as many as five synapses, thus allowing polysynaptic circuits to be imaged. Manganese gives good T1-based contrast for neuronal pathways in MRI under non-toxic low doses that might possibly be acceptable to humans. In addition, manganese can act as a long lasting MRI stain for neuronal activity after temporary blood brain barrier removal with mannitol or other appropriate agents. In this way, it might be used as a functional rather than purely anatomical tracer of connections that are more or less active under different cognitive conditions. This is a secondary application that will probably be limited to animal studies. The ability to do non-invasive studies of anatomical connections in humans could offer an enormous advance in understanding the way that the brain is connected and the anatomical constraints on functional architectures.

Conventional fMRI currently relies upon intrinsic signals mediated hemodynamically throughout the brain. But it is possible to use relatively low-abundance tracers – such as specific neurotransmitters, receptors and antagonists – by enhancing their signal. The use of labelled organic compounds – para-hydrogen-based hyper-polarized carbon-13 – is being explored as one way of doing this. This could

endow functional neuroimaging with a neurotransmitter or neuroreceptor specificity with enormous implications for pharmacological research on brain processes that relate to cognition.

Researchers can also use functional Magnetic Resonance Spectroscopy (MRS) using oxygen-17, carbon-13 and nitrogen-15 labelling and proton spectroscopy to investigate in vivo metabolic pathways in the human brain. This is potentially an extremely important area of research. The key issues are the cost of labelled substances and the dependency on high-field MRI systems.

The use of targeted MR contrast agents that can map gene expression and calcium concentration is a further area of future research. Although there is no current research in this area in the UK, there has been some progress in the United States. The issues here are obtaining synthesis recipes and development of good organic chemistry at MRI sites in the UK.

It is possible that dendritic currents induce magnetic fields that could cause intra-voxel de-phasing and generate MRI signals by themselves. If this is true, MRI could detect the magnetic changes associated directly with neuronal responses (c.f. MEG). In principle, this would give the spatial resolution of MRI with the temporal resolution of MEG. This is potentially a very exciting prospect. However, the issues here are the size of the magnetic signal in relation to noise and the enormous signal averaging that would be required to measure an evoked response.

2.8 Interim conclusion

In summary, many open questions centre on integrating different measurement modalities, or bringing together different disciplines in a common research endeavour, either to understand the nature of measured brain signals or to finesse the spatio-temporal resolution of those measurements. Both are predicated on more refined and plausible mathematical models of neuronal dynamics, for which advances in machine learning and computational neuroscience will be extremely valuable.

All these endeavours require a close link between imaging neuroscience, biomathematics and physics. New technologies are primarily aimed at conferring a pharmacological or anatomical specificity on the relatively non-specific techniques that are now available. This specificity will lend our understanding of brain function a much more mechanistic basis that will be central to developments in basic cognitive and clinical neuroscience.

3 Multiple single-neuron recording: How information is represented in the brain – the neural code

Edmund Rolls

3.1 Summary

While non-invasive functional brain imaging has made remarkable progress, it is sobering to reflect on the uncomfortable fact that they provide indirect measurements of brain activity, such as hemodynamic signals. This is not quite the same as recording from neurons themselves.

In order to understand better how the brain operates, we need to know how information is represented in the brain. Rapid advances in understanding the neural code are now on the horizon, because new techniques enable recordings from large numbers of single neurons simultaneously (e.g. 100), and because new techniques in information theory enable quantitative understanding of the neural code from such recordings.

Interdisciplinary teams of empirical neuroscientists and theoreticians trained in quantitative approaches in the physical sciences or mathematics need to come together to address the issue of neural encoding. Considerable resources are needed to record the activity of large numbers of single neurons simultaneously while large numbers of different stimuli are presented.

3.2 How do populations of neurons represent information?

Single neurons are the computational elements of the brain. They transmit information to other neurons by sending 'all-or-none' action potentials along axons to other neurons. Recording the activity of many neurons simultaneously would enable us to answer questions of the following type:

To what types of input do different neurons respond by altering their spiking activity? To perform computations, brain areas receive different types of input, with some inputs represented by the activity of a quite small proportion of neurons of each type. For example, the orbitofrontal cortex contains only 3.5% of neurons whose spiking activity is related to a mismatch between an expected reward and the actual reward obtained. However, while these neurons may be a small proportion, mixed with many others that respond to visual and taste stimuli, they appear to be an important part of its computation.

Timing At what time do neurons start to respond after a stimulus? And for how long do they respond? Do neurons in certain cortical areas maintain their activity in a stimulus-selective way after a stimulus has been removed, thus implementing short-term memory?

Information content How much information is in the number of spikes emitted by each neuron, and how much by synchronization? Is information contained primarily in the number of spikes that each neuron emits in a short time period (a rate code), or is there additional information present in the relative time of firing of different neurons, as has been championed by Singer (e.g. 1999)? For example, if two neurons fired together (i.e. became 'synchronized') during stimulus 1 but not stimulus 2, could this provide information that only stimulus 1 had occurred? The relative amount of information contributed in these two ways can now be addressed quantitatively.

The answer is to use information theory, originally developed by Shannon (1948). This is the only way to measure on an equal basis what we can learn about a stimulus or event from different sources in the spike code. In doing this, it is essential to measure how much evidence stimulus-dependent synchronization adds to the spike count code. Any synchronization information present may be redundant or quantitatively small compared to the information encoded in the spike trains of different neurons in an ensemble of neurons.

Applying information theory to the question of how information the number of spikes of different neurons contains is difficult. The sampling problem of obtaining enough trials of data to estimate accurately all the probabilities involved for all neurons having particular rates and degrees of synchronization for each stimulus in the set. Until recently, this prohibited the application of information theory techniques for rate vs synchronization to cases involving more than a very few neurons (Rolls et al, 2003). However, a new approach uses decoding from neuronal responses and their relative times of firing to each stimulus of which stimulus has been seen (Franco et al, 2003). The mutual information can then be calculated straightforwardly between the decoded stimulus, and that which was actually shown, in the way described by Rolls et al (1997).

This new approach can apply to very large numbers of neurons, each with as many spikes as wished. The background to the measurement of information from populations of neurons, and many of the results obtained so far, are described by Rolls and Deco (2002).

Population coding How much information is encoded by populations with different numbers of neurons? The fundamental issue here is whether and how information increases as more neurons are added to the ensemble. Is there redundancy across the activity of different neurons, or does information increase monotonically (even linearly?) with the number of neurons in the ensemble.

Notwithstanding the excitement many feel and we have expressed above about non-invasive human brain imaging, it is also right to bring into the open the concerns of scientists who actually record signals from neurons. Specifically, neuroimaging with techniques such as fMRI does not, indeed cannot, answer issues of the timing of firing of coupled neurons (hence order), redundancy between neurons, the numbers of neurons activated for a given type of input, and response synchronization – none of which fMRI can measure. For this reason, fMRI, and neuroimaging in general, may not provide quite the hoped for route to understanding the neuronal network mechanisms that underlie neural computation. To understand how the computation works, it is necessary to know the details of the spiking activity of large numbers of neurons in each brain region.

3.3 New methods for recording spiking activity of many neurons

Recent developments enable simultaneous recording from many neurons to address the above issues. Some of these build on the 'stereotrode' or 'tetrode' developed by O'Keefe and his colleagues at University College London. One method provides up to 240 independently movable microelectrodes with the associated electronics, the Cyborg drive, <http://www.neuralynx.com>. This technique has successfully analysed the activity of neurons in the parietal cortex (Hoffman and McNaughton, 2002).

Another method uses fixed arrays of 100 silicon-mounted electrodes for cortical recording (Donoghue, 2002; Nicolelis and Ribeiro, 2002). These developments reach beyond what is now being achieved in the UK (e.g. Baker and Lemon, 2000; Lenck-Santini et al, 2002; Rolls et al, 2003). Both systems, however, involve considerable logistics. If the field advances to the point where these technologies are essential, teams in the UK will need adequate support to enable them to perform such investigations.

3.4 Understanding the computational properties of population codes

Advances in neuron recording techniques will not only allow the issues raised above to be investigated, but will also enable us to understand the computational properties of the code used by the brain.

Robustness seems to be a key property of the population coding strategy that appears to be used by the cerebral cortex. Damage to a single cell will not, in general, have a catastrophic effect on the encoded representation because the information is encoded across many cells. This is not to deny that the activity of single or small numbers of cells can be important, as has been established by work on the perception of motion after microstimulation of neurons in area MT (V5).

However, population codes turn out to have other computationally desirable properties, such as mechanisms for noise removal, generalization to similar patterns, completion from a part, and the instantiation of complex, nonlinear functions. Understanding the coding and computational properties of population codes has therefore become a main goal of computational neuroscience.

A concept of interest, which requires further development, is that if neurons have smooth Gaussian-like tuning to a stimulus dimension, these tuning functions may provide a basis for a non-linear mapping, which is the type of computation that is very important in brain function (Pouget, Dayan and Zemel, 2000).

Another concept of interest is how the neurons that receive the activity in a cortical code can decode, or interpret, it. It appears that an operation by a neuron of a function as simple as forming a weighted sum of its inputs (dot-product decoding) can extract much of the information that is available in a population code (Robertson et al, 1999; Panzeri et al, 1999). It will be important in future to determine whether neurons could, and do, make more use of information that may be available in the firing of the sending neurons.

4 Visualizing molecular events and intrinsic signals in living neurons

Andrew Matus and Bashir Ahmed

4.1 Introduction

The brain is alive, yet one might not realise this from looking at textbook pictures of stained neurons. Just as non-invasive brain imaging opened up a new world to cognitive neuroscientists ten years ago, optical imaging could be about to usher in a new revolution.

From its very beginning, the invasive investigation of brain function has depended on visualizing how nerve cells are arranged into functional circuits. The modern era in neuroscience began in the late 19th century with the first histological techniques for seeing individual neurons and the fibre-like extensions, axons and dendrites that connect them.



Much of our current understanding of brain function has come from progressive exploration of neural circuits in different parts of the brain. These measurements use a combination of electrophysiological techniques to record the activities of individual neurons coupled with anatomical methods for tracing their connections.

Until recently, the anatomical part of this enterprise was limited to dead tissue. Although microelectrodes were used to record the activities of living neurons, the tracing of connections was always performed on dead tissue that had been fixed using chemical preservatives and then stained with coloured dyes to make nerve cells and their connections visible. These are severe limitations because of the most important features of the brain's functional anatomy can be observed only in the living state. In particular, brain circuits are characterized by the property of plasticity by which functional relationships between nerve cells change, either at the level at which information is transmitted through existing connections or by the breaking and reforming of connections in new patterns. The lack of methods for visualizing the structure of living neurons has hitherto made it impossible to investigate this second, anatomical, aspect of plasticity – a crucial element in the brain mechanism of learning and memory.

Within the past few years, this situation has changed dramatically thanks to methods for making individual protein molecules visible within living cells, including brain neurons. Central to this development has been the discovery of genes, from marine animals such as jellyfish, which produce brightly fluorescent proteins inside living cells. Techniques from molecular genetics allow the introduction of these genes into the cells of mammalian species such as mice. There they produce fluorescent proteins that allow us to follow anatomical changes in live neuronal circuits.

More importantly, these same genes allow us to follow individual molecules inside nerve cells, opening up the possibility of discovering where and when molecular events underlying learning and memory take place in the adult brain. The potential of these new techniques for exploring the molecular and cellular mechanisms of brain function is vast. Equally promising is their scope for investigating disease states such as mental retardation or schizophrenia, where disturbances of nerve cell anatomy are implicated but still barely understood.

4.2 Basic aspects of the technical advances

Several advances in different fields embracing molecular biology and microscopy have come together to make possible live cell imaging of brain neurons.

4.2.1 'Tagging' neuronal structures with fluorescent proteins

Genes derived from marine organisms that produce fluorescent proteins are indispensable for these new techniques. The archetype is green fluorescent protein (GFP), derived from the jellyfish *Aequorea victoria*. As its name implies, GFP radiates green light when illuminated at an appropriate frequency. The molecular mechanism underlying this effect, now well understood, is the basis for producing mutated versions of GFP with improved spectral properties. Within the past few months, the first satisfactory red fluorescent protein (RFP) has been described (Campbell et al., 2002).

Equally important for the use of fluorescent proteins is the unexpected discovery that these genes can be joined to a wide range of non-fluorescent genes without disturbing their natural function. This makes it possible to produce *gene fusions* – that is, a fusion protein in which a neuronal protein is joined directly to the GFP protein. The result is a combination of the two proteins in which the GFP works as a fluorescent 'tag' that makes the tagged neuronal process visible in a microscope designed to capture fluorescent light (see below).

An unexpected benefit of this approach is that even difficult proteins such as actin – difficult because it is highly sensitive to changes in its chemical structure – appear to work normally inside nerve cells when joined to GFP (Fischer et al., 1998). Proof of concept has recently been obtained that we can use GFP and RFP to visualize simultaneously two proteins associated with different *cytoskeletal* structures inside live nerve cells that are associated with separate phases of circuit plasticity. The potential for extending this approach to visualize a larger number of gene products is clear.

4.2.2 Recent advances in microscopy techniques

Fluorescent staining has been used to study cell structure for almost 50 years. However, capturing images from the minuscule amounts of light emanating from GFP-tagged proteins inside living cells

makes special demands. This challenge has been met with advanced, and in some cases radically new, microscopy techniques. The most important of these is the use of highly sensitive electronic cameras based on sophisticated versions of the same charge-coupled devices (CCDs) used in modern video cameras.

Compared to conventional colour film, the sole capture medium available until a few years ago, electronic capture devices have several important advantages:

- They are far more sensitive than film to low levels of light, allowing the capture of faint images from living objects (cells) that are quickly damaged when illuminated by the strong light sources needed to produce images on conventional film.
- The signals produced by CCD cameras allow for electronic compensation to subtract the *noise*, the large constant background in a faint image. The remaining *signal* – the part of the image that actually represents the object being studied – can be amplified even before the image is recorded. Conventional film records all the light emerging from an image source. The weak image signal from a single GFP-tagged protein inside a fluorescent cell would be lost against the high background noise.
- Following on from the use of electronic capture devices, computer based techniques have been developed, and are constantly being improved, that allow automated processing of raw image data to improve image quality and extracts precise measurements.

Another advance with great potential, arising from the application of electronic image capture devices, is the development of new types of microscopes that can form images from light emitted from cells deep inside brain tissue. These instruments operate in the principle of confocal microscopy and can form high definition images of nerve cells inside brain tissue while ignoring out of focus light from other fluorescent cells above or below the cell being studied. The most advanced of these devices uses a quantum effect known as two-photon microscopy.

The principle of the two-photon microscope is that photons of light within a powerful illuminating beam combine at the focal point within the tissue to form photons at half the wavelength of the original. This has two decisive advantages. First, because the wavelength of the illuminating beam does not excite GFP, interference from out-of-focus fluorescent light above and below the point of focus is minimized. Second, the double-wavelength illuminating light used in two-photon microscopy is in the infrared region, where brain tissue is significantly more transparent compared to illuminating light used in conventional confocal microscopy. In combination, these two factors allow the imaging of structures significantly deeper within brain tissue than has previously been possible.

4.2.3 Time-lapse imaging

An additional feature of modern microscopy – made possible by the widespread use of computer systems to capture, store and process biological images – is time-lapse recording. In this procedure images of living cells are taken repeatedly at set intervals. ‘Played back’ as a continuous ‘movie’ they can reveal dynamic changes in cell morphology or molecular dynamics. Examples of rapid changes in molecular dynamics in nerve cells or slow changes in cell shape in synaptic connections appear on the Matus and Svoboda websites: <http://www.fmi.ch/members/andrew.matus/>

4.2.4 Biosensors

Several of the fluorescent proteins we have discussed are natural biosensors. They respond to physiological events with changes in fluorescence intensity. For example, some mutant forms of GFP are pH sensitive and are less fluorescent in the acidic environment of an intracellular compartment such as a synaptic vesicle. Pilot studies have demonstrated the potential of this property for visualizing synaptic vesicle release (Miesenbock et al., 1998). Another fluorescent protein, DsRed, changes colour from green to red as it ‘matures’ over a period of about 24 hours after synthesis suggesting that it may be useful as a ‘fluorescent timer’ for measuring turnover rates of physiologically important proteins or for tracing the ‘history’ of structures with which they are associated (Terskikh et al., 2000).

Based on these naturally occurring examples, researchers have set out to design biosensors with enhanced sensitivities and with specificities. Among those that may prove valuable in neurobiological applications are engineered proteins for detecting local changes in membrane potential or the activity states of ion channels, for visualizing intracellular signalling molecules such as calcium and for determining the redox or the phosphorylation states of proteins (for further discussion (see Zhang et al., 2002). These applications still require extensive development and optimization before we can use them routinely in simple cell systems. It is presently impossible to predict when, if at all, they will be ready for analysing neuronal circuitry.

4.3 Optical imaging of intrinsic signals in the brain

Many of the techniques used on brain tissue *in vitro* can also apply *in vivo* to the living, dynamic brain. These generally use intrinsic signals, although the *in-vivo* imaging of mice with GFP engineered into a subset of neurons is underway (Trachtenberg et al, 2002). Technological advances, coupled with mathematical and statistical techniques, permit the detection of intrinsic signals where the signal magnitude embedded in noise is extremely small (< 0.01%). The relevant imaging technologies are based on detection of signals emanating from optical variations (e.g., Intrinsic Signal Optical Imaging), magnetic perturbations (e.g., MEG), or radiotracer emissions (Single Photon Emission Tomography).

Intrinsic imaging exploits changes in the properties of reflected or transmitted light that can be observed through processes in the tissue. The earliest experiments involved light scattering from bundles of axons during the passage of action potentials. More recently, intrinsic signal imaging has been applied to exposed cortical tissue to delineate functional activations and has gained prominence in brain research through its association with fMRI. Extrinsic imaging is based on optical measurements that follow changes, such as the wavelength of reflected light, in the physical properties of a substance, usually a dye, as a function of stimulation of a tissue.

A new method for optical imaging, exploited in the Cognitive Neuroscience Research Centre at Oxford and other research laboratories, uses intrinsic signals to monitor activity over a wide area of the cerebral cortex (Grinvald et al., 1986). In this method, light from a halogen source, a microscope lamp, passes through a narrow-band filter and illuminates the surface of the cortex. The reflected light focuses on a detector, originally a matrix of photodiodes. More recently, video cameras have become popular. They provide many more near simultaneous images (see figure in reference Bonhoeffer and Grinvald, 1996).

Put these basic units together with computer controlled stimulation and recording equipment, and it is possible to record optical images that reflect changes in the absorption of the light by the brain tissue. This absorption depends on external stimuli and is thus a way of looking at the brain's response to those stimuli. This system has been used *in vivo* in both anaesthetized and behaving animals (Grinvald et al., 1999).

The primary advantage of these techniques is that they provide an overview of functional organization from multiple sites at a fine spatial resolution. In some cases, recordings are from a broad area of tissue, as much as tens of square mm, in an attempt to show the spatial relationship between many active neurons.

In other cases, we can make measurements at much higher magnification and can show regional variations in activity within a neuron, or between small numbers of neurons. In the latter configuration, the neurons are typically incubated in a dye that is sensitive to voltage, pH or an ion (e.g. Ca^{++}). These dye-related signals can be very fast, with time constants as short as 1-2 microseconds. They thus offer advantages over other recording methods, such as intrinsic signal imaging.

A further advantage of optical image can be its non-invasive nature. In this respect, intrinsic signal imaging is clearly preferable. Some intrinsic signal experiments have been performed on the exposed human cortex.

A final advantage of optical imaging is the possibility of addressing many questions in a single experiment. In contrast to some methods (e.g. 2-DG, c-fos), researchers can present some stimulus, observe the response, then change the configuration of the stimulus. Experiments can also combine optical imaging with other methods. Various studies have observed brain dynamics using single-unit recording, and local drug or electrical stimulus application, in addition to natural stimulation.

Presently, with intrinsic signal imaging, resolution is approximately $50\ \mu\text{m}$ by $50\ \mu\text{m}$ over $8\ \text{mm}$ by $8\ \text{mm}$ of cortex, but with poor temporal resolution (sub-seconds range). Newer protocols, for example, presentation of temporally periodic stimuli or time reversed stimuli, are increasing the spatial resolution and dramatically reducing the acquisition times (Kalatsky and Stryker, 2003).

With the use of dyes – voltage sensitive dyes, for example (Wenner et al., 1996; Antic et al., 1999; Zochowski et al., 2000; Arieli and Grinvald, 2002; Slovin et al., 2002) – temporal resolution has reached the sub-millisecond range but a smaller region of cortex has to be imaged, a few mm square. In both cases, though, only a surface view is possible and events through the cortical depth are combined over a depth of $500\ \mu\text{m}$ to $800\ \mu\text{m}$. Developments in camera/photodiode arrays, together with depth scanning based technology, would greatly aid this area of research, possibly allowing depth sectioning from the surface to the white matter. This will give a 3-D image of activity within cortical tissue together with the temporal order of activation ('4-D' images). There has already been some success in this direction (Maheswari et al., 2003).

4.4 Non-invasive optical techniques: near-infrared spectroscopy

A narrow beam of light introduced at a point can penetrate the surface. The light is scattered within the medium, with a tiny proportion of this light emitted from the surface. The emitted light takes a quasi-semicircular path through the medium. A number of techniques exploit this phenomenon to detect anatomical landmarks or functional changes without invasive procedures. Complex time-resolved measurements of light intensity at the surface – to continuously monitor cerebral oxygenation and haemodynamics non-invasively, for example (Jobsis, 1977) – allows us to study cerebral oxygenation in newborn infants (Brazy et al., 1985). Furthermore, greater depth of penetration is possible with light wavelengths in the near infrared ($750\ \text{nm}$ to $1000\ \text{nm}$) (<http://www.medphys.ucl.ac.uk/research/borg/>).

One non-invasive optical imaging technique is Optical Coherence Tomography (OCT). Laser-diodes, emitting light at wavelengths around $690\ \text{nm}$ to $850\ \text{nm}$ and at power levels from a few milliwatts to tens of milliwatts. The light from the diodes is intensity modulated at high frequency (100 to $250\ \text{MHz}$). Optical fibres with a diameter less than $1\ \text{mm}$ take the laser light to the surface: additional fibres a few mm in diameter convey the light emitted from the surface back to photomultipliers, CCD cameras or photodiodes to detect the emitted light.

There are two basic methods of OCT. A continuous method emits a constant light and detects changes in total light. The second, time-resolved, method uses light that is either intensity modulated at radio-frequency, the frequency domain method, or intensity modulated in the picosecond range, the time-domain method.

In the time-resolved methods, the frequency domain case measures the modulation amplitude and phase delay in response to an intensity modulated signal. The time domain technique measures the temporal distribution of photons emitted between points on the surface in response to illumination by an impulse of light (Gratton et al., 1994; Gratton and Fabiani, 2001; Obrig and Villringer, 2003; Hebden et al., 2002). The measurement apparatus is relatively light and can be made into a head device, e.g., MONSTIR (multi-channel opto-electronic near-infrared system for time-resolved image reconstruction) (Hebden et al., 2002).

To obtain high resolution with this approach, three-dimensional images will require a very large number of recording channels, increasing the number of source-detector pairs, refined arrangement of probe arrays (Obrig and Villringer, 2003). With refinement of recording and analysis methods (TOAST, temporal optical absorption and scattering tomography (Arridge and Schweiger, 1997)), it may be possible to attain on-line, high-temporal and spatial resolution of functional activity through a full cranial helmet (Gratton and Fabiani, 2001; Hebden et al., 2002).

4.5 State of the art in optical imaging

Although commercial instruments for two-photon microscopy are becoming available, the technique's use in cutting edge applications requires substantial investment in materials and manpower. It could benefit substantially from continued technical development.

Recent imaging studies in American laboratories have shown that it is possible to follow experience-induced changes in numbers of synapses in the brains of living mice over periods of days (Trachtenberg et al., 2002). While exciting, this time scale is still slow compared to the dynamic events thought to un-

derlie learning and memory mechanisms in the brain. Moreover, the techniques can as yet be applied only to anaesthetised and restrained animals. However, the rapid pace of development suggests that successors to these techniques will increasingly allow realistic exploration of dynamic events in brain circuits. For example, techniques are being developed for attaching a miniature fibre-optic confocal microscope to the skull of a live rat (Helmchen et al., 2001). The technical challenges are formidable, but the potential for illuminating, literally, molecular events inside the brains of freely moving animals suggests that, if successful, such techniques will revolutionize our understanding of brain function.

Developments that may contribute significantly to progress will be the adaptation of techniques using green fluorescent protein to animals biologically and behaviourally more closely related to humans. For example, a programme to develop transgenic primates, such as marmosets, for GFP-based imaging in neurons in the central nervous system via collaboration between a dedicated Brain Neuron Imaging Group, and a consortium working with tractable primates, such as the European Marmoset Research Group, may provide considerable benefits such as bringing diverse groups together:
<http://www.dpz.gwdg.de/emrg/emrgcons.htm>.

Such an initiative may also provide early identification of opportunities for diagnostic and therapeutic approaches. We should not discount the possible application of fluorescent imaging in human surgical procedures, complementing present diagnostic technology such as fMRI.

It is impossible to predict either the ultimate form nor the likely success of such advanced techniques. However, the power and scope of the overall approach argues strongly for an investment in the underlying imaging technology.

4.6 Further reading on optical imaging

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Zhang, J., Campbell, R. E., Ting, A. Y., and Tsien, R. Y. (2002). Creating new fluorescent probes for cell biology. *Nat Rev Mol Cell Biol* **3**, 906-918.

Trachtenberg, J. T., Chen, B. E., Knott, G. W., Feng, G., Sanes, J. R., Welker, E., and Svoboda, K. (2002). Long-term in vivo imaging of experience-dependent synaptic plasticity in adult cortex. *Nature* **420**, 788-794.

5 Glossary of terms

biosensor A biological molecule, usually a protein, that produces a measurable signal, usually optical, in response to a change in cell physiology.

confocal microscopy

A system of illumination that rejects out-of-focus light from biological images. There are several different confocal systems that varying in technical complexity and suited to different applications.

cytoskeleton/cytoskeletal

Structures composed of protein filaments that support and control the shapes of cells. The cytoskeleton is important in nerve cells for its role in controlling circuit connectivity.

ensemble recording

Electrophysiological recordings from a large number of single-cells simultaneously. This is not to be confused with multi-unit recording in which single-cells cannot be identified.

fixed/fixation

Fixation is the process of chemically treating biological tissue to preserve their structure after death. Fixed tissue can be stored for long periods and, with appropriate techniques the detailed anatomy of cells can be preserved down to the level of molecular structure.

functional Magnetic Resonance Imaging (fMRI)

A neuroimaging method that uses the different magnetic susceptibility of deoxyhaemoglobin compared to oxyhaemoglobin to measure changes in the activity of a

brain area, which are reflected in its metabolism and this need for oxygen. Typical spatial resolutions in human studies are 1-3 mm x 1-3 mm x 1-3 mm, and the temporal resolution is in the order of seconds. The method is non-invasive, and can be repeated on an individual subject many times.

MONSTIR Multi-channel Opto-electronic Near-infrared System for Time-resolved Image Reconstruction images brain tissue non invasively

Positron Emission Tomography (PET)

A neuroimaging method that uses radioactive isotopes to estimate the blood flow in a brain region, which reflects the metabolism of the brain region and through this the neural activity. Typical spatial resolutions in human studies are 5 mm x 5 mm x 5 mm, and the temporal resolution is in the order of 90 seconds.

staining With a few notable exceptions, the molecules that make up biological tissue are colourless. To render them visible in the microscope, fixed tissues are treated with reagents that selectively stain individual structures within a tissue, usually by reacting specifically with a particular molecule such as a protein.

synchronisation

A brain-state in which neurons that are spatially separated show firing patterns over time that are solely coupled. The existence of synchronised states is widely thought to be relevant to the binding problem in cognitive neuroscience.

TOAST A short form of 'temporal optical absorption and scattering tomography'

Two-photon microscopy

A system of confocal microscopy that exploits a quantum effect to enhance the selective imaging of in-focus light while additionally increasing the penetration of illuminating light into biological tissue.

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6.1 Web sites

<http://svobodalab.cshl.edu/>

This site contains time-lapse recordings of changes in dendritic spine numbers in brains of living mice over periods of several days.

<http://www.dpz.gwdg.de/emrg/emrgcons.htm>

The URL of the European Marmoset Group

<http://www.opt-imaging.com>

An optical imaging website