Molecular neuroimaging – A proposal for a novel approach to high resolution recording of neural activity in nervous systems

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Summary
Neuroimaging is a key methodology for the understanding of how neural systems work and how neurodynamics leads to neurally controlled behavior in animals. Current neuroimaging methods (e.g. fMRI, MEG, voltage-sensitive dye imaging) provide a critical contribution towards the current understanding of nervous systems, but they all have severe limitations in terms of temporal or spatial resolution or in terms of ability to access deep structures of nervous systems.

Here a novel molecular neuroimaging method is proposed. The method is based on using molecular components to record neural activity of individual neurons at high temporal resolution and at any depth within the nervous tissue. The proposed method is presented in terms of conceptual designs of mechanisms that are expected to work as described. However, and this should be emphasized, there is no current physical realization of the proposed molecular neuroimaging method.

The paper presents a conceptual design of the proposed molecular neuroimaging method. The aim of the paper is to stimulate thinking and experiments that may lead to the realization of the proposed method.

Introduction

Nervous systems deal with information collection, integration, analysis in animals and act as command and control centers of these organisms [1]. Understanding how the nervous system works is critical for the understanding of animals as biological organisms. Due to the complex nature of the nervous system (e.g. large number of neurons, many and various kinds of connections, various short- and long-term adaptations of connections) the current understanding of nervous system is relatively limited [1–3]. Neuroimaging methods allow the collection of detailed activity data about working neural systems and thus are critical for the progress of the understanding of nervous systems [4–7].

Current neuroimaging methods use a variety of techniques to collect data about the activity of neurons. Some methods record variations in the electrical or magnetic activity of the nervous system that are recordable on the surface of the nervous tissue (EEG, MEG) [8,9], others consider variations in magnetic signals caused by the activity of neurons (fMRI) [6] or variations in the emission of positrons due to variability of neural activity (PET) [7]. A range of methods use visible and near-visible light to detect variations in intensity of transmitted, reflected or emitted light caused by variations of the activity of neurons (e.g. Ca^{2+}-imaging, voltage-sensitive dye imaging, two-photon imaging, second harmonic imaging) [4]. Some of these methods provide very good time resolution (sub-millisecond range – e.g. EEG), others have very good planar spatial resolution (micron and even sub-micron range – e.g. optical imaging), and others can see deep into the nervous system (e.g. MRI) [4–7]. However, currently there is no effective neuroimaging method that would have very good temporal and spatial resolution, and would be able to image deep layers of three-dimensional neural systems.

Here a new proposal for high resolution neuroimaging is described. This proposal is based on the concept of molecular imaging, which means that the data collection step uses specially designed recording molecules to gather the data about neural activity. The design of the proposed method is presented in details. However, it has to be emphasized that the proposed method has no current physical realization. The aim of the paper is to stimulate interest and contribute in this way to the development of improved neuroimaging techniques.

The rest of the paper is structured as follows. First I review briefly the current range of most common neuroimaging methods. This is followed by a detailed presentation of the concepts and designs that are used in the proposed molecular neuroimaging method. In the discussion section a few critical aspects of the practical realization of the proposed method are discussed. The paper is closed by the conclusions section.
Neuroimaging methods

Neuroimaging methods in general collect data about the activity of neurons and of the neural system, which is analyzed in order to derive a representation of the recorded neural activity. The recorded activity is usually some correlate of the actual neural activity, e.g. the rate of usage of some molecules, the concentration of ions, fluorescence induced by the electrical field generated around the cell membrane of neurons [4–6]. The key parameters of neuroimaging methods are the temporal resolution (i.e. how frequent are the recording steps), the spatial resolution (i.e. what is the size of recorded surface or volume for each recording unit), the depth of the recording (i.e. how deep into the nervous tissue can the recording go), and the signal-to-noise ratio of the recording (i.e. how noisy is the recorded signal). Ideally the temporal resolution should be in the sub-millisecond range, the spatial resolution should be at the sub-micron level, the method should be able to penetrate deep into the tissue and the recorded signal should contain little noise. Usually methods that have very good temporal resolution have poor spatial resolution (e.g. millimeters or centimeters), methods that can penetrate deep into the tissue usually have relatively poor temporal and spatial resolution (e.g. seconds and millimeters or centimeters), and methods that have good spatial and temporal resolution cannot penetrate deep into the tissue (e.g. they may work only on the surface of the tissue). Most of the methods generate data that contain relatively large noise component compared to the actual signal component (e.g. the neural signal induced variation in the recorded data is in the range of a few percents or tenths of percents of the undisturbed recorded values) [4–6].

Electro-encephalography (EEG) [8] and magneto-encephalography (MEG) [9] record small changes in the electrical and magnetic fields created by neurons in the brain. The recordings are collected from a number of points on the surface of the skull or on the surface of the cortex (invasive EEG). The number of recording points may vary between a few tens to a few hundreds. The recordings can be collected with very high temporal resolution (e.g. over 20 kHz – i.e. over 20 samples in a millisecond). However, to calculate the location of origin of signals that are recorded a complex mathematical optimization problem has to be solved, which usually can be solved only numerically and may have multiple solutions. This means that the spatial features of the EEG and MEG data are relatively poor. Note that in case of cortex surface EEG with many electrodes and with strong signals (e.g. epileptic activity in the brain) the recordings may be sufficiently good to localize the source of the strong signal [10]. A related technique is the use of microelectrode arrays (MEA) to record from small volumes of neural tissue (e.g. cultured neural cells, retina) [11]. In this case the electrodes are micron-scale pin tips arranged in a regular pattern (20–200 microns apart). MEA recording is usually used with flat neural systems that allow the relatively good identification of the source of the recorded signals. However this limits the applicability of MEAs, and highlights the limitation of this technique in terms of recording from deep neural tissues.

Functional magneto-resonance imaging (fMRI) [5] and positron emission tomography (PET) [6] are based on the recording of the level of metabolic activity in the brain using detected deviations in magnetic signals (dependent on the local level of oxygen in the blood) or indirectly detected emission of positrons (dependent on the local usage of radioactively labeled metabolites). Both methods offer the possibility of deep imaging of the brain, but their temporal and spatial resolutions are poor (seconds to minutes and millimeters to centimeters). Some very high power MRI machines (e.g. having magnets with over 7 Tesla magnetic field strength) can achieve better spatial resolution (e.g. tens of microns) in small animals [12]. The signal-to-noise ratio can be improved by using contrast agents in case of fMRI, however most contrast agents are toxic, which limits their usability [13]. A related method uses optical imaging with reflected near infrared light to detect blood oxygenation changes [14], making it an alternative of the fMRI. The temporal and spatial resolution of the optical imaging is comparable to that of the fMRI due to the scattering of the light in the tissue, while the depth of the imaged tissue is in the range of centimeters, which is less than the depth of the fMRI imaging.

Optical imaging methods use transmitted or emitted light to detect the activity of neurons or of the nervous tissue [4]. While these methods can achieve very good spatial resolution (micron and sub-micron range) and very good temporal resolution (sub-millisecond range) the most significant limitation of these methods is the depth constraint of them (i.e. usually they can record only from the surface of the neural system – similar to the MEA recording) [4]. Optical imaging using fluorescent light includes for example voltage-sensitive dye imaging (i.e. the dye molecule bound to the neural membrane fluoresce and the intensity of the fluorescence depends on the strength of electrical field across the neural membrane) [15] and calcium-imaging (the fluorescence strength depends on the concentration of Ca2+ ions) [16]. A variant of voltage-sensitive dye imaging uses the measurement of the intensity of the transmitted light – in this case the electrical field across the neural membrane influences the amount of the absorbed light by the dye molecules [17]. It has to be noted that most dyes used in optical imaging are toxic, which limits the applicability of this imaging method. Recent advances in optical imaging using genetically encoded fluorescence sources have the potential to improve considerably the features of optical imaging methods (e.g. less toxicity, possibility to see deeper into the nervous tissue) [18]. Another possibility to extend the use of optical imaging to deep brain structures is to use thin light guides [19], which penetrate the brain, however, even this method is limited to be used only on the internal surface that is illuminated by the light guides. Optical imaging using laser scanning microscopes combined with voltage-sensitive dyes (two-photon laser scanning microscopy – 2PLSM [20], second harmonic imaging microscopy – SHIM [21]) allows very high spatial and temporal resolution (sub-micron and sub-millisecond range) of individual neurons, even in deeper areas of brains. The limitation of these methods is that they are essentially constrained to the imaging of very small areas, possibly parts of single neurons due to process of data collection (i.e. laser scanning along lines).

Molecular neuroimaging

The aim of the proposed novel molecular neuroimaging method is to provide detailed recording of neural activity at sub-neuron resolution (i.e. separate recording of dendrites, axons, soma and their parts), at high temporal resolution with good signal-to-noise ratio (i.e. to be able to detect details of neural activity), and at any level of depth within the nervous tissue. The key idea of the proposed method is that the activity of neurons can be recorded by appropriate macro-molecules that get attached to the membrane surface of neurons and undergo electrical field induced conformational changes in their parts that represent the recorded neural activity.

The components of the proposed molecular neuroimaging system are: (1) the recording macro-molecule; (2) the activation head molecule; (3) the start complex; (4) the termination complex; (5) the recording molecular units; and (6) the readout sub-system (parts (3)–(5) are components of part (1)). The recording components (parts (1) and (2)) are delivered to the analyzed neural system through the blood stream, assuming that they
can cross the blood–brain barrier. The components (1)–(5) are shown in Fig. 1.

The activation head molecule gets anchored into the cell membrane of neurons. This is delivered separately in advance of the imaging experiment. Specific brain localization of anchoring may be driven by conditional activation of the membrane anchoring, which may depend on the presence of specific neurotransmitters, channel molecules in the neural membrane, or other factors (e.g. localized induction of strong change in the magnetic field that may deliver the energy to activate the anchoring only in a specific spatial area within the brain). The anchoring of the activation head molecule involves the penetration of the cell membrane of the neuron and the extension into the interior of the neuron of an energy harvesting appendage of the activation head molecule. When required, the energy harvesting appendage works as an ATPase [22], gaining energy by linking to ATP molecules inside of the neuron. After anchoring the activation head molecule is ready to bind to an activated starting complex of a recording macro-molecule. The brain or recorded neural system is placed in a modified MRI machine which sets a strong magnetic field with changing direction such that each spatial position within the machine has a unique direction of the magnetic field. By modulating the amplitude of the magnetic field, the created variation in the magnetic field induces a conformational transformation in the activation head molecule. For example, this may be realized through the movement of a triplet of charged spots to new positions (see for example the mechanism of voltage-sensitive dyes [23]), indicating the direction of the magnetic field that sets their position. Following the position recording by activation head molecules the positioning magnetic field is switched off. This mechanism of localization, anchoring and position recording by the activation head molecule is depicted in Fig. 2.

The recording macro-molecule has a backbone formed of small molecular units (e.g. similar to the sugar–phosphate backbone of RNA and DNA [22]). This backbone has attached a string of recording molecular units. The beginning of the molecule has a start complex attached to it, while the other end has a termination complex attached to it. The start complex is originally partly inactive. It can attach to the activation head molecule but its conformation is such that it prevents dissociation from the activation head. The full activation of the start complex needs a change in a strong magnetic field of appropriate orientation. The analyzed brain or neural system is positioned in the modified MRI machine which delivers the localized change in the appropriate magnetic field which fully activates the start complex of recording molecules attached to activation head molecules. Recording molecules that are not attached to activation head do not get activated. Depending on the maximal amplitude of the activation signal a movable charged spot moves within the start complex. If start complex activation signals are delivered multiple times their maximal amplitude is changed such that the starting complex can record the time of activation through the movement of the charged spot. The activation of the recording molecule – activation head complex is presented in Fig. 3.

After the full activation of the start complex the link with the activation head is released and the activation head gets attached to the first recording molecular unit. This also leads to the adoption of an altered inactive conformation by the start complex, which prevents it from linking again to an activation head molecule. The activation head jumps from one recording unit to the next one starting from the start complex and moving towards the termination complex. The energy for these jumps is provided through the energy harvesting appendage of the molecule, for example a mechanism like in the case of kinesin and tubulin may work to support these jumping movements [24]. The duration of the steps is expected to be very uniform, and in the range of tenth of milliseconds. The position of the recording unit along the molecular backbone in the recording molecule indicates the time of the recording since the start of the full activation of the recording molecule – activation head molecule complex (the start time is recorded in the start complex). The process of the progress of the activation head molecule along the recording molecule is shown in Fig. 4.

The recording molecular units get activated when they get attached to the activation head molecule. Following the activation, a charged spot within the recording unit moves in response to the electric field, that is generated across the membrane of the neuron [23]. After the jump of the activation head to the next recording unit, the activation of the previous recording unit stops. The previously active unit keeps the record (the moved position of the charged spot) of the strength and direction of the electric field generated by the neuron at the time of the recording. This process of recording of neural electrical activity is shown in Fig. 5.

The activation head molecule reaches the end of the recording molecule by attaching to the termination complex. The termination complex is transformed through this attachment such that its transformation involves the movement of a triplet of charged spots to match the location of the positional indicator charged spots in the activation head molecule. After this transformation the termination complex is detached from the activation head molecule. The detached recording molecule cannot be reactivated since its start complex is in inactive form that cannot attach again to an activation head molecule. The activation head molecule anchored in the membrane of the neuron may attach to a new, previously not activated, recording molecule that may get fully activated at the next round of the magnetic stimulation that can activate recording molecule – activation head molecule complexes. Following the recording session, in absence of magnetic stimulation, the activation head molecule adopts a new conformation that

![Fig. 1. The components of the proposed molecular recording system.](image-url)
Fig. 2. The mechanism of localized activation and position recording of activation head molecules.

Fig. 3. The process of activation of the recording molecule – activation head molecule complex.
leads to its auto-cleaving such that its components are released from the neuron. The termination of the recording by a recording molecule is depicted in Fig. 6.

The recording molecules re-enter the blood stream of the organism. They are collected from the blood. The collected recording molecules are analyzed in the readout sub-system. This system uses analogues of recent DNA reading technologies that allow the reading the identity of single nucleotides in single DNA molecules using fluorescence inducing labeling [25]. It is expected that this system makes possible to read the positioning of charged spots in the start complex, recording units and termination complex through fluorescence (either directly or through appropriate fluorescent labeling). The reading from the start complex will indicate the amplitude of the magnetic field that set the position of the charged spot, and knowing this amplitude it is possible to determine the start time of the recording. The readings from the recording units will determine the strength and direction of the electric field that acted upon the recording unit, and the position of the recording unit in the sequence will determine the time of recording relative to the start time. The reading of the termination complex will require three separate readings to determine the three directional positions of the three charged spots within the molecule. These three readings will determine the direction of the position setting magnetic field at the recording site, allowing the determination of the spatial position of the recording site. In all measurements the intensity or the wavelength of the fluorescence are expected to be used as the basis of measurement. The readout methodology is represented in Fig. 7.
Discussion

The proposed molecular neuroimaging system does not exist at the moment of writing of this paper. It is described as a proposal of such system with the aim of stimulating work in the proposed direction. However, there are a few issues already that merit some brief discussion.

One issue is the delivery of the molecules into the brain or nervous system of the animal that is investigated. It is critical that all molecules are capable of passing the blood–brain barrier in both directions. Considering that these molecules are likely to be relatively large this may constitute some impediment. However, it is expected that there are molecular transit facilitation techniques that can be used to make the proposed system work (e.g. using immune cells or appropriately designed nano-particles that can pass through this barrier, as carriers).

The recording of signals may be noisy (e.g. noisy reading of the strength of the electrical field, small deviations in the times between jumps of the activation head molecule) and the readout process may add further noise to the signal that is recorded (e.g. noise in the readout of the intensity of fluorescence). To deal with this, it is expected that recording of neural activities goes on for relatively long time (e.g. minutes), and that the activity of each neuron is recorded by many recording molecules. This will allow to calculate...
are different from the electrical field generated by neurons. Such
hood of the first one.
place), and the anchoring of activation head molecules into the
short (i.e. they may record for a number milliseconds or possibly
process). This means that sufficiently many recording molecules
neural membrane may need to prevent the simultaneous anchoring
of another activation head molecule in the too close neighborhood
of the first one.
Finally, the recording units may be tuned to record signals that are
different from the electrical field generated by neurons. Such
alternative signals can be ion concentrations (e.g. Na⁺, K⁺, Ca²⁺, Cl⁻), the presence and concentration of certain neurotransmitters (e.g. dopamine, serotonin), or the presence of signaling molecules or channel molecules on the surface of neurons. These alternative recording units could extend the use of the proposed technique to allow the dissection of mechanisms contributing to neural activities of interest.

Conclusions

This paper describes a proposal for a novel molecular imaging method for neuroimaging. The proposed method is presented in terms of detailed mechanisms that are expected to work as described. Assuming that the method works as expected, it will provide a data about dynamics of neural systems at an unprecedented level of detail, allowing high temporal resolution simultaneous monitoring of the activity of very many single neurons at any level of depth in the nervous tissue.

The proposed molecular imaging method relies on the use of a recording molecule that has a similar chain-like structure as the RNA and DNA [22], and is able to record its spatial position, the start time of the recording and the electrical activity of a recorded neuron, as its recording molecular units pass through the interaction with an activation head molecule [24] anchored into the neuronal membrane. The recording mechanism is based on the use of induced dislocation of charged spots within molecules [23]. The readout conceptually is based on recent methods of reading the DNA sequence using single molecules [25].

The proposed molecular imaging method has no physical realization at the moment. The aim of this paper is to provide stimulation to thinking and experimenting that may lead to an actual implementation of the proposed molecular neuroimaging method.

Conflicts of interest statement

None declared.

References

[5] Benecke R, Nyberg L. Imaging cognition II: an empirical review of 275 PET and
[6] Logothetis NK, Pauls J, Augath M, Trinath T, Oeltermann A. Neurophysiological
functional brain imaging with positron emission tomography. London: Academic
Press; 1998.
[8] Freeman WJ. Mass action in the nervous system: examination of neurophysiological
derived from subdural strip and grid electrodes: a simulation study. Clin
[12] Cyr M, Caron MG, Johnson GA, Laakso A. Magnetic resonance imaging at
[13] Caravan P. Strategies for increasing the sensitivity of gadolinium based MRI
infants by near-infrared optical topography. Proc Natl Acad Sci 2003;100:
10722–7.
imaging of neocortical activity. In: Yuste R, Konnerth A, editors. Imaging in
neuroscience and development. Cold Spring Harbor: Cold Spring Harbor
Imaging in neuroscience and development. Cold Spring Harbor: Cold Spring
Harbor Laboratory Press; 2005. p. 239–44.
combined intracellular and optical recording of invertebrate neural network
Genetically encoded fluorescent sensors of membrane potential. Brain Cell
[19] Helmsen F, Denk W. A two-photon fiberscope for imaging in freely moving
animals. In: Yuste R, Konnerth A, editors. Imaging in neuroscience and
photon excitation of photometric probes enables optical recording of action potentials from mammalian nerve terminals in situ. J Neurophysiol
2008;99:1545–53.
imaging of live cells by second harmonic generation. Biophys J 1999;77:
3341–9.
[22] Fluhler E, Burnham VG, Loew LM. Spectra, membrane binding and
potentiometric response of new charge shift probes. Biochemistry 1985;24:
5749–55.
mode waveguides for single-molecule analysis at high concentrations. Science