Research proposal

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Overview

Systems neuroscience studies the links between (1) an animal's behaviour, (2) the activity of its neurons, and (3) how these neurons are connected. Currently, only the first two can be observed simultaneously, using *in vivo* recordings of neural activity. Observing the connections between neurons, on the other hand, requires imaging brain slices, and thus killing the animal. In addition, such a wire-tracing process is costly and time-consuming.

We aim to develop an algorithm that infers the connections between neurons based on recordings of their voltages, instead of post-mortem imaging. We believe this is possible because the activity of neurons is mainly determined by the connections between them, and because recent advances in recording technology are yielding, for the first time, the necessary quality of data to solve this problem.

Such an algorithm then allows for fast and cheap estimation of the neural wiring in behaving animals, throughout their lifetime and across experiments. This will allow systems neuroscientists – whether they study vision, memory, or movement disorders – to find answers to their questions linking brain wiring and brain (dys)function in a manner more straightforward than ever before.

Background

This section is based on references [1]–[5].

Recording neural activity

There are currently two methods in popular use to record the activity of multiple individual neurons, *in vivo*: calcium imaging and extracellular electrode recordings. The strength of each method is the weakness of the other. Both are performed through a small, surgically created hole in the skull.

In calcium imaging, neurons are filled with a calcium indicator or "dye" – a molecule that becomes fluorescent when calcium binds to it. When a neuron sends an action potential (a "spike"), its cell body is briefly flooded with calcium. A dyed neuron that has just sent a spike thus becomes fluorescent for a short while. To record activity, laser light is focused in a point and scanned across a volume of brain tissue. Neurons that recently

spiked will send light back, which is captured to yield a three-dimensional movie of neural activity. This allows scientists to observe large numbers of neurons – namely all active neurons in the volume. An additional advantage is that calcium indicators can be genetically targeted so that they only occur in specific neuron types of interest, providing a focused view. A major disadvantage however is that multiple spikes fired from a neuron in rapid succession cannot be easily distinguished, as the calcium effects of each spike are slow and combine non-linearly.

Extracellular electrode recordings on the other hand have a much finer time resolution and every spike is individually distinguishable. They work by inserting long, thin implants in the brain, that have many electrodes exposed on their surface. Each electrode measures the local electric field potential, and thereby picks up the spikes from nearby neurons. The increased time resolution comes at the cost of only sampling a small subset of the neurons in the areas of interest, not knowing exactly where those neurons are, and not being able to target neural subtypes specifically.

Calcium imaging thus provides good spatial information but has a low temporal resolution, whereas extracellular electrode recordings present the opposite tradeoff: precise spike counts and timings, but limited spatial information and sampling of neurons. Recently, a recording technique is emerging that combines the advantages of both.

Voltage imaging

Voltage imaging is very similar to calcium imaging: all or a genetically selected subset of neurons are made fluorescent, and these are scanned with a focused laser, to yield three-dimensional movies of neural activity. The difference is that the indicator molecules used in voltage imaging fluoresce in direct proportion to the membrane potential of the cell, instead of its calcium concentration. This then allows to directly observe the membrane potential of all neurons of interest in the field of view.

Although voltage imaging has existed for a long time, the recorded signal has long been too weak to distinguish it from background noise (unless animals with very large neurons are used, or the activity of many co-firing neurons is pooled together). In recent years however, multiple labs have been iteratively refining the voltage indicator molecules. Together with the improvements in fluorescence imaging technology, driven by calcium imaging, this has made voltage imaging now powerful enough to image multiple individual neurons *in vivo* in common model animals. The signal-to-noise ratio has improved to the point that not only individual spikes, but also subthreshold voltage fluctuations can be observed [7]–[9]. As explained next, it is precisely this level of detail that we believe enables *in vivo* connection mapping.

Inferring wiring from activity

The potential to infer the wiring from neural activity rests on the basic link between the two: an excitatory neuron that sends a spike will slightly increase the voltage of all its

downstream neurons (this small increase is called the excitatory postsynaptic potential, or EPSP). When a neuron has received enough spikes, its voltage crosses a threshold and it will send a spike itself. [1] To estimate neural wiring, the idea is then to invert this reasoning: if neuron B often shows activity right after neuron A has fired, then neuron A is likely to be connected to neuron B. (See the “Methods” section for a formalisation of this idea).

As both calcium imaging and extracellular electrode recordings yield (at best) spike timing data only, existing activity-to-wiring approaches have been based only on spike timing, and not on more detailed measurements of neural activity [4]. The problem with this is that the correlation between two neurons being connected and them spiking together close in time is quite tenuous. For one, most neurons need to receive many spikes – each of which can come from any of its hundreds to tens of thousands of input neurons – before it fires a spike itself. Second, many neurons have long time constants, meaning that a spike can influence spiking in its receiving neurons up to hundreds of milliseconds later. [6]

As a result, spike-based wiring inference methods require very long recording durations to obtain some confidence on the wiring between even small numbers of neurons. During these long recordings, the connectivity may have already changed. And long recordings are not possible for fluorescence imaging, as dyes require recovery after each recording session.

When we can observe the subthreshold increases in voltage occurring directly after each spike however, we might be able to accurately reconstruct connectivity from recordings on the timescale of individual *in vivo* experiments. The recent advances in voltage imaging provide exactly this kind of data.

Aims

The overall goal is to test whether subthreshold voltage recordings indeed allow *in vivo* connection mapping.

Phase 1: Design algorithm using simulations

The first step is to implement a subthreshold voltage-based algorithm for connection mapping, and evaluate it on simulated neuronal networks. These have the advantage of having a known ground truth – the connectivity is part of the simulation design – and of allowing many experiments in parallel, on a scale and speed not attainable by wet lab experiments. We will iteratively improve the new algorithm based on these test results, and compare it to existing spike-based approaches to connectivity inference.

An important part of algorithm design will be to efficiently explore the search space of possible wirings. This space grows exponentially large in the number of neurons[1] and the optimisation problem of equation (2) (see the "Methods" section) is thus not suited to exhaustive search for even relatively small numbers of neurons. Another design goal is robustness to noise. Even though it has increased considerably, the signal-to-noise ratio of voltage imaging is often still not close to that of the golden standard of intracellular voltage recordings.

Phase 2: Test algorithm on recordings

Next, the new algorithm must be tested on *in vivo* recordings with known neural connectivities. Simulations cannot consider all possible biological complexities, and tests on real data will expose the algorithm to those. A proof-of-concept on real recordings establishes a credibility that is essential to bring connectivity inference to the attention of the broader systems neuroscience community. As it stands, activity-to-wiring algorithms have only rarely been featured in the 'Methods' section of systems neuroscience papers. Besides their need for long recordings, this might be due to the fact that none of these spike-based methods have been shown to reconstruct real anatomical connectivity [7]–[11].[2]

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[1] N neurons can have N^2 directed connections between them, and for the simplest case when only the existence of connections is considered (as opposed to their weights or dynamical properties e.g.), the number of possible connectivities is 2^{N^2} (for a given connectivity, every connection is either there or not).

[2] To their credit, most of the cited studies include an application to real physiological data (mostly from ex-vivo slices and cell cultures). Sometimes, summary statistics of the calculated network (such as the connection probability between neurons) are then compared with known properties

There are as of yet no *in vivo* voltage imaging studies with corresponding anatomical wiring diagrams. The second step, with a longer-term time frame, is therefore to establish a collaboration with an imaging lab to obtain such data. One approach is through the roundworm *C. Elegans*, for which all connections between its three hundred neurons are known. *In vivo* voltage imaging has been demonstrated in *C. Elegans*, albeit only with specific subtypes of neurons [12]–[14]. With the recent developments of high-quality voltage indicators and recording technology [15]–[17], the complete recordings necessary to truly test our proposed algorithm are a small step.

In the meantime, besides testing on simulated neuronal networks, the proposed new algorithm can be applied to *in vivo* recordings without corresponding anatomical connectivity, and to recordings from small-scale neuronal cell cultures.

Methods

Inference framework

To recap, the potential for inferring the wiring from neural activity rests on the experimentally shown relationship between the two (the "forward model"), in which a connection between neurons causes spikes in the upstream neuron to affect activity in the downstream neuron. The idea is then to invert this reasoning: if neuron B often shows activity right after neuron A has fired, then neuron A is likely to be connected to neuron B. We can formalize such inverse reasoning using Bayes' theorem:

$$\underbrace{p(\text{wiring} \mid \text{activity})}_{\text{posterior}} \propto \underbrace{p(\text{activity} \mid \text{wiring})}_{\text{likelihood}} \underbrace{p(\text{wiring})}_{\text{prior}} \tag{1}$$

i.e. the probability that neurons are connected in a certain way, given recorded activity traces from these neurons, is proportional to how likely these recorded traces would be were the neurons indeed connected in this way, and to how likely such a wiring is in itself.

The likelihood is the forward model, expressed as a composition of probability distributions. The prior makes it possible to incorporate expert knowledge and provides regularisation. For example, a network where every neuron is connected to every other may be assumed less likely than one with a bit sparser connectivity.

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of the recorded-from neural tissue. However, the calculated network is never directly compared with the anatomical connectivity.

Given these two parameterised probability distributions, equation (1) provides the inverse model, or the posterior probability of any given wiring. This can be used to find the most likely wiring given the recorded activity traces:

$$\text{most likely wiring} = \underset{\text{wirings}}{\arg\max}\ p(\text{wiring} \mid \text{activity}) \tag{2}$$

One advantage of a probabilistic framing like this is that we can calculate uncertainty estimates for inferred wirings, and that we can test whether our forward model and assumptions are plausible by sampling wirings from the prior, and activity traces from the likelihood.

Forward modelling

Modelling neural activity based on their wiring is a well-established field [18]. A common model is that of the (stochastic) leaky integrate-and-fire neuron, although biophysically more detailed models are possible too. The transfer function from neural voltages to the video signal of voltage imaging can be modelled akin to how calcium imaging is modelled (as in [10] e.g.).

Equation (1) can then be used to derive the required inverse model (which, in addition to the unknown wiring, will likely also contain free parameters).

Optimisation & inference

The thusly found inverse model will likely not be analytically tractable, and equation (2) therefore not classically optimisable. Approximate inference methods like numerical sampling and variational inference can then be used to still calculate satisfactory solutions, uncertainty estimates, and model diagnostics [19]. Specifically, recent advances in machine learning allow efficient variational inference if we can specify the inverse model as a differentiable function or computer program [20]. There are multiple frameworks for the programming language Python that make this possible [21].

To address the exponentially large search space of possible neural wirings, we will need to design strong priors. In addition, known heuristic algorithms from global optimisation, such as simulated annealing or evolutionary programming, could be applied.

Model evaluation

Brian [22] and NEST [23] are two mature frameworks that can simulate subthreshold neuron voltages from given network topologies.

To obtain physiological recordings, we can contact the authors of existing voltage imaging studies, and make use of Professor Humphries' professional network. In addition, open imaging and connectomics data can be used, such as the WormAtlas (wormatlas.org) and the Allen Brain Atlas (brain-map.org).

Timeline

The following is a possible timeline.

2020	Q1	Simulate neuronal networks. Contact imaging labs.
	Q2	Implement simple baseline EPSP-based algorithm.
	Q3	Implement spike-based algorithms from literature, compare.
	Q4	Iterate on algorithm design. Write on findings.
2021	Q1	*idem*
	Q2	Apply algorithm to voltage imaging recordings.
	Q3	Iterate on algorithm design. Write on findings.
	Q4	*idem*
2022	Q1	*idem*
	Q2	Compile doctoral thesis.
	Q3	*idem*
	Q4	*idem*

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