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UNIVERSITY OF BRISTOL

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DOCTORAL THESIS

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Investigating, implementing, and creating methods for analysing large neuronal ensembles

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for the degree of Doctor of Philosophy*

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in the

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Biological Intelligence & Machine Learning Unit
Department of Computer Science

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July 8, 2020

¹⁵ Declaration of Authorship

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¹⁷ creating methods for analysing large neuronal ensembles" and the work presented in it are
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Abstract

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**Investigating, implementing, and creating methods for analysing large neuronal
ensembles**

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by Thomas J. DELANEY

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The Thesis Abstract is written here (and usually kept to just this page). The page is kept centered vertically so can expand into the blank space above the title too...

44

Acknowledgements

45 The acknowledgments and the people to thank go here, don't forget to include your project

46 advisor...

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³⁹⁹ **List of Abbreviations**

COMb Conway-Maxwell-binomial (distribution)

OASIS Online active set method to infer spikes

⁴⁰⁰ **List of Symbols**

$[Ca^{2+}]$	Free calcium concentration	mol
$[BCa]$	Fluorescent indicator bound calcium	mol
$[ECa]$	Endogenous mobile buffer bound calcium	mol
$[ImCa]$	Immobile mobile buffer bound calcium	mol
$[BCa^*]$	excited fluorescent indicator bound calcium	mol
k_{X_f}	binding (affinity) rate	s^{-1}
k_{X_b}	unbinding (dissociation) rate	s^{-1}

For/Dedicated to/To my...

402 **Chapter 1**

403 **Introduction**

404 **1.1 Overview**

405 Since Hodgkin and Huxley's squid experiments featuring a single axon (Hodgkin and Hux-
406 ley, 1939), to more recent research with spike sorted data from ~ 24000 neurons from 34
407 brain regions from 21 mice (Allen et al., 2019), the number of neurons contributing to elec-
408 trophysiological datasets has been growing. Recording methods using two-photon calcium
409 imaging have also been used to extract data from populations containing over 10000 neurons
410 (Peron et al., 2015). This dramatic growth in the number of neurons available for analysis
411 requires a dramatic change in analysis methods. In this project, we have attempted to address
412 some of the difficulties in collecting data from these large ensembles, and analysing these
413 data.

414 To focus on calcium imaging for a start, a neuron that contains a fluorescent calcium
415 indicator in its cytoplasm will fluoresce when bombarded with photons. The amount that the
416 cell will fluoresce is dependent on the concentration of fluorescent indicator within the cell,
417 and the concentration of calcium within the cell. When a neuron fires an action potential, the
418 influx of free calcium ions causes an increase in fluorescence when those ions bond with the
419 fluorescent indicator and those bounded molecules are bombarded with photons. After the
420 action potential, as calcium is extruded from the cell the fluorescence returns to a baseline
421 level. This is the basic mechanism of fluorescent calcium indicator based imaging.

422 This method has some advantages over electrophysiology as measure of neuronal ensem-
423 ble activity. Isolating individual neurons is easier and more reliable than identifying unique
424 spike sources in electrophysiology (Buccino et al., 2019). Also, spike sorting methods can
425 only detect spikes, but imaging methods can also detect cells that are not spiking, because
426 cells will emit a baseline level of fluorescence when not firing action potentials. Calcium
427 imaging sites can be re-used for weeks for longitudinal studies (Chen et al., 2013). The

428 fluorescent indicator is delivered to the cell by adeno-associated viruses, consequently there
429 can be problems with indicator gradients around the infection site, and expression levels
430 will change in individual cells over weeks (Tian et al., 2009; Chen et al., 2013). This de-
431 livery method can also cause cell pathology, and nuclear filling (Zariwala et al., 2012), but
432 these problems can be solved by using lines of transgenic mice (Dana et al., 2014). The
433 fluorescence signal itself can serve a a good indicator of cell activity, but similarly to electro-
434 physiology, the aim of calcium imaging is often spike detection.

435 If the imaging data is collected at a high enough frequency, and the signal-to-noise ratio
436 of the fluorescence trace is high enough, it should be possible to infer the spike times to some
437 level of accuracy. For example, the calmodulin based indicator GCaMP6s has a sufficiently
438 high signal-to-noise ratio that isolated action potentials can be detected and inferred (Chen
439 et al., 2013). Many spike inference algorithms exist (Vogelstein et al., 2010; Pnevmatikakis
440 et al., 2016; Friedrich and Paninski, 2016; Pnevmatikakis et al., 2013; Pnevmatikakis et al.,
441 2014; Deneux et al., 2016; Greenberg et al., 2018), and some of these can perform both cell
442 isolation and spike detection simultaneously (Vogelstein et al., 2010; Pnevmatikakis et al.,
443 2016; Pnevmatikakis et al., 2014; Deneux et al., 2016). But the relationship between spik-
444 ing and fluorescence change is not fully understood. For example, the fluorescent indicator
445 will act like an additional calcium buffer within the cell cytoplasm and will compete with
446 the other endogenous buffers to bind with free calcium cells. Therefore, the concentration of
447 those endogenous buffers, and the binding dynamics of those buffers will have an effect on
448 the change in fluorescence in response to an action potential. Furthermore, the binding dy-
449 namics of the fluorescent indicator itself will have an effect on the change in fluorescence. For
450 example, the GCaMP series of fluorescence indicators are based on the calcium buffer pro-
451 tein *calmodulin*. This protein has four binding sites, whose affinities interact non-linearly.
452 But most of the spike inference algorithms model the fluorescence as a linear function of a
453 calcium trace, and they model this calcium trace as a first or second order autoregression with
454 a pulse input to represent action potentials. Deneux et al. (2016) developed a spike inference
455 algorithm with a bit more biological inspiration, but this amounted to a very similar process.
456 While this autoregression idea appears to be a reasonable approximation, the algorithms that
457 use this approximation are outperformed by the most recently published spike inference al-
458 gorithm to be cited here (Greenberg et al., 2018). This algorithm does take into account the
459 binding dynamics of both the endogenous buffers and fluorescent calcium indicator, and the
460 concentrations of free calcium, indicator, and endogenous buffer within the cell cytoplasm.
461 This shows that there is value in more biologically inspired models of fluorescent calcium

462 indicators.

463 In light of the growing popularity of two-photon calcium imaging, and the lack of biolog-
464 ically inspired spike inference algorithms (Greenberg et al. developed their spike inference
465 algorithm in parallel to our work), we decided to develop a biologically inspired model for
466 fluorescent calcium indicator fluorescence. The idea being that our model would take a spike
467 train, or simply spike times, provided by the user, and return the fluorescence trace that would
468 be induced by this spike train or spike times. The model contains parameters for concentra-
469 tions of indicator and endogenous buffers, as well as affinity and unbinding rates for these
470 buffers. There are also parameters for the baseline concentration of free calcium in the cell
471 cytoplasm, and the cell radius (as a means for calculating the cell volume). With this model,
472 we hoped that experimentalists would be able to test out different calcium indicators on the
473 types of spike trains that they expect to encounter. This way they could decide ahead of time
474 which indicator suited their situation best. Since the output of our model is a fluorescence
475 trace, the spike inference models mentioned above can be applied to the modelled fluores-
476 cence. This means that the model could also be used to benchmark the performance of these
477 spike inference algorithms, and to investigate the impact of variations in the model on spike
478 inference accuracy.

479 We have outlined some of the advantages that calcium imaging has over electrophysiolog-
480 ogy. But electrophysiology is more useful in some situations. One particular drawback for
481 two-photon calcium imaging is that it can only be used for imaging near to the surface of the
482 brain. Although imaging with three (or presumably more) photons may solve this problem
483 in the future (Ouzounov et al., 2017). A better option for reading activity from neurons be-
484 yond the surface of the brain is to use Neuropixels probes (Jun et al., 2017). These probes
485 can be used to read from thousands of neurons simultaneously in many different areas of the
486 brain (Allen et al., 2019; Stringer et al., 2019; Steinmetz, Carandini, and Harris, 2019; Stein-
487 metz et al., 2019). This brings us to another problem for which we require new innovations
488 in our analysis methods. Specifically, analysing correlated behaviour in neural ensembles
489 consisting of neurons from many different brain regions.

490 Until the invention of new technologies such as the Neuropixels probes, most elec-
491 trophysiology datasets read from neurons in only one or two regions. Therefore most of
492 the research on interactions between neurons in different regions is limited to two regions
493 (Wierzynski et al., 2009; Patterson et al., 2014; Girard, Hupé, and Bullier, 2001). In chapters
494 3 and 4 we used datasets with neurons from 9 and 5 different brain regions respectively.

495 In light of recent findings based on correlated behaviour showing that spontaneous be-
496 haviours explain activity in many different parts of the brain that would otherwise be regarded
497 as noise (Stringer et al., 2019), that satiety is represented brain wide Allen et al., 2019, and
498 that exploratory and non-exploratory states are represented in the amygdala Gründemann
499 et al., 2019, it was clear that state representation or motor control had an influence on cor-
500 related behaviour in areas of the brain not usually associated with these tasks. Also, given
501 differences in timescales of fluctuations in different brain regions Murray et al., 2014, and
502 different timescales for event representation in different brain regions Baldassano et al., 2017,
503 we decided to investigate brain wide correlated behaviour at timescales ranging from 5ms up
504 to 3s.

505 We started off measuring the correlations in spike counts between individual neurons in
506 our ensemble. These measurements induced a weighted undirected graph where each node
507 represented a neuron, and the weight of each edge was the strength of the correlation be-
508 tween the neurons represented by the nodes at either end of that edge. In order to put the
509 neurons into groups with correlated behaviour, we applied a novel community detection al-
510 gorithm (Humphries et al., 2019) to this graph. We repeated this analysis for timescales from
511 milliseconds to seconds. Bear in mind that our correlation based graph was completely ag-
512 nistic of the anatomical regions in which our cells resided. We then compared our correlated
513 communities to their anatomy at each timescale. In this way, we used a novel method, never
514 applied neuronal data before, to analyse the makeup of correlated communities across dif-
515 ferent regions at different timescales. We found that for short timescales $< 50\text{ms}$ correlated
516 communities tended to exist within anatomical regions, and for longer timescales $> 100\text{ms}$,
517 the correlated communities tended to exist across anatomical regions. This is broadly in
518 agreement with a similar finding for EEG data from humans performing semantic or memory
519 tasks (Stein and Sarnthein, 2000). Von Stein et al. (2000) found that visual processing taking
520 place locally in the visual system was captured in the gamma frequency range (25 – 70Hz),
521 while semantic processing was captured in the beta range (12 – 18Hz), and tasks involving
522 mental imagery and working memory retention were captured in the theta and alpha ranges
523 (4 – 8Hz, and 8 – 12Hz respectively).

524 Many important findings have been made by measuring the correlations between binned
525 spike counts, but there are some problems with this method of analysis. Firstly, the width
526 of the bins used to bin spike times into spike counts has an effect on the magnitude of the
527 correlations measured. Using a short bin width can cause your measurements to be artificially
528 small (Cohen and Kohn, 2011). This may not be an issue if one is considering relative size of

correlations when using the same bin width, but it is still not ideal. Secondly, while pairwise correlations can capture most of the information in a small network (up to 40 cells) of highly correlated cells (Schneidman et al., 2006), a model based on pairwise correlations alone will fail to capture the activity of larger (~ 100 cells) networks, higher order correlated activity is required (Ganmor, Segev, and Schneidman, 2011). One problem with these higher order correlations is that they may be defined in different ways (Staude, Grün, and Rotter, 2010). Furthermore if we want to include them in a model this usually involves greatly increasing the number of parameters to fit, which increases the dimension of the parameter space leading to the ‘curse of dimensionality’. Some models attempt to sidestep these problems while still capturing higher-order correlations. These models attempt to capture the relationship between each individual neuron in the ensemble, and the ensemble as a whole. Okun et al (2015) called the strength of this relationship the ‘population coupling’, and demonstrated that this quantity can predict an individual neuron’s response to optogenetic stimulation of the whole ensemble. They also showed that this quantity was an indicator of the neuron’s synaptic connectivity (Okun et al., 2015). With the ‘population tracking model’, O’Donnell et al. (2016) linked the probability of firing an action potential for each individual neuron with the distribution of the number of active neurons. This allowed model fitting for a large number of neurons, as well as calculation of full pattern probabilities, and population entropy (O’Donnell et al., 2017).

In this work, we also aimed to capture correlated behaviour between the neurons in a neuronal ensemble without measuring correlations directly. Correlation coefficients capture the linear component of the relationship between two random variables, but will not measure any relationship beyond linearity. Also, measuring correlation coefficients using short timebins can be difficult for neuronal data (Cohen and Kohn, 2011). We decided to abandon correlation, and we aimed to quantify a more general concept of association by modelling the number of active neurons in the ensemble using a Conway-Maxwell-binomial (COMb) distribution (Kadane, 2016).

The COMb distribution is a probability distribution over the number of successes in a sequence of Bernoulli trials, where these trials can be associated in some way. The COMb distribution is an extension of the standard binomial distribution, with an additional parameter to model association between the Bernoulli variables. Using this additional parameter the distribution can capture positive association, where the Bernoulli variables tend to take the same value, negative association, where the Bernoulli variables tend to take opposite values, or no association i.e. the standard binomial distribution.

563 We fit a COMb distribution to spike sorted electrophysiological data taken from five
564 different regions in the brain of an awake mouse exposed to visual stimuli Steinmetz et al.,
565 2019. We examined whether or not a model based on the COMb distribution was able to
566 capture changes in the number of active neurons in these neuronal ensembles in response to
567 the stimuli. We also investigated the relationship between the changes as captured by the
568 COMb model and the change in neural variability as measured by Churchland et al. in their
569 famous paper (Churchland et al., 2010).

570 Our overall aim was to investigate some of the challenges in analysing large ensembles
571 of neurons present today. That included collecting the data to analyse (via calcium imaging),
572 and subsequently analysing these data. We felt that this was a worthwhile project because
573 the size of datasets, in terms of numbers of neurons and data collected, is growing rapidly.
574 Consequently these challenges will only become greater unless they are addressed. This is
575 our attempt at addressing them.

576 **Chapter 2**

577 **Sensitivity of the**
578 **spikes-to-fluorescence transform to**
579 **calcium indicator and neuron**
580 **properties**

581 *Abstract*

582 Fluorescent calcium indicators such as GCaMP are widely used to monitor neuronal activity.
583 However the relationship between the fluorescence signal and the underlying action potential
584 firing is poorly understood. This lack of knowledge makes it difficult for experimenters
585 to decide between different indicator variants for a given application. We addressed this
586 problem by studying a basic biophysical model of calcium dynamics in neuronal soma. We
587 fit the model parameters to publicly available data where GCaMP6s fluorescence and whole-
588 cell electrophysiological recordings were made simultaneously in the same single neurons.
589 We systematically varied the model's parameters to characterise the sensitivity of spike train
590 inference algorithms to the calcium indicator's main biophysical properties: binding rate,
591 dissociation rate, and molecular concentration. This model should have two potential uses:
592 experimental researchers may use it to help them select the optimal indicator for their desired
593 experiment; and computational researchers may use it to generate simulated data to aid design
594 of spike inference algorithms.

595

2.1 Introduction

596 Although fluorescent calcium indicators such as GCaMP are widely used to monitor neuronal
597 activity, the relationship between the fluorescence signal and the underlying action potential
598 firing is imperfect. The fluorescence signal has a low signal-to-noise ratio, and most indica-
599 tors' kinetics are slow relative to the millisecond-timescale dynamics of the membrane volt-
600 age (example in figure 2.1A). This makes spike inference difficult. Furthermore, the effects
601 of the indicator and cell properties on the fluorescence signal are unknown. For example,
602 genetically encoded indicators can accumulate within neurons over weeks and months (Chen
603 et al., 2013). Studies using calcium-sensitive fluorescent dyes have shown that indicator con-
604 centration has substantial effects on the spike-to-fluorescence relationship (Maravall et al.,
605 2000). Therefore spike rates inferred from GCaMP fluorescence signals may give mislead-
606 ing results if comparing across imaging sessions. More generally, the poor understanding of
607 the spike-to-fluorescence transform means experimenters may not know whether to trust the
608 outputs of spike train inference methods in any given application.

609 Spike trains are usually inferred from the time series of intensity values of one pixel of the
610 fluorescence image, where the pixel is located at the cell's soma. The problems of identifying
611 these pixels, and inferring spikes from their time series can solved separately or together.
612 When attempting to infer spikes, the fluorescence trace is modelled as a linear combination of
613 calcium concentration dynamics, a baseline calcium concentration, and some Gaussian noise.
614 The calcium concentration dynamics are modelled as an autoregressive process of degree 1
615 or 2 with a pulse input corresponding to the spike train, or the number of spikes fired in a
616 time step. The model includes no dynamics for the fluorescent indicator itself. Furthermore,
617 in order to make this model into an easily solvable linear programming problem the number
618 of spikes fired in a timestep is not restricted to non-negative integers but to arbitrary non-
619 negative values (Vogelstein et al., 2010; Pnevmatikakis et al., 2016; Friedrich and Paninski,
620 2016; Pnevmatikakis et al., 2013; Pnevmatikakis et al., 2014). More biologically inspired
621 spike inference models do exist (Deneux et al., 2016), but their fundamentals are very similar.
622 In this work, we investigated the effect of changing dynamics and buffer concentrations on
623 the accuracy of the inference algorithms based on these models.

624 The aim of this project was to model the fluorescence traces produced by a fluorescent
625 calcium indicator in a neuron soma resulting from a specific spike train, given calcium indi-
626 cator parameters such as binding rate, dissociation rate, and molecular concentration. Such

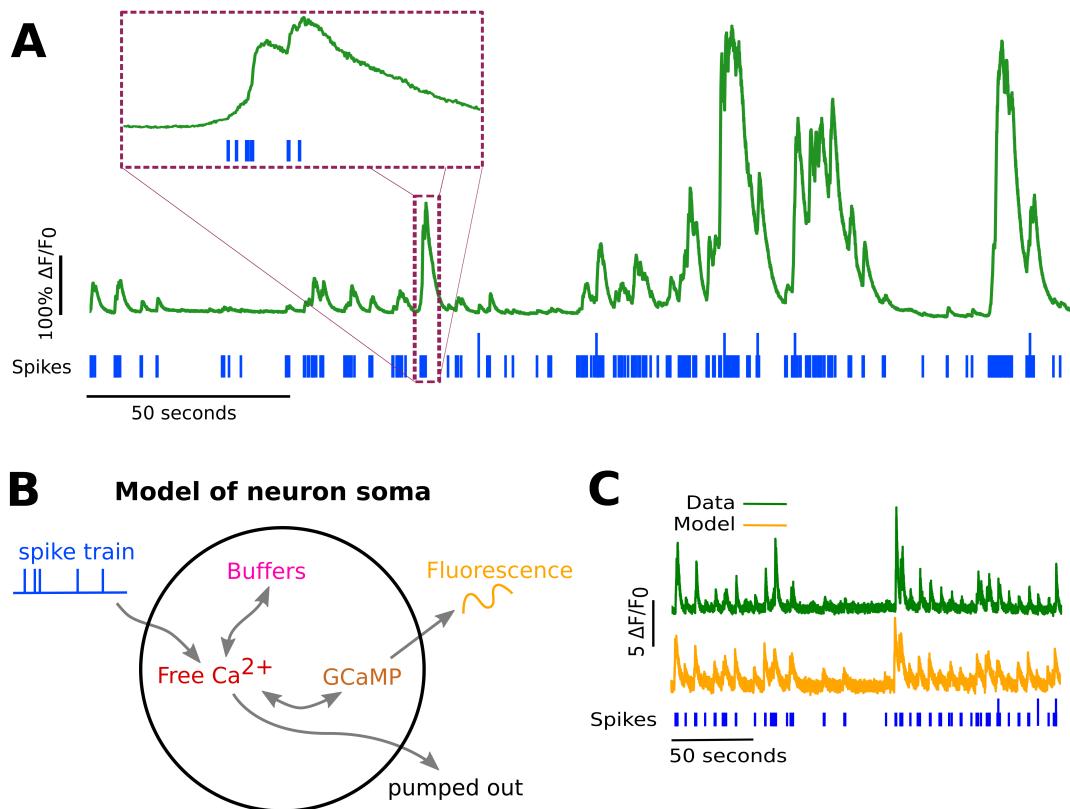


FIGURE 2.1:

A: Example spike train (blue) and the corresponding GCaMP6s fluorescence trace (green), data replotted from (Berens et al., 2018). Inset shows zoomed section of traces to highlight slow decay of GCaMP6s fluorescence relative to spike time intervals.

B: Schematic diagram of the neuron calcium and GCaMP computational model.

C: Good visual match of data fluorescence trace (green) and model simulated fluorescence (orange) in response to an identical spike train (blue).

627 a model would allow benchmarking of various spike inference algorithms, and enable under-
628 standing of how indicator characteristics affect the quality of spike train inference.

629 The model we developed consisted of free calcium, fluorescent indicator molecules, and
630 mobile and immobile endogenous calcium buffers. The indicator molecules which were
631 bound to a calcium molecule could be either excited, i.e. able to release a photon, or relaxed.
632 In order to reproduce the noise inherent in the data collection, we modelled the release of
633 photons from the excited indicator bound calcium as a stochastic process.

634 The fluorescence traces produced by the simulation were calibrated to reproduce the
635 signal-to-noise ratio observed in experimental data. Previously published spike inference
636 algorithms were then used to infer spike trains from the experimental fluorescence traces and
637 the modelled fluorescence traces. The parameters of the model were then varied in order to
638 determine the effect on the system dynamics and the effects on spike inference.

639 2.2 Methods

640 2.2.1 Calcium dynamics model

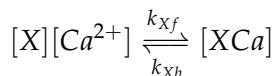
641 We wrote a biophysical model of the calcium dynamics within a cell soma. When a neu-
642 ron fires an action potential, voltage-dependent calcium ion-channels open up that allow a
643 current of Ca^{2+} to flow into the neuron (Koch, 1999). The increase in the free calcium ion
644 concentration inside of the cell, along with changes in the concentration of potassium and
645 sodium, causes the change in cell membrane potential, which must be depolarised. The de-
646 polarising process consists of free calcium ions leaving the cell through open ion channels,
647 or binding to molecules within the cell called buffers, or calcium storage by organelles such
648 as the endoplasmic reticulum. A diagram illustrating the cell, its channels, and its buffers
649 can be seen in figure 2.1A. There are several different types of calcium buffer, each with
650 different dynamics and different concentrations within different types of excitable cell. The
651 fluorescent calcium indicator is another calcium buffer, with the useful property that when it
652 is bound to a calcium ion, the bound molecule may become excited by a photon and release
653 a photon in return. This is what creates the fluorescence. After the action potential has taken
654 place, the free calcium concentration within the cell will return to a baseline level (Maravall
655 et al., 2000).

656 We modelled the the dynamics of five molecular concentrations,

- 657 • Free calcium ion concentration, $[\text{Ca}^{2+}]$

- 658 • Fluorescent indicator bound calcium, $[BCa]$
 659 • Endogenous mobile buffer bound calcium, $[ECa]$
 660 • Endogenous immobile buffer bound calcium, $[ImCa]$
 661 • Excited buffered calcium, $[BCa^*]$

The term ‘buffering’ refers to free calcium ions coming into contact with buffer molecules and binding together. Diagrammatically:



662 where $[X]$ represents any buffer molecule, and k_{X_f} and k_{X_b} represent the binding and un-
 663 binding (dissociation) rates in units of per molar concentration per second ($M^{-1} s^{-1}$) and per
 664 second (s^{-1}) respectively. The speed of this chemical reaction is determined by the binding
 665 and unbinding rates.

666 There are a number different endogenous buffers in any neuron. Which buffers are
 667 present, and the buffers’ concentrations vary from cell to cell. In order to capture the ef-
 668 fects of mobile and immobile endogenous buffers without introducing several parameters,
 669 they were modelled as two buffers. One representing mobile buffers and the other represent-
 670 ing immobile buffers. Each with their own binding and unbinding rates.

The fluorescent calcium indicator behaves similarly to the other calcium buffers. The calcium is buffered by the indicator in the same way. But an indicator bound calcium molecule can become excited by absorbing the energy from a photon. An excited indicator bound calcium molecule can then release a photon to go back to its ‘relaxed’ state.



671 The released photons are captured by a photon collector. This gives us the fluorescence trace.
 672 Ignoring the baseline level of free calcium in a neuron, the system of equations we used
 673 to model all of these interactions is as follows:

$$\begin{aligned} \frac{d[Ca^{2+}]}{dt} = & k_{Bb}[BCa] + k_{Eb}[ECa] + k_{Imb}[ImCa] \\ & - k_{Bf}[B][Ca^{2+}] - k_{Ef}[E][Ca^{2+}] - k_{Imf}[Im][Ca^{2+}] \\ & + \beta([Ca_0^{2+}] - [Ca^{2+}]) \end{aligned} \quad (2.1)$$

$$\frac{d[BCa]}{dt} = k_{Bf}[B][Ca^{2+}] - k_{Bb}[BCa] + r[BCa^*] - e[BCa] \quad (2.2)$$

$$\frac{d[ECa]}{dt} = k_{Ef}[E][Ca^{2+}] - k_{Eb}[ECa] \quad (2.3)$$

$$\frac{d[ImCa]}{dt} = k_{Imf}[Im][Ca^{2+}] - k_{Imb}[ImCa] \quad (2.4)$$

$$\frac{d[BCa^*]}{dt} = \eta[BCa] - r[BCa^*] \quad (2.5)$$

674 where $[Ca_0^{2+}]$ is the baseline calcium concentration within the cell soma, β is a rate defining
675 how quickly free calcium enters or leaves the cell in the absence of an action potential, η
676 is the excitation rate for indicator bound calcium, r is the photon release rate for the excited
677 indicator bound calcium, and f and b are used to indicate the forward and backward rates
678 for chemical reactions respectively. The excitation rate defines the proportion of indicator
679 bound calcium that becomes excited at each time step. The photon release rate defines the
680 proportion of excited indicator bound calcium that releases a photon and returns to its relaxed
681 state at each time step. An action potential is modelled as a discontinuous increase in the free
682 calcium concentration to an appropriate value (Maravall et al., 2000).

683 Note that each of the three pairs of binding and unbinding terms in the first equation has a
684 corresponding pair in one of the subsequent three equations. Binding removes a free calcium
685 molecule and adds a bound calcium molecule, and unbinding does the opposite.

686 When using this model to simulate a fluorescence trace, the system of equations above are
687 first solved over a period of 25s without action potentials. This lets each of the five tracked
688 chemical concentrations reach their steady state. Then we use the given spike train and the
689 parameters to model the fluorescence trace.

690 Note that since the model has no spatial component, the mobile and immobile buffers
691 only differ in their binding and unbinding rates.

692 Photon release & capture

693 We used a simple model for the photon release. The number of photons released at each time
694 step was controlled by the number of excited indicator bound calcium molecules in the cell
695 and a parameter called the ‘release rate’. The release rate is an optimised free parameter of
696 the model.

697 As for the photon capture, in two-photon excitation microscopy the photons scattered
698 by the fluorescent indicator get scattered in all directions. Therefore the number of photons
699 detected is stochastic. This made the process for capturing photons the natural source of
700 noise in the system. The number of photons captured, and therefore the intensity of the

701 fluorescence, is modelled using a binomial distribution. The number of photons released was
702 used as the number of trials. The probability of success, or ‘capture rate’ was a free parameter
703 of the model that we optimised.

704 **2.2.2 Parameter optimisation**

705 The free parameters of the model are as follows:

706 **Calcium rate, β** Controls how quickly the concentration of free calcium will be driven to
707 the baseline concentration.

708 **Capture rate, p** The average proportion of photons captured by the photon detector.

709 **Excitation rate, η** The number of indicator bound calcium molecules that become excited
710 by photon bombardment at each time step.

711 **Release rate, r** The number of excited indicator bound calcium molecules that release a
712 photon at each time step.

713 To optimise the free parameters given a fluorescence trace, we applied the following proce-
714 dure:

- 715 1. The frequency power spectrum of the trace was measured.
- 716 2. The power spectrum was smoothed using a boxcar smoother (aka. sliding average, box
717 smoother).
- 718 3. The log of the smoothed power spectrum was measured.
- 719 4. Use the model to create a modelled fluorescence trace.
- 720 5. Apply steps 1, 2, and 3 to the modelled fluorescence trace.
- 721 6. Calculate the root mean squared difference between the log power of the actual fluo-
722 rescence trace, and the log power of the modelled fluorescence trace.
- 723 7. Calculate the root mean squared difference between the actual fluorescence trace and
724 the modelled fluorescence trace.
- 725 8. Use an optimisation algorithm to reapply this process, attempting to minimize the sum
726 of the two root mean squared differences at each iteration.

727 Using the root mean squared difference of the log power spectra as part of the objective
728 function forces the model to match the noise frequency of the actual fluorescence. Using
729 the root mean squared difference of the traces themselves forces the model to match the
730 amplitude of the fluorescence trace more accurately.

731 In order to minimise the objective function, a suite of meta-heuristic optimisation (aka.
732 black-box optimisation) algorithms were implemented on each of the traces in the dataset.
733 These methods were chosen because they don't require a gradient for the objective function
734 (gradient-free) and they are particularly useful for minimising stochastic objective functions
735 like the one we used here. The free parameters were optimised for each individual fluores-
736 cence trace. The most successful method for each trace was recorded. The method that was
737 most often successful was probabilistic descent, and the second most successful method was
738 generating set search. Both of these methods are examples of pattern search. These two
739 methods were the best optimisers on about 75% of the traces in the dataset.

740 Although this optimisation procedure minimises the value of the optimisation function,
741 the value never reaches zero for a number of reasons. Firstly, the fluorescence traces carry low
742 frequency fluctuations that cannot be captured by the model. Secondly, the model assumes
743 that the process of calcium binding to the fluorescent indicator is linear in time (see equation
744 1), but there are more complicated dynamics involved here. Fluorescent calcium indicators
745 are often built upon the calcium binding protein called 'calmodulin'. This protein has four
746 calcium binding sites. These sites are locally split into two pairs. Each pair has a different
747 affinity for calcium, and the affinity of the binding sites is affected by the occupancy of
748 the other binding sites (Kilhoffer et al., 1992). So the calcium to calcium indicator binding
749 process is non-linear, but the model does not take this into account.

750 **Fixed parameters**

751 As well as the optimised parameters mentioned in section 2.2.2, the model also has thirteen
752 fixed parameters. Please see table 2.1 for details of these parameters and their values. In
753 an application of the model, these parameters can be changed in order to model any given
754 fluorescent calcium indicator.

755 **2.2.3 Julia**

756 The programming language used to write and execute the model was 'Julia'. Julia is a dy-
757 namic programming language designed for technical computing. Julia was designed specif-
758 ically to provide a convenient high-level dynamic language similar to MATLAB, or Python,

Parameter	Description	Value	Source
baseline	The baseline concentration of free calcium within the cell soma	$4.5 \times 10^{-8} M$	(Maravall et al., 2000)
cell radius	The radius of the soma (assumed to be spherical)	$10^{-5} M$	(Fiala and Harris, 1999)
endogenous	The concentration of endogenous mobile buffer within the cell soma	$10^{-4} M$	(Faas et al., 2011)
frequency	The frequency at which the spike trains are sampled.	100Hz	
immobile	The concentration of endogenous immobile buffer within the cell soma	$7.87 \times 10^{-5} M$	(Bartol et al., 2015)
indicator	The concentration of fluorescent indicator within the cell soma	$10^{-4} M$	(Maravall et al., 2000)
k_{Bb}	The unbinding rate of the fluorescent calcium indicator	$160 s^{-1}$	(Bartol et al., 2015)
k_{Bf}	The binding rate of the fluorescent calcium indicator	$7.77 \times 10^8 s^{-1} M^{-1}$	(Bartol et al., 2015)
k_{Eb}	The unbinding rate of the endogenous mobile buffer	$10^4 s^{-1}$	(Bartol et al., 2015)
k_{ef}	The binding rate of the endogenous mobile buffer	$10^8 s^{-1} M^{-1}$	(Bartol et al., 2015)
k_{Imb}	The unbinding rate of the endogenous immobile buffer	$524 s^{-1}$	(Bartol et al., 2015)
k_{Imf}	The binding rate of the endogenous immobile buffer	$2.47 \times 10^8 s^{-1} M^{-1}$	(Bartol et al., 2015)
peak	The increase in free calcium concentration within the cell induced by an action potential	$2.9 \times 10^{-7} M$	(Maravall et al., 2000)

TABLE 2.1: **Fixed parameters** A table of the parameters fixed before optimising the model. The values of these parameters could be changed to model different fluorescent calcium indicators.

759 with improved performance. Julia’s type system and Julia’s direct interfaces with C and
760 Fortran allow this aim to be achieved (Bezanson et al., 2012). The Julia version of the
761 ‘Sundials’ package for ODE solving was used to solve the system of equations above. The
762 BlackBoxOptim.jl package for Julia was used to perform the optimisation.

763 **2.2.4 Spike inference**

764 We used spike inference algorithms to compare the quality of spike inference using the mod-
765 elled traces to the quality of spike inference using the observed traces. We also used the
766 spike inference algorithms to assess the effect of parameter perturbation on the spike infer-
767 ence. Three algorithms were used:

768 **Constrained non-negative deconvolution algorithm (aka Pnevmatikakis algorithm)** This
769 algorithm uses a constrained version of non-negative Weiner deconvolution to infer a
770 calcium signal and a ‘spiking activity signal’ from the fluorescence trace (Vogelstein
771 et al., 2010; Pnevmatikakis et al., 2016). The spiking activity signal is a non-negative
772 vector of real numbers reflecting the cell’s activity rather than an actual spike train. We
773 inferred a spike train by choosing an optimised threshold for the spiking activity sig-
774 nal. Whenever the spiking activity signal exceeded that threshold, an action potential
775 was inferred. The threshold was optimised by minimising the difference between the
776 number of spikes observed and the number of spikes predicted.

777 **ML-Spike algorithm** This algorithm uses a generalised version of the Viterbi algorithm to
778 return the spike train that maximises the likelihood of producing the given fluorescence
779 trace. The Viterbi algorithm is an algorithm for estimating the most likely sequence
780 of hidden states resulting in a sequence of observed states in a discrete-time finite-
781 state Markov process (Forney, 1973). In this case, each hidden state is defined by the
782 presence or absence of an action potential, and each observed state is the value of the
783 fluorescence trace at each time step. This algorithm assumes that the concentration of
784 calcium within the cell will decay to a drifting baseline, rather than a fixed baseline
785 (Deneux et al., 2016).

786 **Online Active Set method to Infer Spikes (OASIS)** This algorithm is once again based on
787 an auto-regressive model of the fluorescence trace, but can be generalised to any or-
788 der. The algorithm itself is a generalisation of the pool adjacent violators algorithm
789 (PAVA) that is used in isotonic regression. The OASIS algorithm works through the

790 fluorescence trace from beginning to end, this combined with the speed of the algo-
791 rithm means that it could be used for real-time online spike inference (Friedrich and
792 Paninski, 2016). Given a fluorescence trace, the algorithm will return the most likely
793 spike train and an inferred denoised fluorescence signal.

794 In order to quantify the quality of spike inference for a given algorithm, we ran that algorithm
795 on all of the fluorescence traces in dataset number eight of the spike finder datasets. Then we
796 measured some binary classification measures on the results. These measures included

- 797 • Accuracy
- 798 • True positive rate (aka recall, sensitivity, hit rate)
- 799 • True negative rate (aka specificity)
- 800 • Precision
- 801 • Negative predicted value
- 802 • False negative rate (aka miss rate)
- 803 • False positive rate (aka fall-out)
- 804 • False discovery rate
- 805 • False omission rate

806 In making these measurements, we allowed a tolerance of two subsequent time bins for spike
807 prediction. For example, the spike train data is a vector of 0s and 1s, with one element for
808 each time bin. A ‘0’ denotes inactivity, a ‘1’ denotes the presence of at least one action
809 potential. The inferred spike trains produced by the spike inference algorithms take the same
810 form. In our analysis, if a spike appeared in the inferred spike train up to two time frames
811 after a spike in the observed spike train, that spike was considered correctly inferred i.e. a true
812 positive. However, once a spike in the inferred spike train was matched to a spike from the
813 observed spike train, the inferred spike could not be matched to another observed spike. To
814 illustrate, if two spikes were inferred in the two time bins following an isolated observed
815 spike, the first inferred spike was considered correctly inferred, but the second inferred spike
816 was considered incorrectly inferred, i.e. a false positive.

817 The most useful measure was the true positive rate. This is because the spiking is sparse
818 and this measurement is sensitive to the number of spikes observed and inferred, but is not
819 affected by the true negative or false negative rates. After optimising the parameters for each

820 fluorescence trace we measured the spike inference quality for the observed fluorescence
821 traces, and compared this to the spike inference quality for the modelled traces.

822 When measuring the spike inference quality for higher frequency spike train (1 – 10Hz),
823 we used the accuracy as our binary classification measure. At these frequencies the variance
824 of the fluorescence trace was much higher than for sparser spiking regimes, therefore we
825 wanted to take into account the number of false negatives inferred by the algorithm.

826 **Comparing spike inference quality**

827 In order to compare spike inference quality we had to use methods for comparing samples.
828 When comparing the true positive rate distributions arising from two different datasets, or
829 two different algorithms on the same dataset, we compared the distributions using a paired
830 t-test.

831 **2.2.5 Perturbation analysis**

832 In order to measure the sensitivity of spike inference to changes in a given model parameter,
833 we perturbed the parameter and compared the quality of spike inference with the perturbed
834 parameters to the quality of spike inference with the experimental or optimised parameters.
835 In order to maximise the possibility of observing a difference due to the perturbation, we
836 perturbed the chosen parameter by a relatively large amount. For example, the experimen-
837 tal value for the molar concentration of the fluorescent indicator within the cell was 10^{-4}M
838 (Maravall et al., 2000). The perturbed values used for this parameter were 10^{-2}M , 10^{-3}M ,
839 10^{-5}M , and 10^{-6}M . The quality of the inference was compared by measuring the true posi-
840 tive rate for each perturbed value and using a t-test to compare the distributions of the results.

841 This analysis was performed firstly without any optimisation of the free parameters for
842 use with the perturbed parameters. Then the analysis was performed after the optimised
843 parameters for each perturbed value were calculated.

844 **2.2.6 Signal-to-noise ratio**

845 To assess the effect of perturbation on the modelled traces, we measured and compared the
846 signal to noise ratio (SNR) on each of the modelled traces. We calculated the SNR as the
847 peak change in fluorescence divided by the standard deviation of the baseline fluctuation of
848 the fluorescence trace (Tada et al., 2014). We measured these values by running the model
849 on a spike train consisting a long period of inactivity followed by one action potential. We
850 ran the model on this spike train one hundred times. We then measured the mean change

851 in fluorescence and standard deviation of baseline activity across the one hundred modelled
852 fluorescence traces, and calculated the SNR.

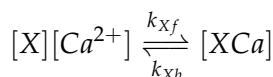
853 **2.2.7 Data sources**

854 All of the data used in this project was sourced from the ‘Spike Finder’ project (spikefinder.codeneuro.org).
855 The data consisted of a collection of datasets with simultaneously measured fluorescence
856 traces and action potentials (Berens et al., 2018).

857 **2.3 Results**

858 **2.3.1 A biophysical computational model can generate accurate fluorescence
859 traces from spike trains**

To study the relationship between action potential firing and calcium fluorescence, we built a computational model of calcium dynamics in a neuronal soma. The model consisted of four dynamic variables: the concentration of free calcium, two types of endogenous buffer, and the calcium-sensitive fluorescent indicator. Each of the buffers and the indicator could independently bind and unbind with calcium. These reactions were modelled as



860 where X is the buffer concentration and Ca^{2+} is the calcium concentration. Each species
861 could therefore exist in two states: either bound with calcium or unbound. To model the
862 imaging process, we also added a third, excited state to the indicator. When in the calcium-
863 bound state, the indicator could be converted to an excited state, corresponding to the absorp-
864 tion of a photon. The rate of this excitation process could be interpreted as the intensity of
865 the light illuminating the sample. Once excited, the species decayed back to the unexcited
866 state at a fixed rate, corresponding to the spontaneous emission of photons. The total emitted
867 fluorescence signal was interpreted as proportional to this de-excitation flux. To represent
868 experimental noise in the photon capture process, we drew a random number of captured
869 photons at each time step from a binomial distribution, parameterised by a number p that
870 corresponds to the mean fraction of released photons that are captured.

871 The model had 17 parameters in total describing the molecules’ concentrations and re-
872 action rates (Methods). We set 13 of these parameters to values from the literature. The
873 remaining 4 parameter values we fit to publicly-available data (Berens et al., 2018), briefly

explained as follows (see Methods for full details). Single neurons from acute rat cortical slices expressing GCaMP6f were imaged with two-photon microscopy while the membrane potentials of the somata of the same neurons were simultaneously recorded via whole-cell patch clamp electrophysiology. In this dataset, the electrical recordings give unambiguous information about neurons' spike times. To do the parameter fitting, we feed these spike trains as inputs to the computational model. After running, the model returns a simulated fluorescence trace. We aimed to find the model parameter values that give the best match between this simulated fluorescence trace and the real fluorescence time series recorded in the corresponding neuron. To do this we used a suite of optimisation procedures to jointly fit both the real neuron's fluorescence time series and power spectrum, which capture complementary information about the spikes-to-fluorescence mapping (Methods). We performed the fitting procedure independently for each of the 20 neurons in the spikefinder dataset (<http://spikefinder.org>). After fitting, the model produced realistic-looking fluorescence time series (Figure 2.1).

2.3.2 Spike inference algorithms perform similarly on real data compared with time series simulated from the model

Researchers often pass the fluorescence time series through a spike inference tool before performing further statistical analyses. These spike inference algorithms take the fluorescence trace as input and attempt to estimate the neuronal spike train that triggered them (Vogelstein et al., 2010; Pnevmatikakis et al., 2016; Friedrich and Paninski, 2016; Pnevmatikakis et al., 2013; Pnevmatikakis et al., 2014; Deneux et al., 2016). Part of our motivation for building this model was to allow us to ask the question on-linearities properties of the cell and the calcium indicator affect the quality of spike inference? In order to trust the conclusions from our model, we should first be confident that spike inference from our simulated fluorescence traces is similar to that from the real data. To test this we passed each of the simulated fluorescence traces through three previously published spike inference algorithms, quantified their performance against the ground-truth electrophysiology data, repeated the procedure for the real calcium fluorescence time series, and compared the accuracy of the inference processes in all cases. The *true positive rate*, also known as the *recall*, the *sensitivity*, or the *probability of detection* of spike inference varied across the three inference algorithms we tried (p value and statistical test here). The constrained non-negative matrix deconvolution algorithm (Pnevmatikakis et al., 2016) (CNMD algorithm) correctly detected approximately 45% of the

906 true spikes, the OASIS algorithm (Friedrich and Paninski, 2016) correctly detected approx-
 907 imately 35% of the true spikes, and the ML spike algorithm (Deneux et al., 2016) correctly
 908 detected approximately 15% of the true spikes (see figure 2.2). Notably, for two of the three
 909 inference algorithms, the quality of inference was also fairly consistent for individual spike
 910 trains, not just the group means ($p > 0.05$, paired t-test). This demonstrates that the models
 911 were generating fluorescence time series that were similarly difficult to decode as the real
 912 data, in ways that were not specific to any one inference algorithm. This is evidence that the
 models captured real aspects of the spikes-to-fluorescence transform.

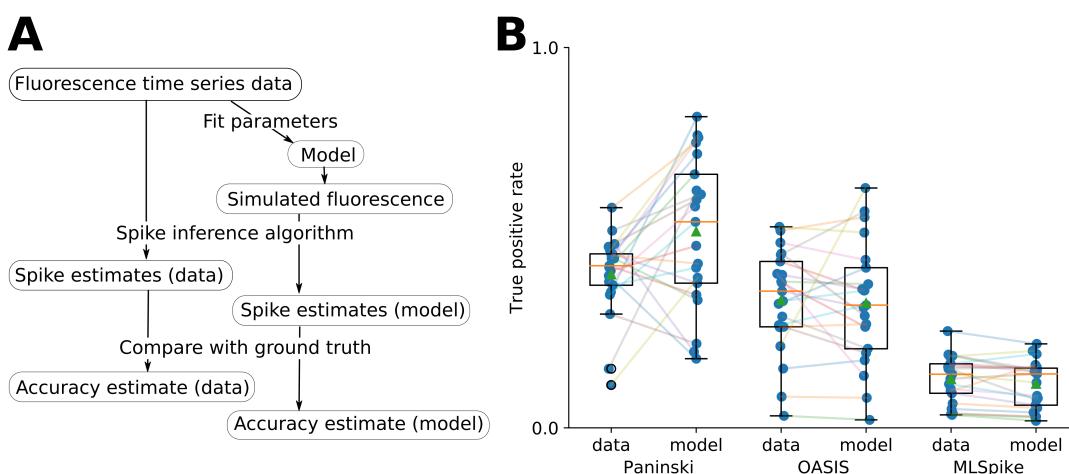


FIGURE 2.2:
 A: Workflow to compare spike inference for real versus simulated fluorescence data.
 B: True positive rates achieved by three different spike inference algorithms when applied to observed spike trains, and simulated spike trains. Data points overlaid as blue circles. The performance is similar from real and simulated data for each of the algorithms.

913

914 2.3.3 Relative effects of various buffers to the fluorescence signal

915 One of the benefits of computational models over laboratory experiments is that we can
 916 observe all the variables in the simulation to gain insight into the system's dynamics, which
 917 can be difficult to do in the lab. We plotted the concentrations of the various species over
 918 time for a version of the model fit to one data set, in response to the same train of spikes used
 919 for fitting (figure 2.3). Figure 2.3a shows the absolute values of the species concentrations,
 920 summed. Consistent with experimental estimates (Maravall et al., 2000), only a small fraction
 921 ($\sim 0.1\%$) of calcium is free and unbound to any buffer. Of the bound calcium, the vast
 922 majority, ($\sim 96\%$) is bound to the GCaMP indicator. The two types of endogenous buffer

923 are bound to the remaining calcium ($\sim 4\%$). An influx of calcium from a single spike adds
924 very little to the total calcium, in relative terms (red line in Figure 3a).

925 When calcium entered the model neuron it was rapidly buffered (Bartol et al., 2015).
926 However the relative fractions of which buffer molecules bound to the influxed calcium was
927 dynamic, and changed over time. Figure 2.3 (b-f) shows the time course of the various
928 species over time in response to a calcium influx event from a single action potential. Cru-
929 cially, the indicator $[BCa]$ competed with the endogenous buffers $[ImCa]$ and $[ECa]$ – all
930 three bind calcium on similar timescales. This implies that the timecourse and amplitude of
931 the $[BCa]$ variable will also depend on the binding rates and availabilities of the endogenous
932 buffers. For example if we decreased the concentration of an endogenous buffer, we might
933 expect both a faster rise time and greater peak amplitude of the $[BCa]$ signal in response to
934 a calcium influx event. The slowest component of the decay had a similar time constant for
935 $[BCa]$, $[ImCa]$ and $[ECa]$, which in turn matched the $[Ca]$ extrusion time constant in our
936 model ($\sim 6.29 \times 10^{-22} \text{Ms}^{-1}$). This implies that the buffers and the indicator had reached
937 a dynamic equilibrium and were jointly tracking the free calcium concentration as calcium
938 was slowly extruded from the cell.

939 Interestingly the excited bound calcium species ($[BCa^*]$) showed a qualitatively different
940 timecourse in response to a calcium influx event. This concentration is subject to the added
941 ‘excitation and release’ dynamic, where a certain proportion of the concentration absorbs the
942 energy from an incoming photon and goes into an ‘excited state’ at each time step. A certain
943 proportion of the concentration releases a photon and reverts to a ‘relaxed state’ at each
944 timestep also. This means that the excited bound calcium lags behind the bound calcium
945 trace. We could think of the excited bound calcium trace as a low pass filtered version of the
946 bound calcium trace.

947 **2.3.4 Spike inference accuracy is sensitive to indicator properties, and likely
948 varies within and between cells**

949 The above results imply that the fluorescence signal depends on the relative properties of
950 both GCaMP and the endogenous buffers. We next used the model to directly ask how
951 sensitive spike inference was to these components. We focused on three key parameters that
952 likely vary from cell to cell and experiment to experiment: GCaMP binding kinetics, GCaMP
953 concentration, and endogenous buffer concentration.

954 Several variants of GCaMP itself have been made that differ in calcium binding kinetics,
955 baseline fluorescence, fluorescence efficiency, and other factors. For example, GCaMP6f has

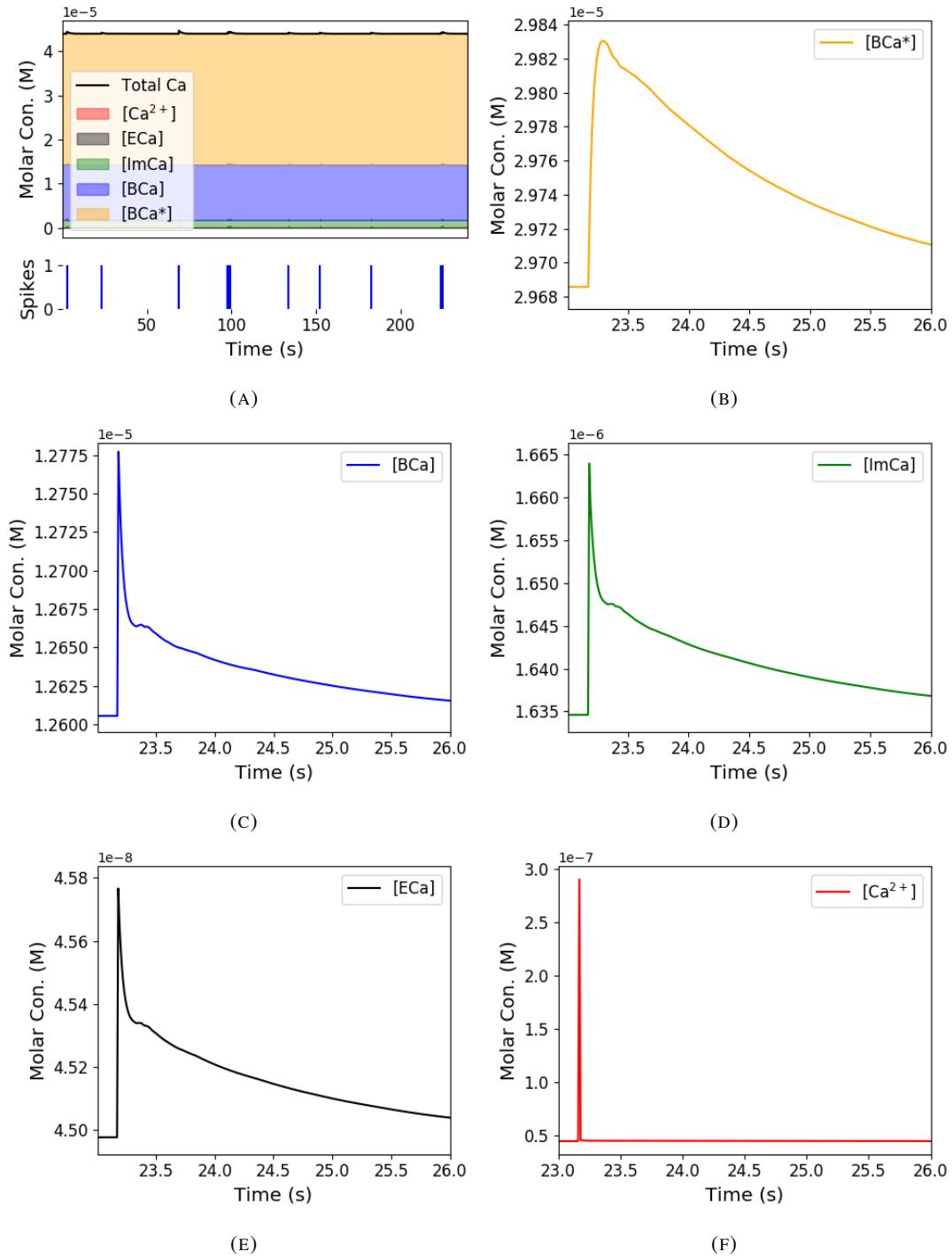


FIGURE 2.3: Calcium Buffering Dynamics (a) The proportions of bound and free calcium concentrations within a cell, with the associated spike train. (b)-(f) The dynamics of the concentration of (b) excited indicator bound calcium, (c) indicator bound calcium, (d) immobile endogenous buffer bound calcium, (e) mobile endogenous buffer bound calcium, and (f) free calcium in response to an action potential at ~ 23.2 s.

956 a decay time constant of \sim 1s, while GCaMP6s has a decay time constant of \sim 2s (Chen
957 et al., 2013). Here we asked how these differences in binding kinetics affect spike inference.
958 We jointly varied the calcium binding and unbinding rates of the indicator by the same factor
959 over a range from 100-fold slower to 100-fold faster from the fitted values, and simulated the
960 fluorescence response for each of the parameter settings in response to the same spike trains
961 as before (figure 2.4). Notably this manipulation does not affect the indicators affinity, and
962 therefore would not affect steady-state responses to prolonged changes in calcium. Instead
963 it is likely to affect its sensitivity to the spike train dynamics. We computed two summary
964 measures from the simulated fluorescence traces: the signal-to-noise ratio for a single spike
965 (Methods, section 2.2.6), and the accuracy of spike inference for each of the spike trains. We
966 observed a reduction in the signal-to-noise ratio and the spike inference quality when we set
967 the binding and unbinding rates were set to one hundredth of their fitted values, and to one
968 tenth of their fitted values. When we increased the value of both binding rates, we observed
969 no change in these measurements. The reduction in both rates lead to smaller increases in
970 fluorescence in response to an action potential and a longer decay time (figure 2.4a), this
971 caused the reduction in signal-to-noise ratio. As both rates were increased, the change in
972 $\Delta F/F_0$ in response to an action potential increased and the decay time decreased slightly,
973 but the fluorescence trace created by these values was very similar to the trace created by the
974 fitted values.

975 Second, the overall concentrations of GCaMP often varies from cell to cell. For exam-
976 ple different cells, even of the same type in the same tissue, can express different levels of
977 GCaMP, due to proximity to the infection site, or the cell becoming ‘nuclear-filled’ (Tian et
978 al., 2009; Chen et al., 2013). Also, GCaMP is often used for longitudinal experiments where
979 the same cells are re-imaged across multiple days or weeks. However since GCaMP expres-
980 sion typically ramps up over time (Chen et al., 2013), the accuracy of spike inference may
981 differ across multiple longitudinal recordings in the same cell. We addressed this by varying
982 the concentration of calcium indicator in the model, simulating spike trains and measuring
983 signal-to-noise ratio and spike inference accuracy on the resulting fluorescence traces. Both
984 increasing and decreasing the concentration of the indicator had effects on the fluorescence
985 trace, signal-to-noise ratio, and spike inference. The signal-to-noise ratio and spike inference
986 quality decreased with decreased indicator concentration, and both showed a decrease when
987 the indicator concentration was increased to 100 times its fitted value (figure 2.5). The signal-
988 to-noise ratio showed an increase when the indicator concentration was increased to 10 times
989 its fitted value, but there was no corresponding change in the spike inference quality. The

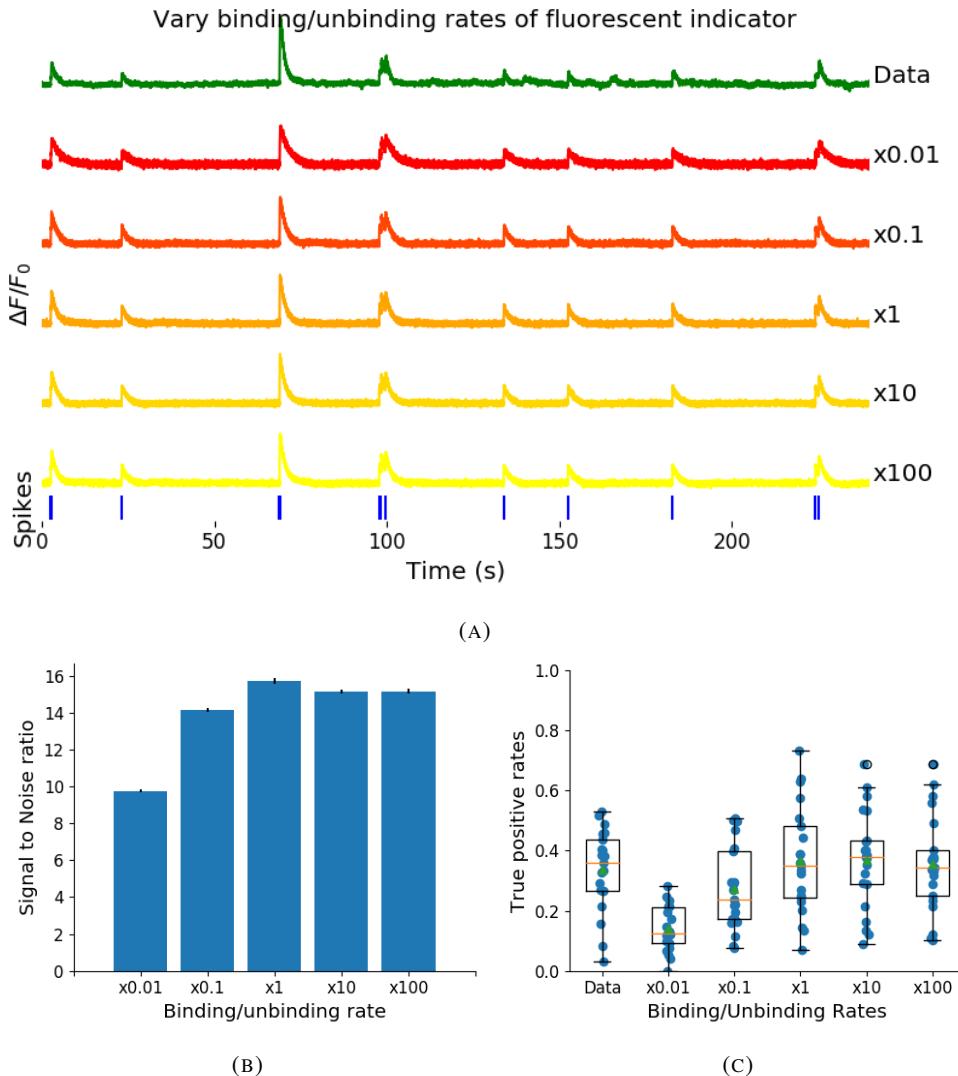


FIGURE 2.4: (a) An example trace for each of the five pairs of values used for the binding and unbinding rates of the fluorescent calcium indicator. (b) The signal-to-noise ratio of the modelled fluorescence traces using each of the four perturbed value pairs, and the experimental value. The SNRs for the value pairs perturbed downward are lower than that for the unperturbed value pair or the higher value pairs. (c) The true-positive rates of the deconvolution algorithm's predictions when inferring from the observed data, and inferring from modelled traces using the perturbed and experimental values.

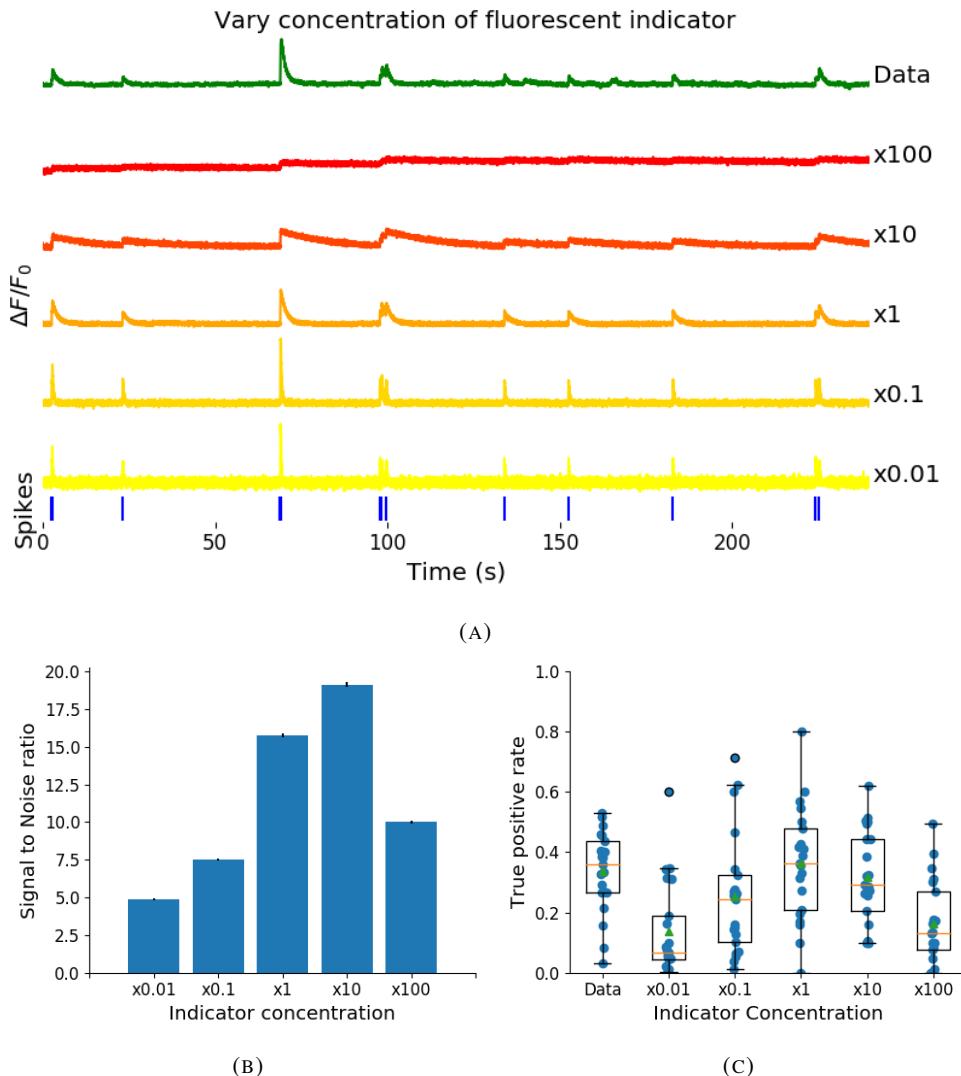


FIGURE 2.5: (a) An example trace for each of the five perturbed values for the concentration of fluorescent calcium indicator. The top two traces are produced by the lower perturbed values, the middle trace is produced by the experimental value, and the lowest two traces are produced when using the higher perturbed values. (b) The signal-to-noise ratio of the modelled fluorescence traces using each of the four perturbed values, and the experimental value. Extreme perturbations of the concentration either above or below the experimental level lowered the SNR. (c) The true-positive rates of the deconvolution algorithm’s predictions when inferring from the observed data, and inferring from modelled traces using the perturbed and experimental values. We found that the algorithms performs equally badly on the two most extreme values, and performs equally well on the experimental value, and the next higher perturbed value.

990 decrease in indicator concentration caused a reduction in the increase in $\Delta F / F_0$ in response
991 to an action potential, and an increase in the decay time of this increase (figure 2.5a). The
992 increase in indicator concentration had the opposite effect, it caused an increase in the change
993 in $\Delta F / F_0$ in response to an action potential, and a decrease in the decay time.

994 Third, the concentration and types of endogenous calcium buffers also vary from neuron
995 to neuron, both within and between cell types (Bartol et al., 2015; Maravall et al., 2000;
996 Neher and Augustine, 1992). Since the calcium buffer capacity of neurons is high, around
997 50-70 (Lee et al., 2000) in excitatory hippocampal pyramidal cells, around 100-250 (Lee et
998 al., 2000) in inhibitory hippocampal pyramidal cells, and 900-200 in Purkinje cells (depend-
999 ing on the age of the subject), these endogenous buffers compete with GCaMP for binding
1000 to calcium, and variations in endogenous buffer concentration may affect GCaMP signal and
1001 therefore spike inference. To address this we varied the concentration of the endogenous
1002 buffer in the model neuron over five orders of magnitude from 0.8 to 8000 μM , simulated
1003 calcium fluorescence traces in response to the same set of spike trains, and performed spike
1004 inference on the resulting fluorescence time series. Increasing the endogenous buffer con-
1005 centration had a substantial effect on the GCaMP fluorescence signal, both decreasing its
1006 amplitude and slowing its kinetics (figure 2.6(a)). This corresponded with a decrease in both
1007 single-spike signal-to-noise ratio (figure 2.6(b)) and spike inference accuracy (figure 2.6(c)).
1008 In contrast, decreasing endogenous buffer capacity from the fitted value had little effect on
1009 either the GCaMP signal or spike inference (figure 2.6).

1010 **2.3.5 Single spike inference accuracy drops for high firing rates, but firing rate
1011 itself can be estimated from mean fluorescence amplitude**

1012 The fluorescence signal recorded from neurons using calcium indicators is typically much
1013 slower than changes in membrane potential for two reasons: first, because the calcium and
1014 the indicator have slow binding and unbinding kinetics, the signal is a low-pass filtered ver-
1015 sion of the membrane potential. Second, neuronal two-photon imaging experiments are often
1016 performed in scanning mode, which limits their frame rate to $\sim 10\text{Hz}$ or slower. This im-
1017 plies that multiple spike events that occur close in time might be difficult to resolve from a
1018 calcium indicator time series. Many cells, especially several types of inhibitory interneurons,
1019 fire tonically at rates higher than 10Hz. We used the model to test whether spike inference
1020 accuracy depended on the neuron's firing frequency by driving the cell with spike trains sam-
1021 pled from a Poisson processes of varying frequency. We simulated a variable firing rate using
1022 an Ornstein-Uhlenbeck process, and simulated the spike trains using a Poisson distribution

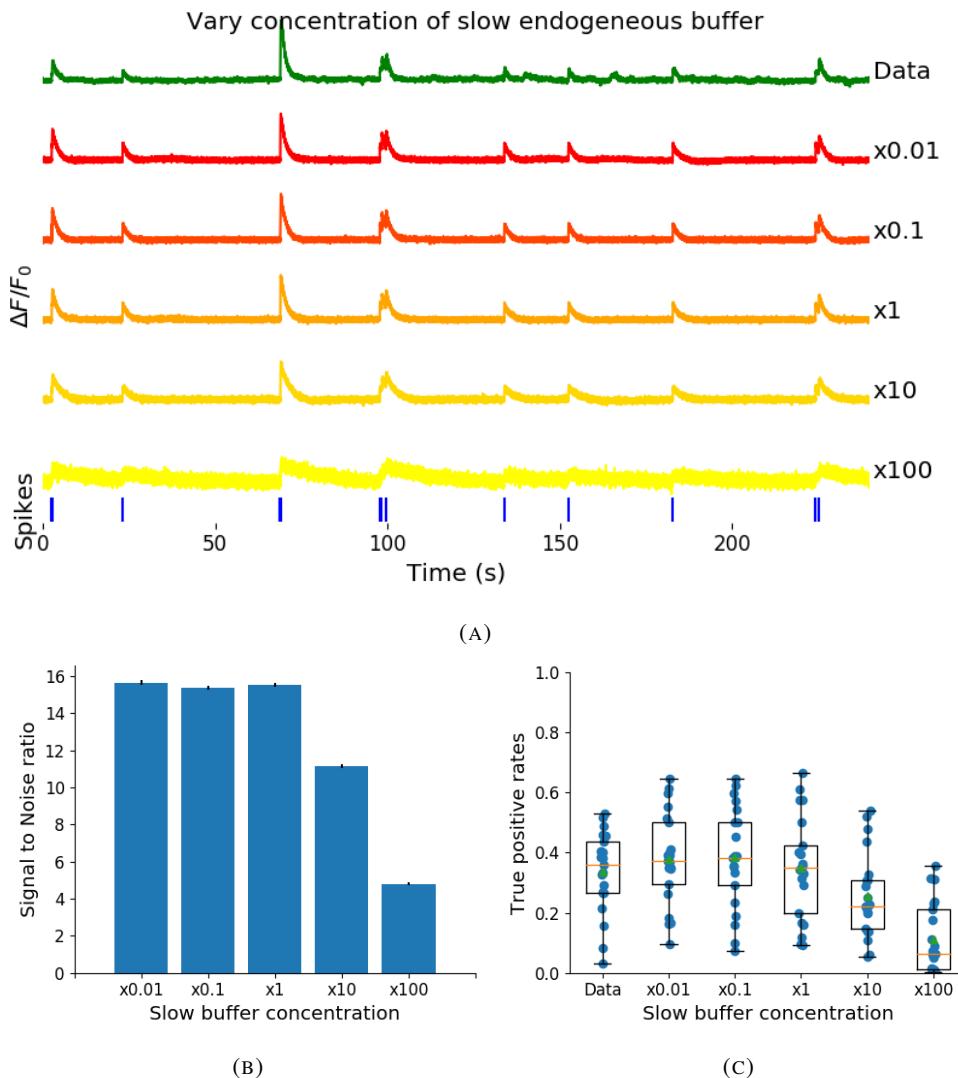


FIGURE 2.6: (a) An example trace for each of the five perturbed values for the concentration of immobile endogenous buffer. (b) The signal-to-noise ratio of the modelled fluorescence traces using each of the four perturbed values, and the experimental value. The lower values for the immobile buffer produce the same SNR as the experimental value. But the higher perturbed values produce fluorescence traces with a lower SNR. (c) The true-positive rates of the deconvolution algorithm's predictions when inferring from the observed data, and inferring from modelled traces using the perturbed and experimental values.

with its rate taken from this process. Because of the high frequency firing rate of these spike trains, we used the accuracy as the measure of spike inference quality. We simulated 30 spike trains at average firing rate of 1, 5, and 10Hz, and measured the spike inference quality of all these traces. Spike inference accuracy decreased with increasing firing rate, for up to 10Hz Poisson spike trains (figure 2.8(left)). Although the accuracy remained above 90% for each of the three frequencies. We also plotted the average $\Delta F/F_0$ as a function of stimulation firing rate. We found that it increased monotonically as a function of firing rate (figure 2.8(right)).

We expected lower spike inference quality as the average spiking frequency increased. Since the fluorescence trace, in some sense, is a low pass filtered version of the spike train, a tightly packed groups of spikes will be more difficult to infer than isolated spikes. However, the increasing amplitude of the fluorescence trace with increasing frequency suggests that some spike inference algorithm could be developed based on this amplitude.

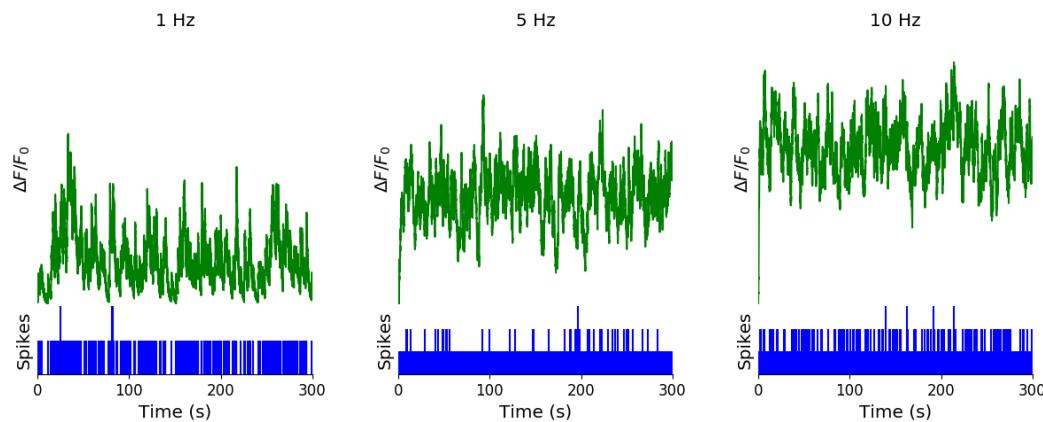


FIGURE 2.7: Simulating fluorescence traces at different firing rates Example modelled traces created using simulated spike trains with a mean firing rate of 1Hz (left column), 5Hz (middle column), and 10Hz (right column). Note the difference in amplitude with different mean firing rates.

2.4 Discussion

We designed a biophysical model for the changes in free calcium and bound calcium concentrations within a cell soma with a fluorescent calcium indicator. We used this model to model the fluorescence trace resulting from a spike train in this cell. We fit the free parameters of the model by matching the power spectrum and amplitude of fluorescence traces with simultaneously measured spike trains. We inferred spikes from real fluorescence traces and modelled fluorescence traces, and measured the quality of the spike inference in both cases.

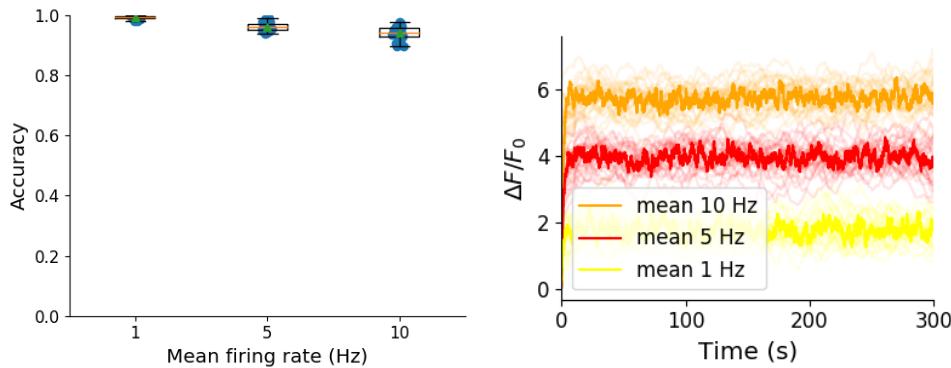


FIGURE 2.8: Inference quality and $\Delta F/F_0$ vs Firing rate (left) The spike inference accuracy when applied to 30 traces created using simulated spike trains with mean firing rates of 1, 5, and 10 Hz. (right) The mean $\Delta F/F_0$ across those 30 traces for each frequency.

1043 We found that the spike inference quality was similar in both cases. We perturbed the concentration of the calcium buffers in the model, and the binding/unbinding rates of those buffers
 1044 in the model, and measured the effect on the signal-to-noise ratio (SNR) of the modelled
 1045 fluorescence traces and the spike inference quality.
 1046

1047 For the fluorescent calcium indicator, we found that any large perturbation away from
 1048 the taken from the literature led to a reduction in SNR, and spike inference quality. For the
 1049 binding/unbinding rates, we kept the ratio of these rates constant, but altered their values in
 1050 parallel. The lower values caused a reduction in SNR, and a reduction in spike inference
 1051 quality. For the endogenous buffer concentration, an increase above the experimental value
 1052 caused a reduction in SNR and spike inference quality.

1053 Although the model produced visually similar time series to the real data, there were a
 1054 few aspects it did not capture. First, the real data featured some low-frequency components
 1055 that did not appear related to the spike events. These were not captured by the models we
 1056 used in this study, but could be added in future by adding a suitable low-frequency term to the
 1057 resulting time series. Second, the real data seemed to have some non-linearities not captured
 1058 in the model, for example the response to two nearby spikes was greater than expected from
 1059 the linear sum of two single spikes. This may be due to the co-operative binding of calmod-
 1060 ulin to calcium, which gives calmodulin a supra-linear sensitivity to calcium concentration
 1061 (Faas et al., 2011). The non-linear dynamics of this binding have been included in a recently
 1062 developed spike inference model (Greenberg et al., 2018). Our model, in contrast, behaved
 1063 much more linearly but could be extended in future to include such non-linearities. Third,
 1064 in the real data the fluorescence peak amplitude seemed to vary from spike to spike, even
 1065 for well-isolated spike events. Recent research has shown that calcium influx due to a single

action potential was quite variable in pyramidal cells, and that this variability had a effect on spike inference (Éltes et al., 2019). However in our model we assumed each spike lead to the same fixed-amplitude injection of calcium to the cell, leading to much greater regularity in fluorescence peak amplitudes. This variability could be added in future versions of the model by making the injected calcium peak a random variable. Fourth, we modelled the soma as a single compartment, but in reality there is likely a non-uniform spatial profile of calcium concentration. This may matter because some endogenous buffers might access calcium right as it influxes from the extracellular space, whereas the majority of the fluorescence signal is more likely coming from the bulk of the cytoplasm. Future models could attempt to model these spatial dependencies to assess whether they affect the overall spike inference procedure.

As well as the optimised parameters, the model has 14 fixed parameters than can be changed to simulate different types of calcium indicators. This model could be used to test the theoretical performance of proposed new types of calcium indicator. The model could also be used by developers of spike inference algorithms to test the effects of changing calcium indicator parameters on spike inference, or to test the affects of changing spiking characteristics on spike inference. For example, high firing rate vs low firing rate, or bursting vs no bursting. Given the increasing amplitude of the fluorescence trace with increasing mean firing rate, it would be possible to build a spike inference algorithm on this principle at least in part.

Our model has already been used as a tool by our colleagues, for simulating fluorescence traces in response to cells that can fire with a continuous rate between 10 and 20Hz, but do not always do so. Our colleagues found that a combination of the amplitude and the variance of the simulated fluorescence trace was the best indicator of firing rate. For example, when a cell was not firing, the amplitude and variance of the fluorescence trace was relatively low. When the cell fired with a low firing rate $\sim 1\text{Hz}$, the mean amplitude was still low but the variance of the fluorescence trace was high, and for high firing rate $\sim 10 - 20\text{Hz}$, the fluorescence amplitude was high, and the variance was low. In this way, our model may be useful for investigating firing rates underlying real fluorescence traces in response to cells which can fire in these rage ranges.

A recent paper by Greenberg et al (2018) described a biophysical model for spike train inference called the ‘Sequential binding model’. Their model for spike inference was similar to our model for fluorescence traces in that their model included parameters for two types of endogenous buffer. But this model also included dynamics for calcium binding to and unbinding from these endogenous buffers. Furthermore, this model included dynamics

1100 for calcium binding to and unbinding from the four binding sites present on a GCaMPs6
1101 molecule. In the accuracy measurements specified in that paper, this model performed better
1102 than the MLspike algorithm, which is also partially a biophysically model, and it performed
1103 better than the constrained non-negative deconvolution algorithm. The sequential binding
1104 model also has biophysically interpretable parameters, and its fitted parameters for quantities
1105 such as buffering capacity and calcium influx upon action potential firing fall in line with
1106 experimental values (Greenberg et al., 2018). Biophysical models like this appear to be the
1107 way forward for spike inference algorithms, and would make a good complimentary tool to
1108 our fluorescence model.

1109 **Chapter 3**

1110 **Functional networks expand across**
1111 **anatomical boundaries as correlation**
1112 **time-scale increases**

1113 *Abstract*

1114 Decades of research has established that correlated spiking plays a crucial role in represent-
1115 ing sensory information. One drawback associated with the recent improvement in recording
1116 technology and consequent large datasets is the difficulty in analysing higher order correla-
1117 tions in large neuronal ensembles. One benefit of these datasets that has not yet been explored
1118 is the opportunity to compare correlations within anatomical regions to correlations across
1119 anatomical regions. In this work, we measured correlations between neurons residing in
1120 nine different brains regions in three awake and behaving mice. Using the these correlation
1121 measurements, we created weighted undirected graph networks and applied network science
1122 methods to detect functional communities in our neural ensembles. We compared these func-
1123 tional communities to their anatomical distribution. We repeated the analysis, using different
1124 timescales for our correlation measurements, and found that functional communities were
1125 more likely to be dominated by neurons from a single brain region at shorter timescales
1126 (< 100ms).

1127 3.1 Introduction

1128 Decades of research has established that correlations play a crucial role in representing sen-
1129 sory information. For example, the onset of visual attention has been shown to have a greater
1130 affect on the correlations in the macaque V4 region than on the firing rates in that region
1131 (Cohen and Maunsell, 2009). Recent findings show that spontaneous behaviours explain cor-
1132 relations in parts of the brain not associated with motor control (Stringer et al., 2019), that
1133 satiety state appears to have a brain wide representation (Allen et al., 2019), and that subject
1134 exploratory and non-exploratory states are represented in the amygdala (Gründemann et al.,
1135 2019). So, behavioural states are likely represented across many regions of the brain, not just
1136 motor related areas. In order to understand the brain, we must understand the interactions
1137 between neurons and regions.

1138 Because of limitations in recording technology almost all research has explored corre-
1139 lations between neurons within a given brain region, or within only two regions at most
1140 (Wierzynski et al., 2009; Patterson et al., 2014; Girard, Hupé, and Bullier, 2001). Rela-
1141 tively little is known about correlations between neurons in many different brain regions.
1142 However, the recent development of ‘Neuropixels’ probes (Jun et al., 2017) has allowed
1143 extracellular voltage measurements to be collected from multiple brain regions simultane-
1144 ously routinely, and in much larger numbers than traditional methods. In this project we
1145 used a publicly-available Neuropixels dataset to analyse correlations between different brain
1146 regions (Stringer et al., 2019).

1147 A drawback associated with the improvement in recording technology is an increase in
1148 the difficulty in analysing these data. For example, analysing the i th order interactions of
1149 N neurons generally requires estimation of N^i parameters. A number that becomes astro-
1150 nomical for large N . New methods are required for analysing these new large datasets. We
1151 attempted to address this requirement in this piece of research by applying a cutting-edge
1152 network science community detection method to neural data.

1153 Another unexplored area of research is the changes in cell interactions at different timescales.
1154 Studies have shown different timescales for fluctuations in spiking activity (Murray et al.,
1155 2014), and different time scales for event representation (Baldassano et al., 2017) across dif-
1156 ferent brain regions. Still most studies focus on quantifying interactions at a given timescale.
1157 But neurons may interact differently, or may interact with different neurons at different
1158 timescales. Here we explore correlated communities of neurons at different timescales.

1159 In this work, we measured correlations between binned spike counts from neurons from

1160 nine different regions of the mouse brain. These measurements induced a weighted undi-
1161 rected graph or network where each neuron is represented by a node, and the strength of
1162 the connection between these nodes/neurons is the strength of the correlation between their
1163 spike counts. We then applied newly invented network methods (Humphries et al., 2019)
1164 to this network to find any community structure, and place the neurons in these correlation
1165 based communities. Finally, we compared these functional communities to the anatomical
1166 membership of the neurons.

1167 To investigate the functional communities and their relationship with anatomy at different
1168 time scales, we repeated these analyses using different length bin widths when binning spike
1169 times.

1170 To find and analyse functional networks while controlling for the subject’s behaviour, we
1171 conditioned the binned spike counts on data from a video of the subject’s face, and repeated
1172 our analysis for spike count correlations (or noise correlations) and signal correlations.

1173 3.2 Data

1174 The data that we used in this project were collected by Nick Steinmetz and his lab members
1175 (Stringer et al., 2019).

1176 3.2.1 Brain regions

1177 Neuropixels probes were used to collect extracellular recordings (Jun et al., 2017) from three
1178 different mice. The mice were awake, headfixed, and engaging in spontaneous behaviour.
1179 The mice were of different sexes and different ages. One mouse was ‘wild-type’, the others
1180 were mutants. Details as follows:

- 1181 1. male, wild type, P73.
- 1182 2. female, TetO-GCaMP6s, Camk2a-tTa, P113
- 1183 3. male, Ai32, Pvalb-Cre, P99

1184 Eight probes were used to collect readings from 2296, 2668, and 1462 cells respectively.
1185 Data were collected from nine brain regions in each mouse:

- 1186 • Caudate Putamen (CP)
- 1187 • Frontal Motor Cortex (Frmocxt)
- 1188 • Hippocampal formation (Hpf)

- 1189 • Lateral Septum (Ls)
- 1190 • Midbrain (Mb)
- 1191 • Superior Colliculus (Sc)
- 1192 • Somatomotor cortex (Sommotcx)
- 1193 • Thalamus (Th)
- 1194 • Primary visual cortex (V1)

1195 Readings were continuous and lasted for about 1 hour (Stringer et al., 2019). Locations of
 1196 each of the probes can be seen in figure 3.1.

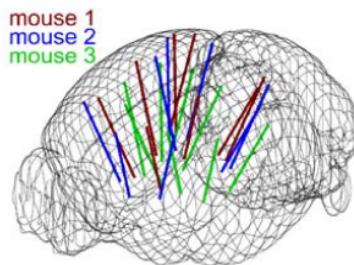


FIGURE 3.1: Probe Locations: The locations of the probes in each of the three mouse brains (Stringer et al., 2019).

1197 3.2.2 Video recordings

1198 Video recordings of the mouse's face were taken during the spontaneous behaviour. We had
 1199 access to the top 500 principle components and top 500 eigenvectors of the processed videos.
 1200 The frequency of recording was slightly less than 40Hz. Each frame contained 327×561
 1201 pixels. These principal components were used as behavioural data. We controlled for these
 1202 components when taking measurements conditioned on behaviour.

1203 3.3 Methods

1204 3.3.1 Binning data

1205 We transformed the spike timing data into binned spike count data by dividing the exper-
 1206 imental period into time bins and counting the spikes fired by each cell within the time period
 1207 covered by each of those bins. The data were divided into time bins of various widths ranging
 1208 from 0.01s to 4s.

If the total length of the recording period was not an integer multiple of the time bin width, we cut off the remaining time at the end of the recording period. This period was at most 3.99s. This is far less than the total recording time of around 1 hour. So, this detail would not affect our results.

3.3.2 Correlation coefficients

We calculated Pearson's correlation coefficient for pairs of spike counts from pairs of neurons. For jointly distributed random variables X and Y , Pearson's correlation coefficient is defined as:

$$\rho_{XY} = \frac{\text{cov}(X, Y)}{\sigma_X \sigma_Y} \quad (3.1)$$

$$= \frac{E[(X - \mu_X)(Y - \mu_Y)]}{\sigma_X \sigma_Y} \quad (3.2)$$

where E denotes the expected value, μ denotes the mean, and σ denotes the standard deviation. The correlation coefficient is a normalised measure of the covariance. It can take values between 1 (completely correlated) and -1 (completely anti-correlated). Two independent variables will have a correlation coefficient of 0. But, having 0 correlation does not imply independence.

If we do not know the means and standard deviations required for equation 3.1, but we have samples from X and Y , Pearson's sample correlation coefficient is defined as:

$$r_{XY} = \frac{\sum_{i=1}^n (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_{i=1}^n (x_i - \bar{x})^2} \sqrt{\sum_{i=1}^n (y_i - \bar{y})^2}} \quad (3.3)$$

where $\{(x_i, y_i)\}$ for $i \in \{1, \dots, n\}$ are the paired samples from X and Y , and $\bar{x} = \frac{1}{n} \sum_{i=1}^n x_i$, and $\bar{y} = \frac{1}{n} \sum_{i=1}^n y_i$ are the sample means.

In practice we used the python function `scipy.stats.pearsonr` to calculate the correlation coefficients.

1223 Total correlations, r_{SC}

The total correlation (r_{SC}) of two cells is the correlation between the spike counts of those cells in response to a given stimulus condition.

1226 **Shuffled total correlations**

1227 We measured the shuffled total correlations between two neurons by randomly permuting one
 1228 of the neuron's spike counts and measuring the total correlations. These shuffled correlations
 1229 were useful when measuring the effect of time bin width on correlations, and when decid-
 1230 ing which correlations should be preserved when creating correlation networks (see section
 1231 [3.3.5](#)).

1232 **Separating Correlations & Anti-correlations**

1233 In order to compare the effect of bin width on measures of negative r_{SC} (anti-correlation) and
 1234 positive r_{SC} separately, we had to separate correlated and anti-correlated pairs. To do this, we
 1235 simply measured the mean r_{SC} , taking the mean across all the bin widths. If this quantity was
 1236 positive or zero we regarded the pair as positively correlated. If this quantity was negative
 1237 we regarded the pair as anti-correlated.

1238 **3.3.3 Conditioning on behavioural data**

Our behavioural data consisted of the top 500 principal components (PCs) of a processed video recording of the mouse's face (see section [3.2.2](#)). Denoting the spike count of a given cell by X , and the PCs by Z_1, \dots, Z_{500} , we wanted to model X as a function of Z_1, \dots, Z_{500} in order to estimate

$$E[X|Z_1, \dots, Z_{500}] = \int_{x \in X} x P(X = x|Z_1, \dots, Z_{500}) dx \quad (3.4)$$

$$= \int_{x \in X} x \frac{P(X = x, Z_1, \dots, Z_{500})}{P(Z_1, \dots, Z_{500})} dx \quad (3.5)$$

1239 Given the 500 components, a naïve estimation of $P(Z_1, \dots, Z_{500})$ or $P(X, Z_1, \dots, Z_{500})$ by
 1240 histogramming was impossible. Therefore we modelled X as a linear combination of the
 1241 PCs.

1242 **Linear regression**

1243 We modelled the spike count of a given cell, X , as a linear combination of the PCs of the
 1244 video of the mouse's face, $\mathbf{Z} = Z_1, \dots, Z_{500}$. We tried three different types of regularization

1245 • *L1* or ‘Lasso’

1246 • *L2* or ‘Ridge regression’

- 1247 • ‘Elastic net’ regularisation (a linear combination of both $L1$ and $L2$ regularisation
 1248 penalties)

1249 The elastic net regularisation performed the best, so we stuck with that.

1250 **Elastic net regularisation**

Suppose we wish to model n observations of a random variable X , $\mathbf{x} = (x_1, \dots, x_n)$ using n instances of m predictors $\mathbf{Z} = (Z_1, \dots, Z_m)$. The naïve elastic net criterion is

$$L(\lambda_1, \lambda_2, \boldsymbol{\beta}) = |\mathbf{x} - \mathbf{Z}\boldsymbol{\beta}|^2 + \lambda_2|\boldsymbol{\beta}|_2 + \lambda_1|\boldsymbol{\beta}|_1 \quad (3.6)$$

where

$$|\boldsymbol{\beta}|_2 = \sum_{j=1}^m \beta_j^2 \quad (3.7)$$

$$|\boldsymbol{\beta}|_1 = \sum_{j=1}^m |\beta_j| \quad (3.8)$$

The naïve elastic net estimator $\hat{\boldsymbol{\beta}}$ is the minimiser of the system of equations 3.6 (Zou and Hastie, 2005)

$$\hat{\boldsymbol{\beta}} = \arg \min_{\boldsymbol{\beta}} L(\lambda_1, \lambda_2, \boldsymbol{\beta}) \quad (3.9)$$

1251 We implemented the model using the `ElasticNetCV` method of Python’s
 1252 `sklearn.linear_models` package.

1253 As well as using the PCs, we also tried fitting the models using the raw video data recon-
 1254 structed from the PCs and eigenvectors. These models performed worse than those using the
 1255 PCs. We expected this because each representation contains the same amount of information,
 1256 but the raw video representation spreads this information across many more components.
 1257 This requires more parameter fitting, but given the same information.

1258 **Conditional covariance**

We calculated the expected value of the conditional covariance using the law of total covariance.

$$\text{cov}(X, Y) = E[\text{cov}(X, Y|Z)] + \text{cov}(E[X|Z], E[Y|Z]) \quad (3.10)$$

1259 where these expected values are calculated with respect to the distribution of Z as a random
 1260 variable.

1261 The law of total covariance breaks the covariance into two components. The first com-
 1262 ponent $E[\text{cov}(X, Y|Z)]$ is the expected value, under the distribution of Z , of the conditional
 1263 covariance $\text{cov}(X, Y|Z)$. This covariance could be interpreted as the unnormalised version
 1264 of what Cohen et al. (2011) call the spike count correlation (Cohen and Kohn, 2011), aka.
 1265 the noise correlation. In particular, this is the covariance of the spike counts in response to
 1266 repeated presentation of identical stimuli.

1267 The second component is analogous to what Cohn et al. (2011) call the *signal correlation*
 1268 (Cohen and Kohn, 2011). In particular, $\text{cov}(E[X|Z], E[Y|Z])$ is the covariance between
 1269 spike counts in response to different stimuli.

Using our linear model, we calculated $E[X|Z_1, \dots, Z_{500}]$ for each cell X . Then we pro-
 ceeded to calculate

$$E[\text{cov}(X, Y|Z_1, \dots, Z_{500})] = \text{cov}(X, Y) - \quad (3.11)$$

$$\text{cov}(E[X|Z_1, \dots, Z_{500}], E[Y|Z_1, \dots, Z_{500}]) \quad (3.12)$$

1270 **Measures of conditional correlation**

As a measure of expected correlation, we measured the ‘event conditional correlation’ (Maugis,
 2014)

$$\rho_{XY|Z} = \frac{E[\text{cov}(X, Y|Z)]}{\sqrt{E[\text{var}(X|Z)]E[\text{var}(Y|Z)]}} \quad (3.13)$$

1271 Although this is not an actual correlation, it is an intuitive analogue to the correlation as a
 1272 normalised version of the covariance.

For comparison, we also measured the ‘signal correlation’

$$\rho_{\text{signal}} = \frac{\text{cov}(E[X|Z], E[Y|Z])}{\sqrt{\text{var}(E[X|Z])\text{var}(E[Y|Z])}} \quad (3.14)$$

1273 this is an actual correlation.

1274 **3.3.4 Information Theory**

1275 **Entropy $H(X)$**

The entropy of a random variable X , with outcomes x_1, \dots, x_N , and corresponding probabilities p_1, \dots, p_N is defined as

$$H(X) = - \sum_{n=1}^N p_n \log_2 p_n \quad (3.15)$$

1276 This quantity is also known as the information entropy or the ‘surprise’. It measures the
 1277 amount of uncertainty in a random variable. For example, a variable with a probability of 1
 1278 for one outcome, and 0 for all other outcomes will have 0 bits entropy, because it contains no
 1279 uncertainty. But a variable with a uniform distribution will have maximal entropy as it is the
 1280 least predictable. This quantity is analogous to the entropy of a physical system (Shannon,
 1281 1948). Note that any base may be used for the logarithm in equation 3.15, but using base 2
 1282 means that the quantity will be measured in ‘bits’.

The joint entropy of two jointly distributed random variables X and Y , where Y has outcomes y_1, \dots, y_M , is defined as

$$H(X, Y) = - \sum_{n=1}^N \sum_{m=1}^M P(X = x_n, Y = y_m) \log_2 P(X = x_n, Y = y_m) \quad (3.16)$$

1283 If X and Y are independent then $H(X, Y) = H(X) + H(Y)$. Otherwise $H(X, Y) <$
 1284 $H(X) + H(Y)$. When X and Y are completely dependent $H(X, Y) = H(X) = H(Y)$.

The conditional entropy of Y conditioned on X is defined as

$$H(Y|X) = - \sum_{n=1}^N \sum_{m=1}^M P(X = x_n, Y = y_m) \log_2 \frac{P(X = x_n, Y = y_m)}{P(X = x_n)} \quad (3.17)$$

1285 When X and Y are independent $H(Y|X) = H(Y)$. Intuitively, we learn nothing of Y by
 1286 knowing X , so Y is equally uncertain whether we know X or not. If Y is totally dependent
 1287 on X , then the fraction in the logarithm is 1, which gives $H(Y|X) = 0$.

1288 These entropy measures are the basis of the mutual information measure.

1289 **Maximum entropy limit**

When spiking data is binned into spike counts there is an upper limit on the entropy of these data. The maximum entropy discrete distribution is the discrete uniform distribution. A

random variable with this distribution will take values from some finite set with equal probabilities. Binned spike count data will take values between 0 and some maximum observed spike count n_{\max} . A neuron with responses that maximises entropy will take these values with equal probability, i.e. if $i \in \{0, \dots, n_{\max}\}$ then $P(X = i) = \frac{1}{n_{\max} + 1}$. The entropy of this neuron will be

$$\begin{aligned} H(X) &= - \sum_{i=0}^{n_{\max}} P(X = i) \log_2 P(X = i) \\ &= - \sum_{i=0}^{n_{\max}} \frac{1}{n_{\max} + 1} \log_2 \left(\frac{1}{n_{\max} + 1} \right) \\ &= - \log_2 \left(\frac{1}{n_{\max} + 1} \right) \\ &= \log_2 (n_{\max} + 1) \end{aligned}$$

1290 Therefore, the maximum entropy of the binned spike counts of a neuron is $\log_2 (n_{\max} + 1)$.
 1291 Of course, it would be very unusual for a neuron to fire in accordance with the discrete
 1292 uniform distribution. Most measurements of entropy taken on binned spiking data will be
 1293 much lower than the maximum. See figure 3.2 to see the maximum entropy as a function of
 1294 the maximum observed spike count.

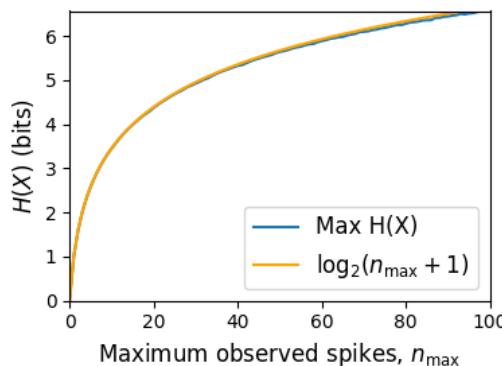


FIGURE 3.2: **Entropy Limit:** The upper limit on entropy of binned spike count data as a function of the maximum observed spike count. The orange line is the analytical maximum. The blue line is the entropy of samples with $N = 1000$ data points taken from the discrete uniform distribution.

1295 **Mutual Information $I(X; Y)$**

1296 The mutual information can be defined mathematically in a number of ways, all of which are
 1297 equivalent. These definitions illustrate the different ways of interpreting the mutual informa-
 1298 tion.

For two jointly distributed random variables X and Y , the mutual information $I(X;Y)$ is defined as

$$I(X;Y) = H(Y) - H(Y|X) \quad (3.18)$$

$$= H(X) - H(X|Y) \quad (3.19)$$

1299 Equation 3.18 fits with the following intuition: The mutual information between X and Y is
1300 the reduction in uncertainty about X gained by knowing Y , or vice versa. We could also say
1301 the mutual information is the amount of information gained about X by knowing Y , or vice
1302 versa.

Another useful entropy based definition for the mutual information is

$$I(X;Y) = H(X) + H(Y) - H(X,Y) \quad (3.20)$$

1303 This definition is useful because it does not require the calculation of conditional probabili-
1304 ties.

The mutual information can also be defined in terms of marginal, joint, and conditional distributions. For example,

$$I(X;Y) = - \sum_{n=1}^N \sum_{m=1}^M P(X = x_n, Y = y_m) \log_2 \frac{P(X = x_n, Y = y_m)}{P(X = x_n)P(Y = y_m)} \quad (3.21)$$

Notice that this can be rewritten as a Kullback–Leibler divergence.

$$I(X;Y) = D_{KL}(P(X,Y) || P(X)P(Y)) \quad (3.22)$$

1305 So, we can also think of the mutual information as a measure of the difference between
1306 the joint distribution of X and Y , and the product of their marginal distributions. Since the
1307 product of the marginal distributions is the joint distribution for independent variables, we
1308 can think of the mutual information as a measure of the variables’ dependence on one another.

1309 The minimum value that $I(X;Y)$ can take is 0. This occurs when the random variables
1310 X and Y are independent. Then we have $H(X|Y) = H(X)$, and $H(Y|X) = H(Y)$, which
1311 according to equation 3.18, gives $I(X;Y) = 0$. We also have that $H(X,Y) = H(X) +$
1312 $H(Y)$ in this case, which according equation 3.20, gives $I(X;Y) = 0$. Finally, we also have
1313 $P(X,Y) = P(X)P(Y)$, which leaves us with 1 in the argument for the logarithm in equation
1314 3.21, which again gives $I(X;Y) = 0$.

1315 The mutual information reaches its maximum value when one of the variables X and
 1316 Y is completely determined by knowing the value of the other. In that case $I(X; Y) =$
 1317 $\min\{H(X), H(Y)\}$.

1318 **Variation of Information** $VI(X, Y)$

The variation of information is another information theoretical quantity based on the mutual information. It is defined as

$$VI(X; Y) = H(X) + H(Y) - 2I(X; Y) \quad (3.23)$$

We can rewrite this as the summation of two positive quantities

$$VI(X; Y) = [H(X) - I(X; Y)] + [H(Y) - I(X; Y)] \quad (3.24)$$

1319 In English, the variation of information is the summation of the uncertainty in the random
 1320 variables X and Y excluding the uncertainty shared by those variables.

1321 This measure will become more relevant when we go on to talk about clusterings because
 1322 $VI(X; Y)$ forms a metric on the space of clusterings.

1323 **Measuring entropies & mutual information**

1324 In practice, we measured the mutual information between spike counts using Python and the
 1325 python package `pyitlib`. We used the PT-bias correction technique to estimate the bias of
 1326 our measurements when measuring the mutual information between the spike counts of two
 1327 cells (Treves and Panzeri, 1995).

1328 When measuring the mutual information between clusterings we used Python, but we
 1329 used the `mutual_info_score`, `adjusted_mutual_info_score`, and
 1330 `normalized_mutual_info_score` functions from the `sklearn.metrics` part of
 1331 the `sklearn` package.

1332 **3.3.5 Network analysis**

1333 **Correlation networks**

1334 In order to analyse functional networks created by the neurons in our ensemble, we mea-
 1335 sured the total correlation between each pair of neurons. These measurements induced an

1336 undirected weighted graph/network between the neurons. The weight of each connection
1337 was equal to the total correlation between each pair of neurons.

1338 We followed the same procedure for total correlations 3.3.2, spike count correlations, and
1339 signal correlations 3.3.3.

1340 **Rectified correlations**

1341 At the time of writing, the community detection method outlined in (Humphries et al., 2019)
1342 could only be applied to networks with positively weighted connections. But many neuron
1343 pairs were negatively correlated. To apply the community detection method, we *rectified* the
1344 network, by setting all the negative weights to zero.

1345 We also looked for structure in the network created by negative correlations by reversing
1346 the signs of the correlations, and rectifying these correlations before applying our network
1347 analysis.

1348 Finally, we used the absolute value of the correlations as the weights for the graph/network.
1349 By doing this, we hoped to identify both correlated and anti-correlated functional communi-
1350 ties of neurons.

1351 **Sparsifying data networks**

1352 When creating our correlation networks, we wanted to exclude any correlations that could
1353 be judged to exist ‘by chance’. To do this, we measured the 5th and 95th percentile of
1354 the shuffled correlations (see section 3.3.2) for the given mouse and time bin width. We
1355 then set all the data correlations between these two values to 0. This excluded any ‘chance’
1356 correlations from our network, and created a sparser network. This allowed us to make use
1357 of the ‘sparse weighted configuration model’ as described in section 3.3.5.

1358 **Communities**

1359 Given some network represented by an adjacency matrix \mathbf{A} , a community within that net-
1360 work is defined as a collection of nodes where the number of connections within these nodes
1361 is higher than the expected number of connections between these nodes. In order to quan-
1362 tify the ‘expected’ number of connections, we need a model of expected networks. This is
1363 analogous to a ‘null model’ in traditional hypothesis testing. We test the hypothesis that our
1364 data network departs from the null network model to a statistically significant degree. For
1365 undirected unweighted networks, the canonical model of a null network is the configuration

model (Fosdick et al., 2016). Since we are working with weighted sparse networks, we used more suitable null models, described below.

1368 **Weighted configuration model**

1369 The *weighted configuration model* is a canonical null network model for weighted networks.
 1370 Given some data network, the weighted configuration model null network will preserve the
 1371 degree sequence and weight sequence of each node in the data network. But the edges will
 1372 be distributed randomly (Fosdick et al., 2016). Any structure in the data network beyond
 1373 its degree sequence and weight sequence will not be captured in the weighted configuration
 1374 model. So, this model can be used in testing the hypothesis that this extra structure exists.

1375 **Sparse weighted configuration model**

1376 The *sparse weighted configuration model* is another null network model. Similar in nature to
 1377 the weighted configuration model (see section 3.3.5), but the sparsity of the data network is
 1378 preserved in the null network. This is achieved by sampling from a probability distribution
 1379 for the creation or non-creation of each possible connection, then distributing the weight of
 1380 the data network randomly in this sparse network (Humphries et al., 2019). This is the null
 1381 network that we used when searching for additional structure in our data networks.

1382 **Spectral rejection**

1383 We made use of the spectral rejection algorithm as outlined in (Humphries et al., 2019). The
 1384 spectral rejection algorithm is a method for finding structure in a network not captured by a
 1385 supposed null model, if such structure exists.

To describe the method, we denote our data network matrix \mathbf{W} , we denote the expected network of our null network model as $\langle \mathbf{P} \rangle$. Then the departure of our data network from the null network can be described by the matrix

$$\mathbf{B} = \mathbf{W} - \langle \mathbf{P} \rangle \quad (3.25)$$

1386 a common choice for $\langle \mathbf{P} \rangle$ in community detection is the ‘configuration model’ (Fosdick et
 1387 al., 2016; Humphries, 2011). The matrix \mathbf{B} is often called the configuration matrix, in this
 1388 context we will use the term ‘deviation matrix’ as it captures the deviation of \mathbf{W} from the
 1389 null model.

1390 To test for structure in the network represented by \mathbf{W} , we examine the eigenspectrum of \mathbf{B}
1391 and compare it to the eigenspectrum of our null model. Firstly, note that since our data model
1392 doesn't allow self loops, and is not directed, the matrix representing the network will be
1393 symmetric and positive semi-definite, and will therefore be invertible with real eigenvalues.
1394 We selected a null model with the same characteristics.

1395 To find the eigenspectrum of the null model, we generated N samples from our null
1396 model P_1, \dots, P_N , and we measured their deviation matrices B_1, \dots, B_N . We then calculated
1397 the eigenspectrum of each of those samples. We calculated the upper bound of the null model
1398 eigenspectrum by taking the mean of the largest eigenvalues of B_1, \dots, B_N . We calculated a
1399 lower bound on the null model eigenspectrum by taking the mean of the smallest eigenvalues
1400 of B_1, \dots, B_N .

1401 We then calculated the eigenspectrum of \mathbf{B} , our data network deviation matrix. If any of
1402 those eigenvalues lay outside of the upper or lower bounds of the null model eigenspectrum,
1403 this is evidence of additional structure not captured by the null model. If we chose the sparse
1404 weighted configuration model (see section 3.3.5) as our null network model, then eigenvalues
1405 lying below the lower bound indicate k -partite structure in the network. For example, if one
1406 eigenvalue lay below the lower bound, this would indicate some bipartite structure in the data
1407 network. If any eigenvalues lay above the upper bound of the null model eigenspectrum, this
1408 is evidence of community structure in the data network. For example, one eigenvalue of \mathbf{B}
1409 lying above the upper bound of the null model eigenspectrum indicates the presence of two
1410 communities in the network (Humphries, 2011).

1411 Node rejection

1412 If there are d data eigenvalues lying outside of the null network eigenspectrum, the d eigen-
1413 vectors corresponding to these eigenvalues will form a vector space. If we project the nodes
1414 of our network into this vector space, by projecting either rows or columns of the data ma-
1415 trix, we can see how strongly each node contributes to the vector space. Nodes that contribute
1416 strongly to the additional structure will project far away from the origin, nodes that do not
1417 contribute to the additional structure will project close to the origin. We want to use this
1418 information to discard those nodes that do not contribute.

1419 We can test whether a node projects *far* away from the origin or *close* to the origin
1420 using the eigenvalues and eigenvectors of B_1, \dots, B_N . The j th eigenvector and eigenvalue
1421 of B_i gives a value for a null network's projection into the j th dimension of the additional
1422 structure vector space. The matrices B_1, \dots, B_N give N projections into that dimension.

1423 These projections are a distribution of the null networks' projections. If the data node's
 1424 projection exceeds that of the null network projections this node is judged to project *far* from
 1425 the origin, and therefore contribute to the additional structure. Otherwise, the node is judged
 1426 to project *close* to the origin, and is therefore rejected (Humphries et al., 2019).

1427 Community detection

1428 Another application for this d dimensional space is community detection. We first project
 1429 all of the nodes into this d -dimensional space, then perform the clustering in this space. The
 1430 clustering and community detection procedure is described in (Humphries, 2011).

1431 In practice, the procedure is carried out n times (we chose $n = 100$ times), this returns n
 1432 clusterings. We resolve these n clusterings to one final clustering using *consensus clustering*.
 1433 We used the consensus clustering method that uses an explicit null model for the consensus
 1434 matrix, as outlined in (Humphries et al., 2019).

1435 3.3.6 Clustering Comparison

A clustering \mathcal{C} is a partition of a set D into sets C_1, C_2, \dots, C_K , called clusters, that satisfy the following for all $k, l \in \{1, \dots, K\}$:

$$C_k \cap C_l = \emptyset \quad (3.26)$$

$$\bigcup_{k=1}^K C_k = D \quad (3.27)$$

1436 If we consider two clusterings, \mathcal{C} with clusters C_1, C_2, \dots, C_K and \mathcal{C}' with clusters
 1437 C'_1, C'_2, \dots, C'_K . There are a number of measurements we can use to compare \mathcal{C} and \mathcal{C}' . In
 1438 the following, the number of elements in D is denoted by n , and the number of elements in
 1439 cluster C_k is n_k .

1440 Adjusted Rand Index

1441 The *adjusted Rand Index* is a normalised similarity measure for clusterings based on pair
 1442 counting.

1443 If we consider the clusterings \mathcal{C} and \mathcal{C}' , and denote

1444 • the number of pairs in the same cluster in \mathcal{C} and \mathcal{C}' by N_{11}

1445 • the number of pairs in different clusters in \mathcal{C} and \mathcal{C}' by N_{00}

- 1446 • the number of pairs in the same cluster in \mathcal{C} and different clusters in \mathcal{C}' by N_{10}

- 1447 • the number of pairs in different clusters in \mathcal{C} and the same cluster in \mathcal{C}' by N_{01}

then the *Rand Index* is defined as

$$RI = \frac{N_{11} + N_{00}}{N_{11} + N_{00} + N_{10} + N_{01}} = \frac{N_{11} + N_{00}}{\binom{n}{2}} \quad (3.28)$$

1448 The Rand Index is 1 when the clusterings are identical, and 0 when the clusterings are com-
1449 pletely different.

The *adjusted Rand Index* intends on correcting the Rand Index for chance matching pairs.

This is defined as

$$ARI = \frac{2(N_{00}N_{11} - N_{01}N_{10})}{(N_{00} + N_{01})(N_{01} + N_{11}) + (N_{00} + N_{10})(N_{10} + N_{11})} \quad (3.29)$$

1450 The adjusted Rand Index is 1 when the clusterings are identical, and 0 when the Rand Index
1451 is equal to its expected value.

1452 Clusterings as random variables

If we take any random element of D , the probability that this element is in cluster C_k of clustering \mathcal{C} is

$$P(K = k) = \frac{n_k}{n} \quad (3.30)$$

1453 this defines a probability distribution, which makes the clustering a random variable. Any
1454 clustering can be considered as a random variable this way.

This means that we can measure any of the information theoretic quantities defined in section 3.3.4 with respect to clusterings. For example, the entropy of a clustering is

$$H(\mathcal{C}) = - \sum_{k=1}^K \frac{n_k}{n} \log \frac{n_k}{n} \quad (3.31)$$

If we have two clusterings, the joint probability distribution of these clusterings is defined as

$$P(K = k, K' = k') = \frac{|C_k \cap C'_{k'}|}{n} \quad (3.32)$$

1455 The joint distribution allows us to define the mutual information between two clusterings,
1456 $I(\mathcal{C}; \mathcal{C}')$ (Meilă, 2007).

1457 **Information based similarity measures**

The mutual information between two clusterings is a similarity measure, with $I(\mathcal{C}; \mathcal{C}') = 0$ if \mathcal{C} and \mathcal{C}' are completely different, and $I(\mathcal{C}; \mathcal{C}') = H(\mathcal{C}) = H(\mathcal{C}')$ if \mathcal{C} and \mathcal{C}' are identical. This can be normalised in a number of different ways to make more similarity measures (Vinh, Epps, and Bailey, 2010)

$$NMI_{joint} = \frac{I(\mathcal{C}; \mathcal{C}')}{H(\mathcal{C}, \mathcal{C}')} \quad (3.33)$$

$$NMI_{max} = \frac{I(\mathcal{C}; \mathcal{C}')}{\max\{H(\mathcal{C}), H(\mathcal{C}')\}} \quad (3.34)$$

$$NMI_{sum} = \frac{2I(\mathcal{C}; \mathcal{C}')}{H(\mathcal{C}) + H(\mathcal{C}')} \quad (3.35)$$

$$NMI_{sqrt} = \frac{I(\mathcal{C}; \mathcal{C}')}{\sqrt{H(\mathcal{C})H(\mathcal{C}')}} \quad (3.36)$$

$$NMI_{min} = \frac{I(\mathcal{C}; \mathcal{C}')}{\min\{H(\mathcal{C}), H(\mathcal{C}')\}} \quad (3.37)$$

We can control for chance similarities between the two clusterings by measuring the *adjusted mutual information* between the clusterings. This is defined as

$$AMI_{sum} = \frac{I(\mathcal{C}; \mathcal{C}') - E\{I(\mathcal{C}; \mathcal{C}')\}}{\frac{1}{2}[H(\mathcal{C}) + H(\mathcal{C}')] - E\{I(\mathcal{C}; \mathcal{C}')\}} \quad (3.38)$$

1458 The first term in the denominator, taking the average of the marginal entropies, can be re-
 1459 placed by taking the maximum, minimum, or the geometric mean (Vinh, Epps, and Bailey,
 1460 2010).

1461 **Information based metrics**

The variation of information between two clusterings $VI(\mathcal{C}; \mathcal{C}')$ (see section 3.3.4) is a metric on the space of clusterings (Meilă, 2007). That is,

$$VI(\mathcal{C}; \mathcal{C}') \geq 0 \quad (3.39)$$

$$VI(\mathcal{C}; \mathcal{C}') = 0 \iff \mathcal{C} = \mathcal{C}' \quad (3.40)$$

$$VI(\mathcal{C}; \mathcal{C}') = VI(\mathcal{C}'; \mathcal{C}) \quad (3.41)$$

$$VI(\mathcal{C}; \mathcal{C}'') \leq VI(\mathcal{C}; \mathcal{C}') + VI(\mathcal{C}'; \mathcal{C}'') \quad (3.42)$$

Another metric is the *information distance* (Vinh, Epps, and Bailey, 2010)

$$D_{max} = \max\{H(\mathcal{C}), H(\mathcal{C}')\} - I(\mathcal{C}; \mathcal{C}') \quad (3.43)$$

Both of these can be normalised

$$NVI(\mathcal{C}; \mathcal{C}') = 1 - \frac{I(\mathcal{C}; \mathcal{C}')}{H(\mathcal{C}, \mathcal{C}')} \quad (3.44)$$

$$d_{max} = 1 - \frac{I(\mathcal{C}; \mathcal{C}')}{\max\{H(\mathcal{C}), H(\mathcal{C}')\}} \quad (3.45)$$

1462 Comparing detected communities and anatomical divisions

1463 In order to quantify the difference or similarity between the communities detected in our
 1464 correlation network and the anatomical classification of the cells in that network, we considered
 1465 the communities and the anatomical regions as clusters in two different clusterings, \mathcal{C}_{comm}
 1466 and \mathcal{C}_{anat} , respectively. We then measured the similarity between the clusterings using the
 1467 mutual information, the adjusted mutual information, and the normalised mutual informa-
 1468 tion. We measured the difference between, or the distance between, the clusterings using the
 1469 variation of information, the normalised variation of information, and the normalised infor-
 1470 mation distance. We also measured the difference between the clusterings using the adjusted
 1471 Rand Index, just to use a non-information based measure.

1472 We took all of these measures for communities detected using different time bin widths.
 1473 This gave us an idea of the effect of time bin width on correlation networks in neural ensem-
 1474 bles relative to anatomical regions within those ensembles.

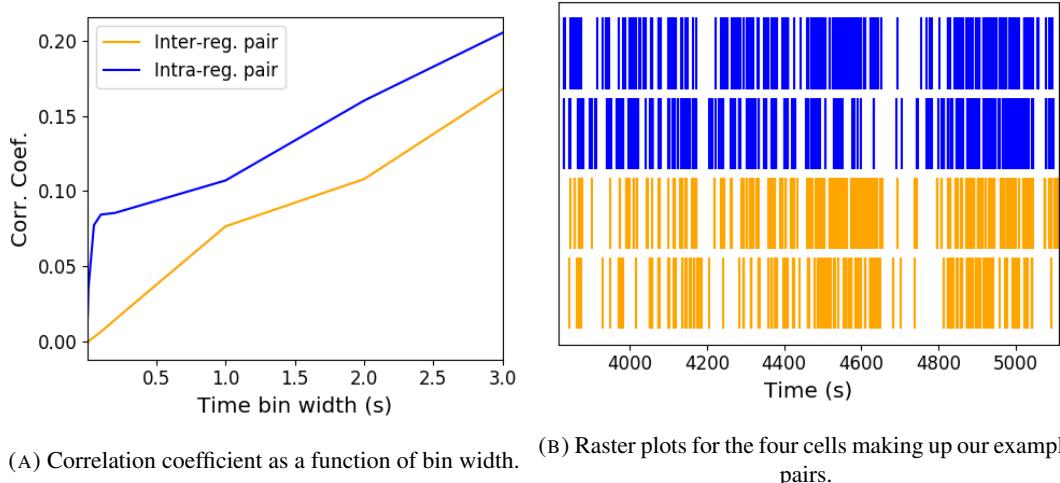
1475 3.4 Results

1476 Note that in the following text, we refer to the correlation coefficient between two sequences
 1477 of spike counts from two different cells as the *total correlation*. We refer to the correlation
 1478 between spike counts in response to a certain stimulus as the *spike count correlation* aka
 1479 *noise correlation*, and we refer to the correlation between mean or expected responses to
 1480 different stimuli as the *signal correlation* (Cohen and Kohn, 2011).

1481 The nine different brain regions from which we had data were the caudate putamen (CP),
 1482 frontal motor cortex (FrMoCtx), hippocampus (HPF), lateral septum (LS), midbrain (MB),
 1483 primary visual cortex (V1), superior colliculus (SC), somatomotor cortex (SomMoCtx), and
 1484 thalamus (TH).

1485 **3.4.1 Average correlation size increases with increasing time bin width**

1486 First we inspected the affect of time bin width on total correlations. We know that using short
 1487 time bins results in artificially small correlation measurements (Cohen and Kohn, 2011), so
 1488 we expected to see an increase in correlation amplitude with increasing time bin width. That
 1489 is exactly what we observed. Taking 50 cells at random, we calculated the total correla-
 1490 tion between every possible pair of these cells, using different time bin widths ranging from
 1491 0.005s to 3s. We found that the longer the time bin width, the greater the correlations (see
 1492 figure 3.4a).



(A) Correlation coefficient as a function of bin width. (B) Raster plots for the four cells making up our example pairs.

FIGURE 3.3: (A) An example of the correlation coefficients between two different pairs of cells, one where both cells are in the same brain region (intra-regional pair), and one where both cells are in different brain regions (inter-regional pair). The correlation coefficients have been measured using different time bin widths, ranging from 5ms to 3s. Note the increasing amplitude of the correlations with increasing bin width. (B) A raster plot showing the spike times of each pair of cells.

1493 We also separated the positively correlated pairs from the negatively correlated pairs
 1494 using the mean correlation of each pair across all bin widths (see section 3.3.2). We found
 1495 that the positively correlated pairs become more positively correlated with increasing time bin
 1496 width, and the negatively correlated pairs become more negatively correlated with increasing
 1497 time bin width (see figures 3.4b and 3.4c).

1498 In figure 3.3a we plot correlations from two example pairs, one pair from within a region,
 1499 and one pair between regions. It can be seen that the correlation coefficient increases with
 1500 bin width. The correlations can be observed by eye in the raster plot for these cells in figure
 1501 3.3b.

1502 When taking the mean across all pairs, the positively correlated pairs dominate in terms
 1503 of both number of pairs, and amplitude of correlations. Therefore the mean across all pairs

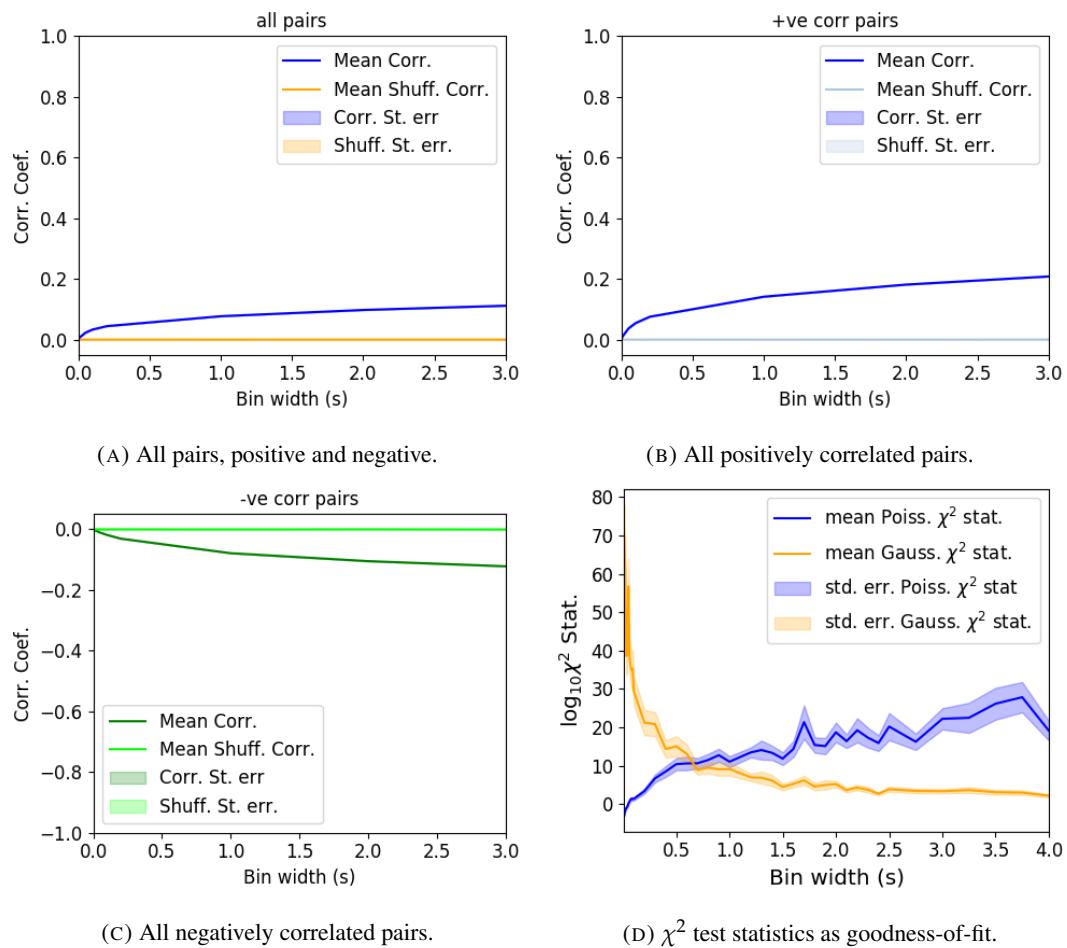


FIGURE 3.4: Mean correlation coefficients measured from pairs of 50 randomly chosen neurons. (A) All possible pairs, (B) positively correlated pairs, and (C) negatively correlated pairs. (D) Mean and standard error of χ^2 test statistics for Poisson and Gaussian distributions fitted to neuron spike counts.

1504 is positive.

1505 These results were observed in each of the three mouse subjects from which we had data.

1506 **3.4.2 Goodness-of-fit for Poisson and Gaussian distributions across increasing
1507 time bin widths**

1508 We wanted to investigate if the width of the time bin used to bin spike times into spike counts
1509 had an effect on the distribution of spike counts. We used the χ^2 statistic as a goodness-of-fit
1510 measure for Poisson and Gaussian (normal) distributions to the spike count of 100 randomly
1511 chosen neurons for a number of bin widths ranging from 0.01s to 4s. For the χ^2 statistic, the
1512 higher the value, the worse the fit.

1513 We expected a Poisson distribution to be a better fit for shorter time bin widths because
1514 spike counts must be non-negative, therefore any distribution of spike counts with mass dis-
1515 tributed at or close to 0 will be skewed. The distribution of spike counts is more likely to be
1516 distributed close to 0 when the time bin widths used to bin spike times into spike counts are
1517 small relative to the amount of time it takes for a neuron to fire an action potential ($\sim 1\text{ms}$ in
1518 the case of non-burst firing neurons).

1519 We expected a Gaussian distribution to be a better fit for longer time bin widths, because
1520 a Poisson distribution with a large rate is well approximated by a Gaussian distribution with
1521 mean and variance equal to the Poisson rate. Therefore, a Gaussian distribution would ap-
1522 proximate the mean of a collection of large spike counts, and have more flexibility than a
1523 Poisson distribution to fit the variance.

1524 We found that that a Poisson distribution is the best fit for shorter time bins less than 0.7s
1525 in length. Then a Gaussian distribution is a better fit for time bins greater than 0.7s in length
1526 (see figure 3.4d).

1527 **3.4.3 Differences between and inter- and intra- regional correlations decrease
1528 with increasing bin width**

1529 We investigated the differences in distribution between inter-regional correlations, i.e. corre-
1530 lations between neurons in different brain regions, and intra-regional correlations, i.e. corre-
1531 lations between neurons in the same brain region.

1532 Firstly, we investigated these quantities for all possible pairs of ~ 500 neurons taken
1533 from across all the 9 brain regions from which we had data. We distributed these neurons as
1534 evenly as possible across all of the regions, so that cells from one region would not dominate
1535 our data. We observed that the mean intra-regional correlations were always higher than the

mean inter-regional correlations for every value of time bin width used. We also observed that as the time bin width increased these mean correlations increased and the difference between the mean inter-regional and intra-regional correlations grew (see figure 3.5 (Left)).

Stringer et al. (2019) had a similar finding using the same data. They used only one value for the time bin width, 1.2s. Using this time bin width to bin spike times and measure total correlations, they found that the mean ‘within-region’ correlations were always greater than the ‘out-of-region’ correlations (Stringer et al., 2019). The figure from their paper showing this result can be seen in figure 3.5 (Right).

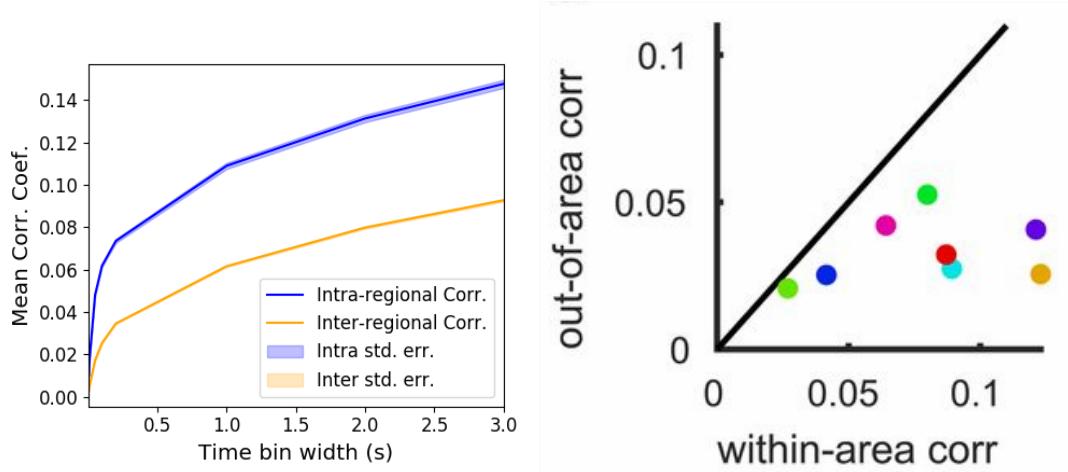
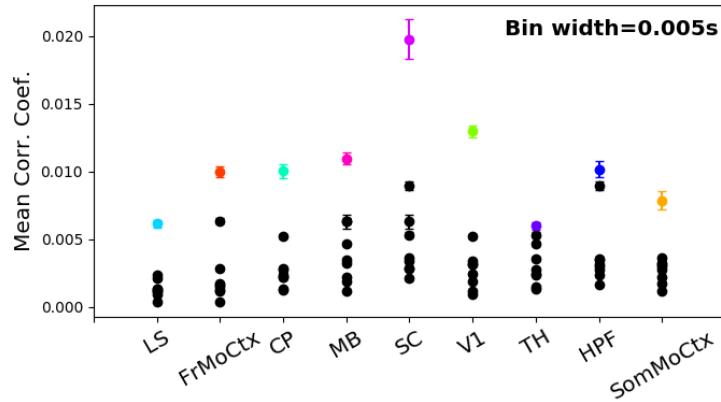


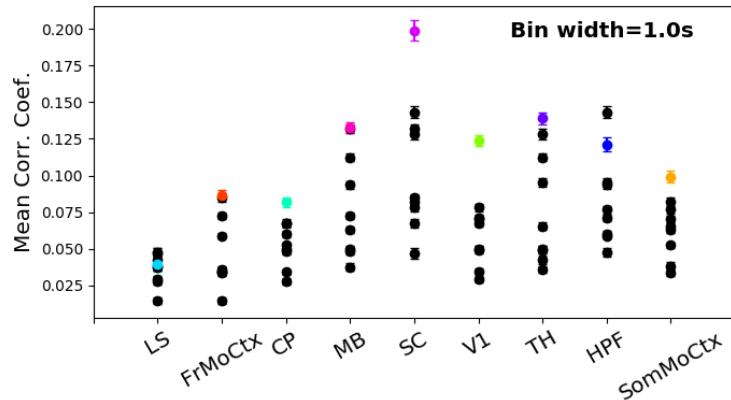
FIGURE 3.5: (Left)The mean intra-region and inter-region correlations using all possible pairs of ~ 500 neurons, spread across 9 different brain regions. (Right) Courtesy of Stringer et al. (2019), mean inter-regional (out-of-area) correlation coefficients vs mean intra-regional (within-area) correlation coefficients for a bin width of 1.2s. Note that the intra-regional coefficients are higher in each case.

Examples of the correlations of one intra-regional pair and one inter-regional pair can be seen in figure 3.3.

Secondly, we separated those pairs into intra-regional and inter-regional groups. We noted that the mean intra-regional correlations (coloured dots in figures 3.6a and 3.6b) for a given region tended to be higher than the mean inter-regional correlations (black dots in figures 3.6a and 3.6b) involving cells from that region. However, in contrast with our previous result, we noted that the difference between the mean intra-regional correlations and most highly correlated inter-regional correlations reduced as we increased the time bin width (see figures 3.6a and 3.6b). This shows that the mean correlations shown in figure 3.5 are not distributed evenly across all region pair combinations.



(A) Mean inter-regional and intra-regional correlations using a time bin width of 5ms.

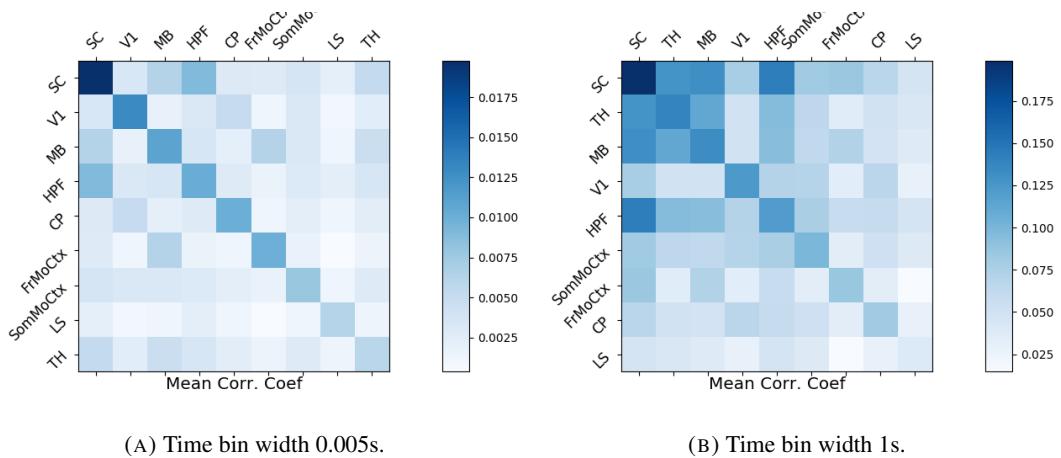


(B) Mean inter-regional and intra-regional correlations using a time bin width of 1s.

FIGURE 3.6: The mean intra-regional correlations (coloured dots) and mean inter-regional correlations (black dots) for a given region, indicated on the x-axis, for different time bin widths. Each black dot represents the mean inter-regional correlations between the region indicated on the x-axis and one other region. (A) shows these measurements when we used a time bin width of 5ms. (B) shows these measurements when we used a time bin width of 1s. Note that the difference between the mean inter-regional correlations and mean intra-regional correlations is smaller for 1s bins.

Finally, to see these regional mean correlations in a bit more detail, to examine the individual pair combinations in particular, we displayed these data in a matrix of mean correlations (see figure 3.7), showing the mean intra-regional correlations on the main diagonal, and the mean inter-regional correlations off diagonal. Comparing a version of this figure created using a short time bin width of 5ms (figure 3.7a) and a version using a longer time bin width of 1s (figure 3.7b) we observed that the mean intra-regional correlations are always relatively high in comparison to the mean inter-regional correlations, but the mean correlations in some inter-regional pairs are relatively much higher when using the longer time bin width.

This could indicate information being processed quickly at a local or within-region level, and the local representations of this information spreading between regions at longer timescales.



(A) Time bin width 0.005s.

(B) Time bin width 1s.

FIGURE 3.7: Mean inter-regional (main diagonal) and intra-regional (off diagonal) correlation coefficients. (A) Shows these measurements when spike times were binned using 5ms time bins. (B) Shows the same, using 1s time bins. Note that the matrices are ordered according to the main diagonal values, therefore the ordering is different in each subfigure.

These results were consistent across the three mouse subjects. But, the relative magnitudes of the mean intra-regional and inter-regional correlations were not consistent. For example, the region with the highest mean intra-regional correlations when using 1s bin widths for subject one is the superior colliculus (SC), but for subject two it is the midbrain (MB).

3.4.4 Connected and divided structure in correlation based networks reduces in dimension with increasing bin width

We used the correlation measurements to create weighted undirected graphs/networks where each node represents a neuron, and the weight of each edge is the pairwise correlation between those neurons represented by the nodes at either end of that edge. We aimed to find

1573 communities of neurons within these networks, and compare the structure of these communities
1574 to the anatomical division of those neurons. The first step of this process involved
1575 applying the ‘spectral rejection’ technique developed by Humphries et al (2019) (Humphries
1576 et al., 2019). This technique compares our data network to a chosen null network model, and
1577 finds any additional structure in the data network beyond that which is captured in the null
1578 network model (if there is any such structure).

1579 By comparing the eigenspectrum of the data network to the eigenspectrum of many samples
1580 from the null network model, this technique allows us to estimate the dimensionality of
1581 the additional structure in the data network, and gives us a basis for that vector space. It also
1582 divides the additional structure into connected structure, and k -partite (or divided) structure.
1583 For example, if our algorithm found two dimensions of additional connected structure, and
1584 one dimension of additional divided structure. We might expect to find three communities,
1585 that is groups more strongly connected within group than without, and we might expect to
1586 find bi-partite structure, that is two sets that are more strongly connected between groups
1587 than within groups.

1588 The technique also finds which nodes contribute to this additional structure, and divides
1589 our data network into signal and noise networks. The details of spectral rejection and node
1590 rejection can be found in sections 3.3.5 and 3.3.5 respectively, and a full overview can be
1591 found in (Humphries et al., 2019).

1592 We chose the sparse weighted configuration model (see section 3.3.5) as our null network
1593 model. This model matches the sparsity and the total weight of the original network but
1594 distributes the weight at random across the sparse network.

1595 We applied the spectral rejection method to our networks based on total correlations using
1596 different values for the time bin width. We observed that for smaller time bin widths, our data
1597 networks had both k -partite structure, and community structure. As the width of the time bin
1598 increased, we found that the k -partite structure disappeared from our data networks, and the
1599 dimension of the community structure reduced in two of the three mice from which we had
1600 data (see figure 3.8).

1601 **3.4.5 Detecting communities in correlation based networks**

1602 We applied the community detection procedure described in section 3.3.5 to our signal net-
1603 works for our various time bin widths. We detected a greater number of smaller communities
1604 at shorter time bin widths, and a smaller number of larger communities for longer time bin
1605 widths (see figure 3.9). This was expected after the results found in section 3.4.4. We found

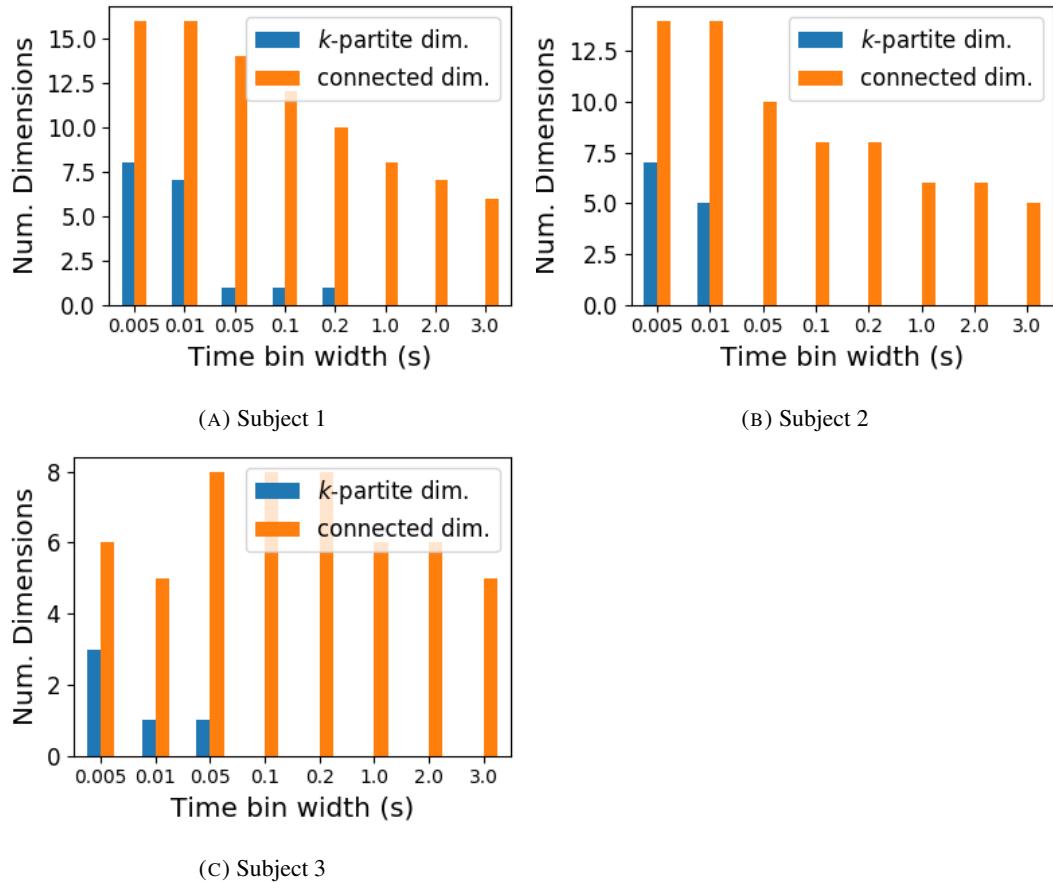


FIGURE 3.8: The number of dimensions in the k -partite and connected structure in the correlation based networks beyond the structure captured by a sparse weighted configuration null network model (see section 3.3.5), shown for different time bin widths. Note that the k -partite structure disappears for time bin width greater than 200ms for all three subjects. The dimension of the connected structure reduces with increasing bin width for 2 of the 3 subjects (top row).

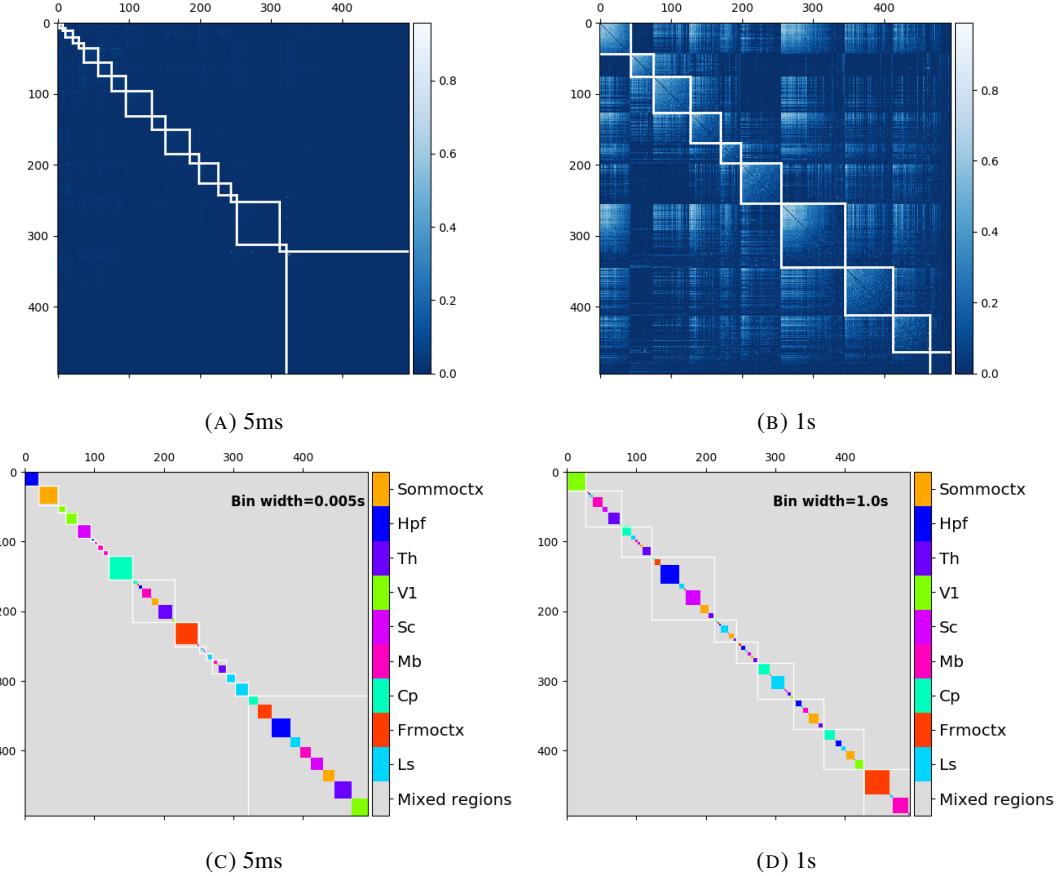


FIGURE 3.9: (A-B) Correlation matrices with detected communities indicated by white lines. Each off main diagonal entry in the matrix represents a pair of neurons. Those entries within a white square indicate that both of those neurons are in the same community as detected by our community detection procedure. Matrices shown are for 5ms and 1s time bin widths respectively. Main diagonal entries were set to 0. (C-D) Matrices showing the anatomical distribution of pairs along with their community membership. Entries where both cells are in the same region are given a colour indicated by the colour bar. Entries where cells are in different regions are given the grey colour also indicated by the colour bar.

1606 more dimensions of additional structure at shorter time bin widths, therefore we found more
1607 communities at shorter time bin widths.

1608 We also noticed that at short time bin widths the communities detected tended to be
1609 dominated by cells from one region. Whereas communities existing in networks created
1610 using wider time bin widths tended to contain cells from many different brain regions. More
1611 on this in the next section.

1612 **3.4.6 Functional communities resemble anatomical division at short timescales**

1613 In order to quantify the similarity of the communities detected to the anatomical division of
1614 the cells. We treated both the anatomical division and the communities as clusterings of these
1615 cells. We then used measures for quantifying the difference or similarity between clusterings
1616 to quantify the difference or similarity between the detected communities and the anatomical
1617 division. Details of these measures can be found in section 3.3.6 or in (Vinh, Epps, and
1618 Bailey, 2010).

1619 We used two different types of measures for clustering comparison; information based
1620 measures (see section 3.3.6) and pair counting based measures (see section 3.3.6). We include
1621 one example of each in figure 3.10.

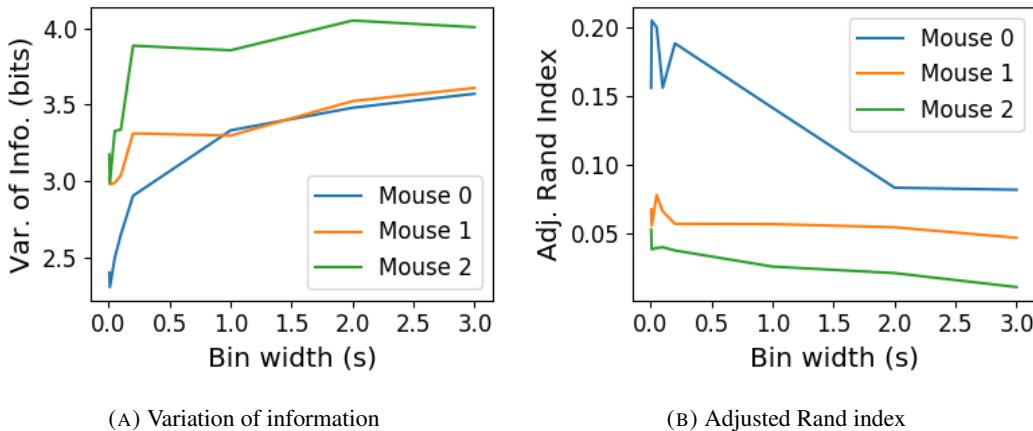
1622 The variation of information is the information based measure included in figure 3.10a.
1623 This measure forms a metric on the space of clusterings. The larger the value for the variation
1624 of information, the more different the clusterings.

1625 The adjusted Rand index is the pair counting based measure included in figure 3.10b. In
1626 contrast with the variation of information, the adjusted Rand index is a normalised similarity
1627 measure. The adjusted Rand index takes value 1 when the clusterings are identical, and takes
1628 value 0 when the clusterings are no more similar than chance.

1629 Both measures indicated that the detected communities and the anatomical division of
1630 the cells were more similar when we used shorter time bins widths (see figure 3.10). This
1631 indicates that correlated behaviour in neuronal ensembles is more restricted to individual
1632 brain regions at short timescales (< 250ms), and the correlated activity spreads out across
1633 brain regions over longer time scales.

1634 **3.4.7 Conditional correlations & signal correlations**

1635 In light of the excellent research of Stringer et al (2019) showing that spontaneous behaviours
1636 can drive activity in neuronal ensembles across the visual cortex and midbrain (Stringer et
1637 al., 2019), we decided to control for the mouse's behaviour when performing our analyses.



(A) Variation of information

(B) Adjusted Rand index

FIGURE 3.10: (A) The variation of information is a measure of distance between clusterings. The distance between the anatomical ‘clustering’ and community detection ‘clustering’ increases with increasing time bin width. (B) The adjusted Rand index is a normalised similarity measure between clusterings. The anatomical and community detection clusterings become less similar as the time bin width increases.

1638 It is possible that our community detection process may be detecting communities across
 1639 multiple brain regions at longer time scales due to aggregating neuronal activity driven by
 1640 several spontaneous behaviours occurring during the time interval covered by a given time
 1641 bin. A time bin of 1s, for example, could contain a spike count where those spikes were driven
 1642 by different spontaneous behaviours. We aimed to investigate this possibility by applying our
 1643 community detection analysis to conditional correlation measures.

1644 We used the top 500 principal components of a video of the mouse’s face as a measure of
 1645 the mouse’s behaviour (see section 3.2.2). We modelled the spike counts as a linear combi-
 1646 nation of the principal components using linear regression with ElasticNet regularisation (see
 1647 section 3.3.3). Using this model, we quantified the expected spike count given the mouse’s
 1648 behaviour $E[X|Z_1, \dots, Z_{500}]$.

1649 We used these expected values to measure $\text{cov}(E[X|Z], E[Y|Z])$, and we used that value,
 1650 the covariance $\text{cov}(X, Y)$, and the *law of total covariance* (see section 3.3.3) to measure
 1651 $E[\text{cov}(X, Y|Z)]$. Here X and Y represent spike counts from individual cells, and Z is short-
 1652 hand for the 500 principal components mentioned above. The two components of the co-
 1653 variance, $\text{cov}(E[X|Z], E[Y|Z])$ and $E[\text{cov}(X, Y|Z)]$, represent a ‘signal covariance’ and ex-
 1654 pected value of a ‘spike count covariance’ respectively, analogous to the signal correlation
 1655 and spike count correlation (Cohen and Kohn, 2011).

1656 We examined the means of these components for different values of the time bin width
 1657 (see figure 3.11). We observed a consistent increase in $E[\text{cov}(X, Y|Z)]$ as the time bin width
 1658 increased. But we saw different trends for $\text{cov}(E[X|Z], E[Y|Z])$ for each mouse.

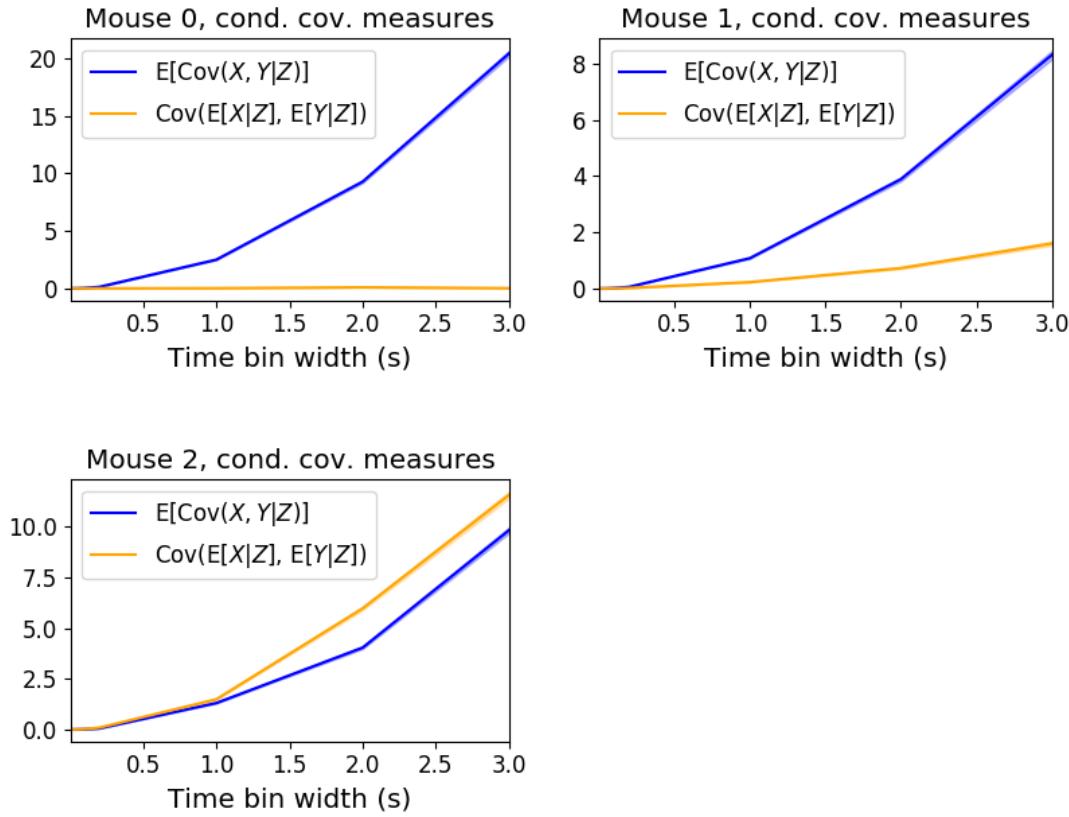


FIGURE 3.11: Comparing the components of the total covariance across different values for the time bin width. We observed a consistent increase in $E[\text{cov}(X, Y|Z)]$ as the time bin width increased. But we saw different trends for $\text{cov}(E[X|Z], E[Y|Z])$ for each mouse.

1659 Using $\text{cov}(E[X|Z], E[Y|Z])$ we measured the signal correlation, ρ_{signal} , and using $E[\text{cov}(X, Y|Z)]$
 1660 we measured the event conditional correlation, $\rho_{X,Y|Z}$ (see section 3.3.3 for more details).
 1661 We saw a consistent increase in $\rho_{X,Y|Z}$ as the time bin width increased, this corresponds to
 1662 the result for $E[\text{cov}(X, Y|Z)]$. We observed different trends for ρ_{signal} for each mouse, this
 1663 corresponds to the result for $\text{cov}(E[X|Z], E[Y|Z])$.

1664 We applied our network noise rejection and community detection process to networks
 1665 based on the spike count correlations $\rho_{X,Y|Z}$ and the signal correlations ρ_{signal} . We noted that
 1666 the community detection on $\rho_{X,Y|Z}$ behaved similarly to the community detection on the total
 1667 correlation. We can see this in figures 3.13a and 3.13b. At very short time bin widths, we
 1668 detect more communities, and those communities often contain cells from one brain region
 1669 only. At longer time bin widths, we detect fewer communities, and those communities tend
 1670 to contain cells from multiple brain regions. When we examine the distance between (or
 1671 similarity between) the anatomical division of the cells, and the detected communities we
 1672 notice that the two clusterings are more similar at shorter time bin widths (see figure 3.14).

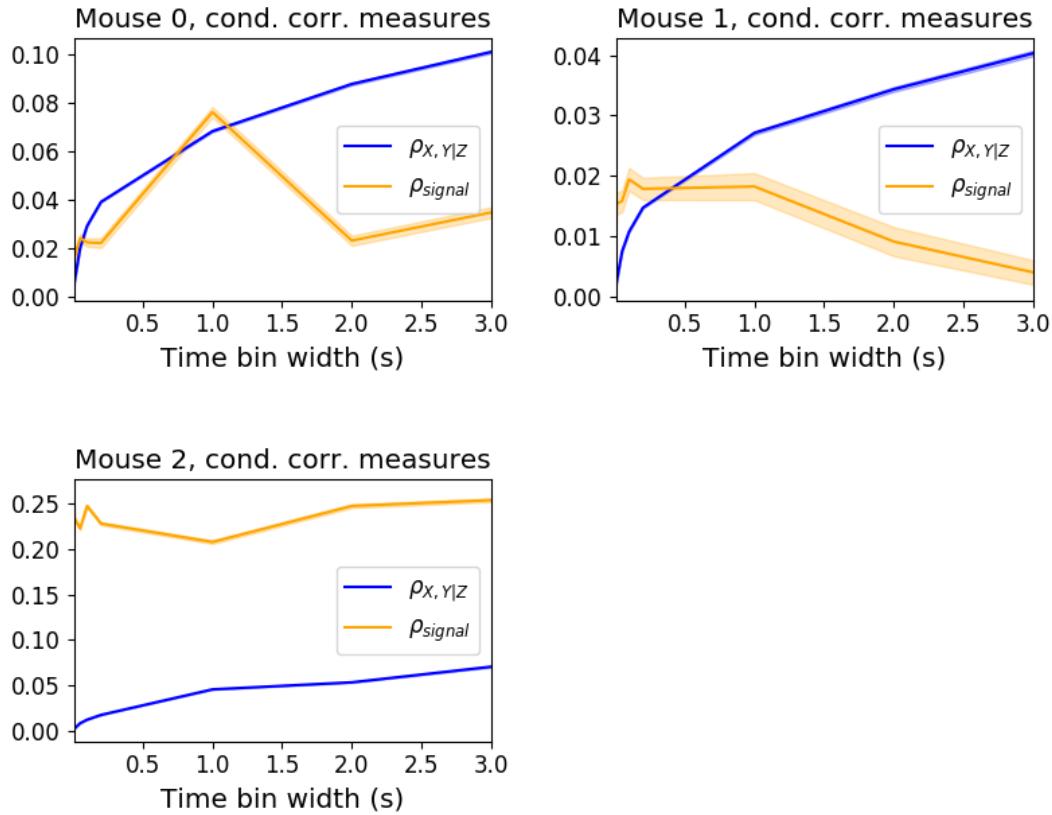


FIGURE 3.12: Comparing the components of the total covariance across different values for the time bin width. We saw a consistent increase in $\rho_{X,Y|Z}$ as the time bin width increased in all three subjects. But we saw different trends in ρ_{signal} for each of the subjects.

When we applied the network noise rejection and community detection process to the networks based on the signal correlations ρ_{signal} we found the number of communities we detected reduced with increasing time bin width. But the number of communities detected was less than that for the total correlations or the spike count correlations. The communities detected always tended to contain cells from multiple regions at both short and long timescales (see figures 3.13c and 3.13d). The communities detected bore very little relation to the anatomical division of the cells. The adjusted Rand index between the community clustering and the anatomical ‘clustering’ is close to zero for every time bin width (see figure 3.15b). This indicates that the similarity between the clusterings is close to chance. We did observe a slight downward trend in the variation of information with increasing bin width (see figure 3.15a), but this is more likely due to a decrease in the number of communities detected rather than any relationship with anatomy.

We also observed that the network noise rejection process rejected some of the cells when applied to the network based on the signal correlations. This means that those cells

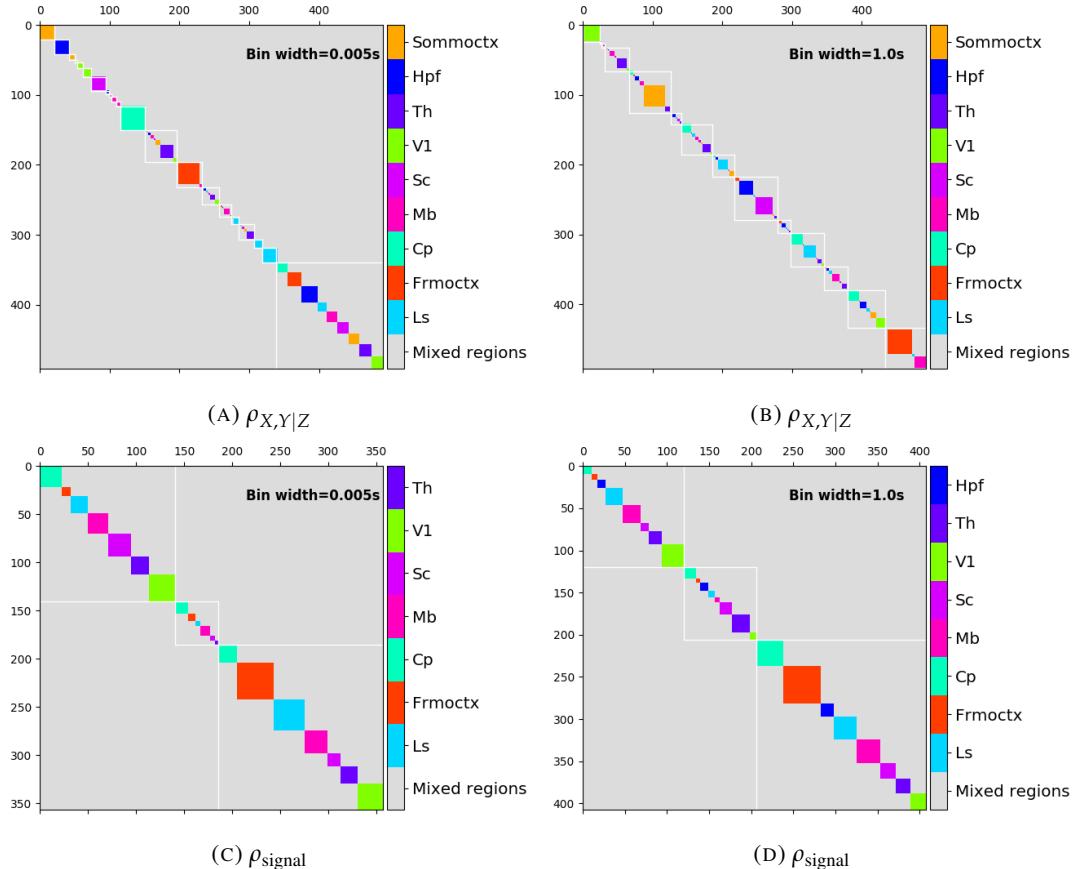


FIGURE 3.13: Matrices showing the regional membership of pairs by colour, and the communities in which those pairs lie. (A-B) Detected communities and regional membership matrix for network based on rectified spike count correlation $\rho_{X,Y|Z}$, using time bin widths of 0.005s and 1s respectively. (C-D) Detected communities and regional membership matrix for network based on rectified signal correlation ρ_{signal} , using time bin widths of 0.005s and 1s respectively.

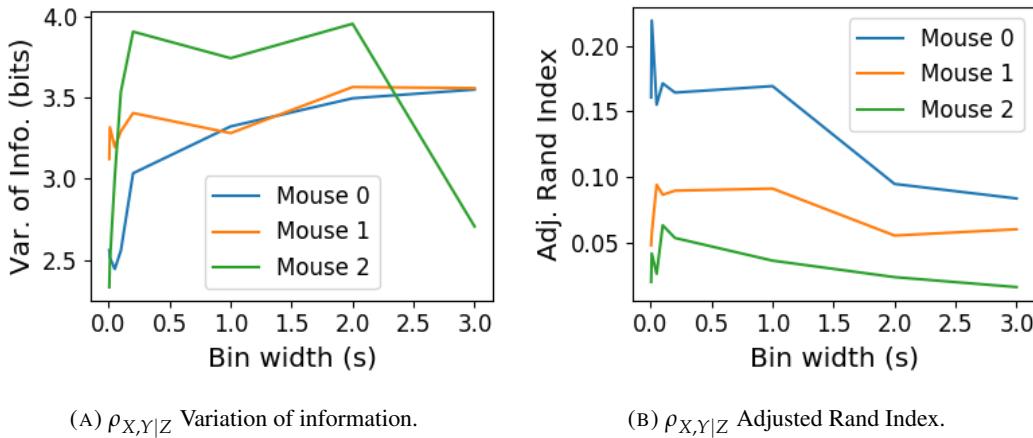
(A) $\rho_{X,Y|Z}$ Variation of information.(B) $\rho_{X,Y|Z}$ Adjusted Rand Index.

FIGURE 3.14: Distance and similarity measures between the anatomical division of the neurons, and the communities detected in the network based on the spike count correlations $\rho_{X,Y|Z}$. (A) The variation of information is a ‘distance’ measure between clusterings. The distance between the anatomical ‘clustering’ and the community clustering increases as the time bin width increases. (B) The adjusted Rand index is a similarity measure between clusterings. The detected communities become less similar to the anatomical division of the cells as the time bin width increases.

1687 did not contribute to the additional structure of the network beyond that captured by the
 1688 sparse weighted configuration model. This is why the matrices in figures 3.13c and 3.13d are
 1689 smaller than their analogues in figures 3.13a and 3.13b.

1690 3.4.8 Absolute correlations and negative rectified correlations

1691 At the moment, the network noise rejection protocol can only be applied to weighted undi-
 1692 rected graphs with non-negative weights. This meant that we had to rectify our correlated
 1693 networks before applying the network noise rejection and community detection process. We
 1694 wanted to investigate what would happen if instead of rectifying the correlations, we used the
 1695 absolute value, or reversed the signs of the correlations and then rectified.

1696 When we used the absolute value of the correlations, we found very similar results to
 1697 those shown above for the rectified total correlations and the rectified spike count corre-
 1698 lations. We detected more communities using shorter bin widths, and these communities
 1699 were more similar to the brain’s anatomy than those communities detected using a longer bin
 1700 width (see figure 3.16). The only exception being that we detected more communities. This
 1701 could indicate that we detected both positively and negatively correlated communities, but
 1702 we haven’t done any further investigation so we cannot say for sure.

1703 When we used the sign reversed rectified correlated networks, we tended to find fewer
 1704 communities. Each community contained cells from many different anatomical regions, at

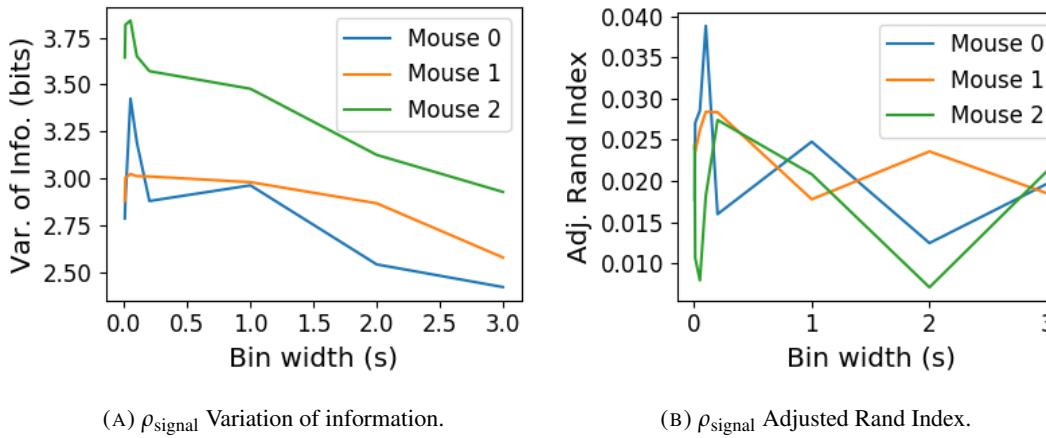
(A) ρ_{signal} Variation of information.(B) ρ_{signal} Adjusted Rand Index.

FIGURE 3.15: Distance and similarity measures between the anatomical division of the neurons, and the communities detected in the network based on the signal correlations ρ_{signal} . (A) The variation of information is a ‘distance’ measure between clusterings. The distance between the anatomical ‘clustering’ and the community clustering increases as the time bin width increases. (B) The adjusted Rand index is a similarity measure between clusterings. The detected communities become less similar to the anatomical division of the cells as the time bin width increases.

1705 both long and short bin widths (see figures 3.17a, 3.17b, 3.17c, 3.17d). The communities
 1706 bore little relation to the anatomical distribution of the cells, this can be seen in figure 3.17f,
 1707 the values close to zero indicate that the similarity between the two clusterings are around
 1708 chance level. This indicates that there was not much structure in the negatively correlated
 1709 networks beyond that captured by the sparse weighted configuration model.

1710 3.5 Discussion

1711 It is well established that the brain uses correlated behaviour in neuronal ensembles to repre-
 1712 sent the information taken in through sensation (Cohen and Maunsell, 2009; Litwin-Kumar,
 1713 Chacron, and Doiron, 2012; deCharms and Merzenich, 1996). However, most studies that
 1714 examine the nature of these correlations in-vivo, study an ensemble of cells from only one
 1715 or two brain regions (Cohen and Kohn, 2011; Wierzynski et al., 2009; Patterson et al., 2014;
 1716 Girard, Hupé, and Bullier, 2001). Furthermore, recent results have shown that behaviour can
 1717 drive correlated activity in multiple brain regions, including those not normally associated
 1718 with motor control (Stringer et al., 2019; Gründemann et al., 2019; Allen et al., 2019). In this
 1719 study, we utilised one of the newly recorded large datasets containing electrophysiological
 1720 recordings from multiple brain regions simultaneously. We investigated correlated behaviour
 1721 in these different brain regions and we investigated correlated behaviour between neurons in
 1722 different regions, during spontaneous behaviour.

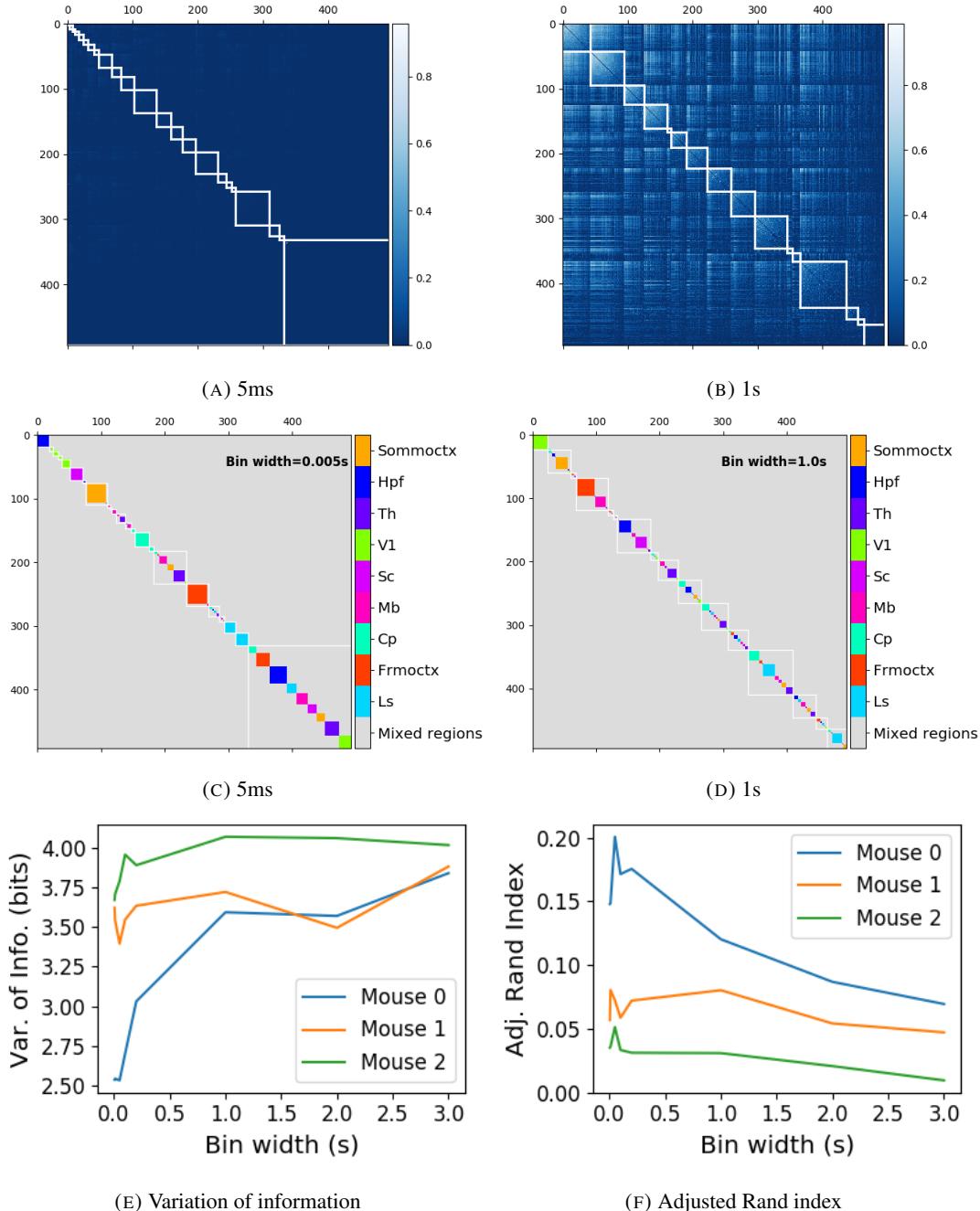


FIGURE 3.16: (A-B) Absolute correlation matrices with detected communities indicated by white lines. These communities are based on the absolute value of the total correlation between each pair of cells. Those entries within a white square indicate that both of those neurons are in the same community. Matrices shown are for 5ms and 1s time bin widths respectively. Main diagonal entries were set to 0. (C-D) Matrices showing the anatomical distribution of pairs along with their community membership. Regional membership is indicated by the colour bar. (E) Variation of information between the anatomical division of the cells, and the detected communities. (F) Adjusted Rand index between the anatomical division, and the detected communities.

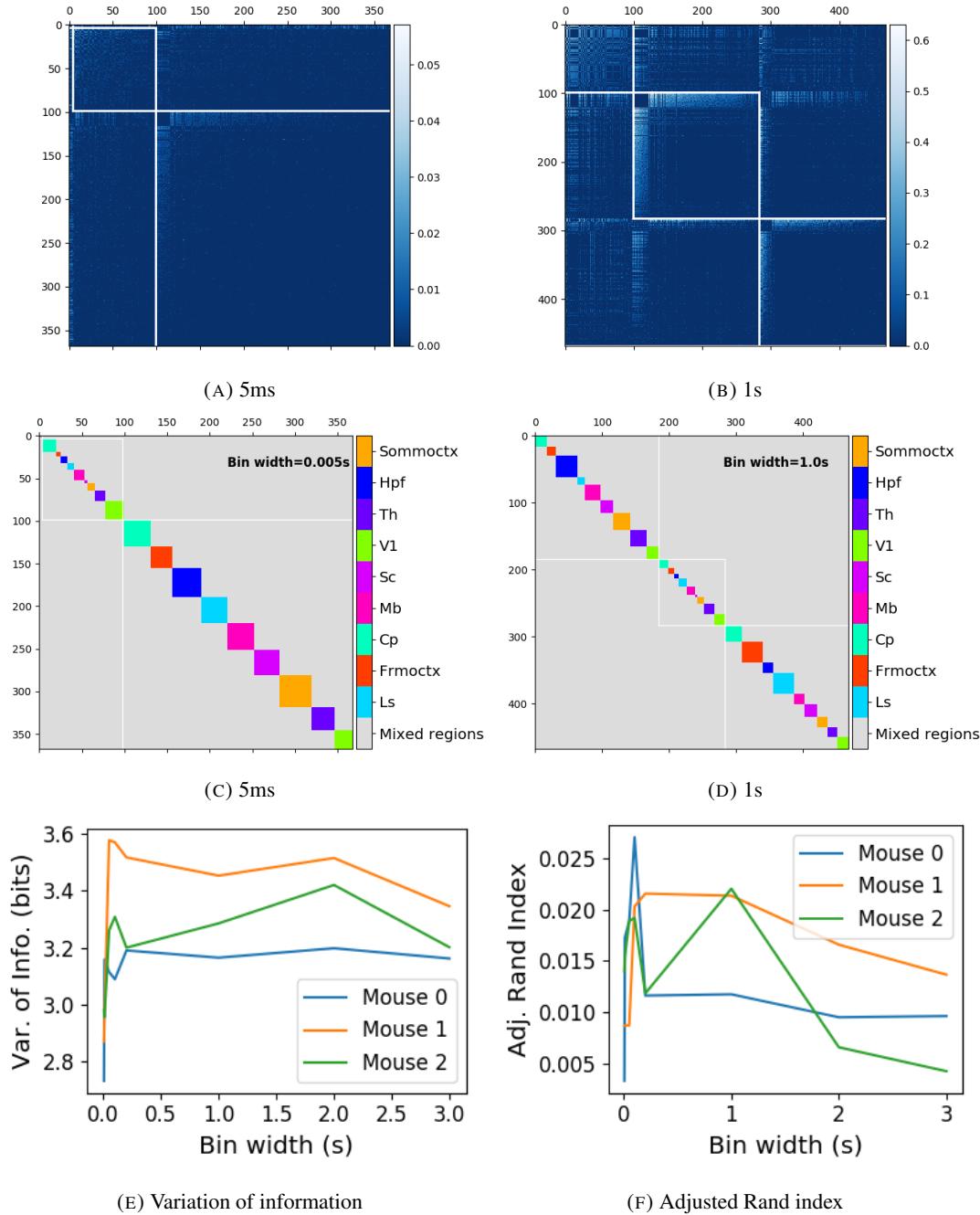


FIGURE 3.17: (A-B) Sign reversed rectified correlation matrices with detected communities indicated by white lines. Those entries within a white square indicate that both of those neurons are in the same community. Matrices shown are for 5ms and 1s time bin widths respectively. Main diagonal entries were set to 0. (C-D) Matrices showing the anatomical distribution of pairs along with their community membership. Regional membership is indicated by the colour bar. (E) Variation of information between the anatomical division of the cells, and the detected communities. (F) Adjusted Rand index between the anatomical division, and the detected communities.

1723 A number of studies have found that the timescale of correlated behaviour induced by a
1724 stimulus can be modulated by the stimulus structure and behavioural context. For example,
1725 the spike train correlations between cells in weakly electric fish are modulated by the spa-
1726 tial extent of the stimulus (Litwin-Kumar, Chacron, and Doiron, 2012), and neurons in the
1727 marmoset primary auditory cortex modulate their spike timing (and therefore correlation) in
1728 response to stimulus features without modulating their firing rate (deCharms and Merzenich,
1729 1996). Furthermore, the width of the time bins over which spike counts are measured has
1730 been shown to have an effect on the magnitude of those correlations (Cohen and Kohn, 2011).
1731 Despite this, very little research has been done comparing correlation measures from the same
1732 dataset at different timescales. We investigated this by varying the time bin width used to bin
1733 spike times into spike counts from as short as 5ms up to 3s.

1734 In order to further investigate the effect of these correlations at different timescales, we
1735 regarded our neuronal ensemble as a weighted undirected graph, where each neuron is rep-
1736 resented by a node, and the weight on each edge is the correlation between the neurons
1737 connected by that edge. We then applied a novel clustering method from network science
1738 (Humphries et al., 2019) to identify communities in these networks. Communities in a net-
1739 work graph refer to sets of nodes that are more strongly connected to each other than the
1740 nodes outside of their set. Another way to put this is to say that the nodes in a community
1741 are more strongly connected than *expected*. What connection strength might be expected is
1742 defined by a null network model. We chose a null network model that matched the sparsity
1743 and total strength of our correlation based data networks. So, if two cells were in the same
1744 community, those cells were more correlated than would be expected given the correlation
1745 strength of their ensemble.

1746 These networks, and the community detection process, were completely agnostic of the
1747 anatomical division of the cells in our ensemble. When we compared the detected commu-
1748 nities with the anatomical division of the cells using distance and similarity measures for
1749 clusterings, we found that the detected communities were more similar to the anatomical
1750 division at shorter timescales. That is, when we used a wider time bin to count spikes, and
1751 computed pairwise correlations with these spike counts, the correlated communities tended to
1752 exist within anatomical regions at shorter timescales, and tended to span anatomical regions
1753 at longer timescales. This could reflect localised functional correlations at short time scales
1754 rippling outwards across brain regions at longer timescales. The brain may be processing
1755 some information quickly locally, and carrying out further, perhaps more detailed, represen-
1756 tation over a longer timescale across many regions using the representations that were just

1757 built locally.

1758 These changes in communities across timescales could also be driven by the anatomy
1759 of the individual cells. For example, it may simply take longer to transmit action potentials
1760 over longer distances, hence correlated activity over longer timescales will exist between
1761 anatomical regions, rather than within. However, the switch to almost exclusively multi-
1762 regional functional networks at 1s bin widths, rather than a mixture of multi-region, and
1763 single-region suggests that the inter-regional correlations either overpower, or inhibit the
1764 local correlations. So there may be more at play than just timescales.

1765 We acknowledged that the region spanning correlated communities that we detected at
1766 longer time scales could exist due to collating activity driven by distinct spontaneous activi-
1767 ties. In order to account for this, we modelled the spike counts as a linear function of the
1768 top 500 principal components of a video of the mouse’s face filmed simultaneously with the
1769 electrophysiological readings. We applied our network noise rejection and community de-
1770 tection process to the weighted undirected networks formed by the spike count correlations
1771 (or noise correlations) and the signal correlations that we calculated using our model. For the
1772 spike count correlation networks, we found much the same results as for the total correlations
1773 as described above. For the signal correlations, the communities detected in these networks
1774 bore little relation to the anatomical division of the cells. Recent findings have shown that
1775 behavioural data accounts for correlations in many brain regions that would otherwise be
1776 dismissed as noise (Stringer et al., 2019), our finding to shows that these correlations are still
1777 governed by the timescale division between local communication and across-region commu-
1778 nication.

1779 There is a lot of room for further investigation based on this research. For a start, the
1780 data that we used here were collected from nine different regions in the mouse brain, but
1781 none of these regions were part of the somatosensory cortex. Given that a mouse experiences
1782 so much of its environment through its sense of smell, some data from this region would be
1783 interesting to investigate. On the same theme, the mice in the experiment from which the
1784 data were collected were headfixed and placed on a rotating ball, but were otherwise behav-
1785 ing spontaneously. Had these mice been exposed to a visual, aural, or olfactory stimulus,
1786 we could have examined the responses of the cells in the brain regions corresponding to vi-
1787 sion, hearing, and olfaction, and compared these responses to the responses from the other
1788 brain regions. Furthermore, we could have investigated the interaction between the sets of
1789 responses.

1790 Another space for further investigation is the community detection. The algorithm that we

1791 used here never detects overlapping communities. But functional communities could indeed
1792 have overlaps. Clustering methods that detect overlapping clusters do exist (Baadel, Thabtah,
1793 and Lu, 2016). Applying one of those algorithms could yield some interesting results. Also,
1794 the community detection algorithm that we used here cannot process graphs with negative
1795 weights, this forced us to separate positive and negative correlations before applying our
1796 network noise rejection and community detections process, or use the absolute value of our
1797 correlations. A community detection algorithm that can work on weighted undirected graphs
1798 with negative weights could yield some interesting results here.

1799 **Chapter 4**

1800 **A simple two parameter distribution
1801 for modelling neuronal activity and
1802 capturing neuronal association**

1803 *Abstract*

1804 Recent developments in electrophysiological technology have lead to an increase in the size
1805 of electrophysiology datasets. Consequently, there is a requirement for new analysis tech-
1806 niques that can make use of these new datasets, while remaining easy to use in practice. In
1807 this work, we fit some one or two parameter probability distributions to spiking data collected
1808 from a mouse exposed to visual stimuli. We show that the Conway-Maxwell-binomial dis-
1809 tribution is a suitable model for the number of active neurons in a neuronal ensemble at any
1810 given moment. This distribution fits these data better than binomial or beta-binomial distribu-
1811 tions. It also captures the correlated activity in the primary visual cortex induced by stimulus
1812 onset more effectively than simply measuring the correlations, at short timescales (< 10ms).
1813 We also replicate the finding of Churchland et al (2010) relating to stimulus onset quenching
1814 neural variability in cortical areas, and we show a correspondence between this quenching
1815 and changes in one of the parameters of the fitted Conway-Maxwell-binomial distributions.

4.1 Introduction

Recent advances in electrophysiological technology, such as ‘Neuropixels’ probes (Jun et al., 2017) have allowed extracellular voltage measurements to be collected from larger numbers of cells than traditional methods, in multiple brain regions simultaneously, and routinely. These larger datasets require innovative methods to extract information from the data in a reasonable amount of time, ‘reasonable’ being subjective in this case.

Theoretically, all the information at any given moment in an electrophysiological dataset with n neurons could be captured by calculating the probability distribution for every possible spiking pattern. This would require defining a random variable with 2^n possible values, a task that quickly becomes impossible as n increases. Attempts at approximating this random variable often involve measuring pairwise or higher order correlations (Schneidman et al., 2006; Flach, 2013; Ganmor, Segev, and Schneidman, 2011). But pairwise correlations may not be enough to characterise instantaneous neural activity (Tkačik et al., 2014). Furthermore, these kinds of models tend to ignore the temporal structure of neuronal data, in favour of smaller model size, and scalability.

Higher order correlations would be helpful here, but defining these correlations can be tricky, never-mind quantifying them. If we use the interaction parameters arising from the exponential family model as measures of higher order correlations, measuring these correlations becomes computationally impractical quite quickly also (the number of ‘three neuron correlations’ to measure scales with $(n)_3$). In this paper, we dispense with measuring correlations directly, and attempt to characterise correlated behaviour by measuring ‘association’; a more general concept that includes correlation.

In this work, we examined the ability of simple distributions to model the number of active (spiking) neurons in a neuronal ensemble at any given timepoint. We compared a little-known distribution named the Conway-Maxwell-binomial distribution to the binomial distribution and the beta-binomial distribution. The binomial distribution is a probability distribution over the number of successes in a sequence of independent and identical Bernoulli trials. The beta-binomial distribution is similar, but allows for a bit more flexibility while still being a model for heterogeneity. Similar to the binomial and beta-binomial, the Conway-Maxwell-binomial distribution is a probability distribution over the number of successes in a series of Bernoulli trials, but allows over- and under-dispersion relative to the binomial distribution. This distribution should therefore be a good candidate for our purposes. We found that Conway-Maxwell-binomial distribution was usually the best candidate of the three that

1849 we examined.

1850 We also observed some interesting changes in the number of active neurons in the primary
1851 visual cortex and hippocampus at stimulus onset and some changes in this activity in the
1852 thalamus which were sustained for the full duration of the stimulus presentation. This let us
1853 know that there were some responses to model.

1854 We found that fitting a Conway-Maxwell-binomial distribution was a better method of
1855 capturing association between neurons than measuring the spike count correlation for the
1856 short time bins that we used (< 10ms).

1857 Finally, we also wanted to investigate parallels between the parameters of the Conway-
1858 Maxwell-binomial distribution and quantities that have been established as relevant to sen-
1859 sory processing. So, we replicated the findings made by Churchland et al. (2010) relating
1860 to a reduction in neural variability at stimulus onset in the macaque cortical regions, but for
1861 data taken from the mouse primary visual cortex. We compared these findings to the values
1862 of the fitted Conway-Maxwell-binomial distribution parameters.

1863 4.2 Data

1864 We used data collected by Nick Steinmetz and his lab ‘CortexLab at UCL’ (Steinmetz, Caran-
1865 dini, and Harris, 2019). The data can be found online ¹ and are free to use for research
1866 purposes.

1867 Two ‘Phase3’ Neuropixels (Jun et al., 2017) electrode arrays were inserted into the brain
1868 of an awake, head-fixed mouse for about an hour and a half. These electrode arrays recorded
1869 384 channels of neural data each at 30kHz and less than $7\mu\text{V}$ RMS noise levels. The sites
1870 are densely spaced in a ‘continuous tetrode’-like arrangement, and a whole array records
1871 from a 3.8mm span of the brain. One array recorded from visual cortex, hippocampus, and
1872 thalamus, the other array recorded from motor cortex and striatum. The data were spike-
1873 sorted automatically by Kilosort and manually by Nick Steinmetz using Phy. In total 831
1874 well-isolated individual neurons were identified.

1875 4.2.1 Experimental protocol

1876 The mouse was shown a visual stimulus on three monitors placed around the mouse at right
1877 angles to each other, covering about ± 135 degrees azimuth and ± 35 degrees elevation.

¹<http://data.cortexlab.net/dualPhase3/>

1878 The stimulus consisted of sine-wave modulated full-field drifting gratings of 16 drift di-
1879 rections ($0^\circ, 22.5^\circ, \dots, 337.5^\circ$) with 2Hz temporal frequency and 0.08 cycles/degree spatial
1880 frequency displayed for 2 seconds plus a blank condition. Each of these 17 conditions were
1881 presented 10 times in a random order across 170 different trials. There were therefore 160
1882 trials with a drifting-grating visual stimulus present, and 10 trials with a blank stimulus.

1883 **4.3 Methods**

1884 **4.3.1 Binning data**

1885 We converted the spike times for each cell into spike counts by putting the spike times into
1886 time bins of a given ‘width’ (in milliseconds). We used time bins of 1ms, 5ms, and 10ms.
1887 We used different time bin widths to assess the impact of choosing a bin width.

1888 **4.3.2 Number of *active* neurons**

1889 To count the number of active neurons in each neuronal ensemble, we split the time interval
1890 for each trial into bins of a given width. We counted the number of spikes fired by each cell
1891 in each bin. If a cell fired *at least* one spike in a given bin, we regarded that cell as active in
1892 that bin. We recorded the number of active cells in every bin, and for the purposes of further
1893 analysis, we recorded each cell’s individual spike counts.

1894 It should be noted that when we used a bin width of 1ms, the maximum number of
1895 spikes in any bin was 1. For the wider time bins, some bins had spike counts greater than
1896 1. Consequently when using a bin width of 1ms, the number of active neurons and the total
1897 spike count of a given bin were identical. But for wider bin widths, the total spike count was
1898 greater than the number of active neurons.

1899 So for the 1ms bin width, the activity of a neuron and the number of spikes fired by that
1900 neuron in any bin can be modelled as a Bernoulli variable. But for wider time bins, only the
1901 activity can be modelled in this way.

1902 **4.3.3 Moving windows for measurements**

1903 When taking measurements (e.g. moving average over the number of active neurons) or
1904 fitting distributions (eg. the beta binomial distribution) we slid a window containing a certain
1905 number of bins across the data, and made our measurements at each window position. For
1906 example, when analysing 1ms bin data, we used a window containing 100 bins, and we slid

Bin width (ms)	Window size (bins)	Window size (ms)	Windows per trial
1ms	100	100ms	296
5ms	40	200ms	286
10ms	40	400ms	266

TABLE 4.1: Details of the different bin width and analysis window sizes used when binning spike times, and analysing those data.

1907 the window across the time interval for each trial moving 10 bins at a time. So that for
 1908 3060ms of data, we made 296 measurements.

1909 For the 5ms bin width data, we used windows containing 40 bins, and slid the window 2
 1910 bins at a time when taking measurements.

1911 For the 10ms bin width data, we used windows containing 40 bins, and slid the window
 1912 1 bin at a time when taking measurements (see table 4.1 for concise details).

1913 By continuing to use windows containing 40 bins, we retained statistical power but sac-
 1914 rificed the number of measurements taken.

1915 There was an interval between each trial with a grey image in place of the moving of
 1916 the moving bar stimulus. This interval varied in time. But we included some of this interval
 1917 when recording the data for each trial. We started recording the number of active neurons,
 1918 and the number of spikes from each neuron from 530ms before each trial until 1030ms after
 1919 each trial. This way, we could see the change in our measurements at the onset of a stimulus
 1920 and the end of stimulus presentation.

1921 As mentioned in section 4.3.2, we recorded the number of active neurons in each bin, and
 1922 the spike count for each neuron in each bin. The actual measurements we took using these
 1923 data in each window were as follows:

1924 **Moving average** The average number of active cells in each window.

1925 **Moving variance** The variance of the number of active cells in each window.

1926 **Average correlation** We measured the correlation between the spike counts of each pair of
 1927 cells in the ensemble, and took the average of these measurements.

1928 **Binomial p** We fitted a binomial distribution to the data in each window and recorded the
 1929 fitted probability of success, p in each case.

1930 **Beta-binomial α, β** We fitted a beta-binomial distribution to the data in each window, and
 1931 recorded the values of the fitted shape parameters, α and β , of each distribution.

1932 **Conway-Maxwell-binomial distribution p, ν** We fitted a Conway-Maxwell-binomial dis-
 1933 tribution to the data in each window, and recorded the fitted values of p and ν for each
 1934 distribution.

1935 **Log-likelihoods** We also recorded the log-likelihood of each of the fitted distributions for
 1936 each window.

1937 **4.3.4 Fano factor**

The *Fano factor* of a random variable is defined as the ratio of the variable's variance to its mean.

$$F = \frac{\sigma^2}{\mu} \quad (4.1)$$

1938 We measured the Fano factor of the spike count of a given cell by measuring the mean and
 1939 variance of the spike count across trials, and taking the ratio of those two quantities. When
 1940 calculated in this way the Fano factor can be used as a measure of neural variability that
 1941 controls for changes in the firing rate. This is similar to the calculation used in (**churchland**).

1942 **4.3.5 Probability Distributions suitable for modelling ensemble activity**

1943 We present here three different probability distributions that could be suitable to model the
 1944 number of active neurons in an ensemble. Each distribution has the set $\{0, \dots, n\}$ as its sup-
 1945 port, where n is the number of neurons in the ensemble. These are simple distributions with
 1946 either two or three parameters each. However, we regard n as known when using these dis-
 1947 tributions for modelling, so in effect each distribution has either one or two free parameters.

1948 **Association**

1949 *Association* between random variables is similar to the correlation between random variables
 1950 but is more general in concept. The correlation is a measure of association; and association
 1951 doesn't have a mathematical definition like correlation does. Essentially, the association
 1952 between two random variables is their tendency to take the same or similar values. Positively
 1953 associated variables tend to take the same value, and negatively associated variables tend to
 1954 take different values. In this research, we work with probability distributions of the number of
 1955 successes in a set of Bernoulli trials. These Bernoulli variables may or may not be associated.

1956 A probability distribution over the number of successes in n Bernoulli trials, where the
 1957 Bernoulli variables may be associated, could constitute a good model for the number of active
 1958 neurons in an ensemble of n neurons.

1959 **Binomial distribution**

The binomial distribution is a two parameter discrete probability distribution that can be thought of as a probability distribution the number of successes from n independent Bernoulli trials, each with the same probability of success. The parameters of the binomial distribution are n the number of trials, and $0 \leq p \leq 1$, the probability of success for each of these trials. A random variable with the binomial distribution can take values from $\{0, \dots, n\}$. The probability mass function of the distribution is

$$P(k; n, p) = \binom{n}{k} p^k (1-p)^{n-k} \quad (4.2)$$

1960 As a model for the activity of a neuronal ensemble, the main problem with the binomial
 1961 distribution is that it treats each neuron, represented as a Bernoulli trial, as independent. It is
 1962 well known that neurons are not independent, and that correlated behaviour between neurons
 1963 is vital for representing sensory information (Cohen and Maunsell, 2009). The binomial dis-
 1964 tribution falls short in this regard, but it is useful as performance benchmark when assessing
 1965 the performance of other models.

1966 **Beta-binomial distribution**

1967 The beta distribution is the conjugate distribution of the binomial distribution. The beta-
 1968 binomial distribution is the combination of the beta distribution and the binomial distribution,
 1969 in that the probability of success for the binomial distribution is sampled from the beta dis-
 1970 tribution. This allows the beta-binomial distribution to capture some over dispersion relative
 1971 to the binomial distribution.

The beta-binomial distribution is a three parameter distribution, n the number of Bernoulli trials, and $\alpha \in \mathbb{R}_{>0}$ and $\beta \in \mathbb{R}_{>0}$ the shape parameters of the beta distribution. The probability mass function for the beta-binomial distribution is

$$P(k; n, \alpha, \beta) = \binom{n}{k} \frac{B(k + \alpha, n - k + \beta)}{B(\alpha, \beta)} \quad (4.3)$$

1972 where $B(\alpha, \beta)$ is the beta function.

This probability distribution can be reparametrised in a number of ways. One of which defines new parameters π and ρ by

$$\pi = \frac{\alpha}{\alpha + \beta} \quad (4.4)$$

$$\rho = \frac{1}{\alpha + \beta + 1} \quad (4.5)$$

1973 This reparametrisation is useful because π acts as a location parameter analogous to the p
 1974 parameter of a binomial distribution. A value of $\rho > 0$ indicates over-dispersion relative to a
 1975 binomial distribution.

1976 As a model for the activity of a neuronal ensemble, the beta-binomial distribution is
 1977 more suitable than a binomial distribution because the over-dispersion of the beta-binomial
 1978 distribution can be used to model positive association between the neurons. An extreme
 1979 example of this over-dispersion/positive association can be seen in figure 4.1b. In this figure,
 1980 the neurons are positively associated and so tend to take the same value, consequently the
 1981 probability mass of the beta-binomial distribution builds up close to $k = 0$ and $k = n$. It is
 1982 worth noting that the location parameter for each distribution has the same value, $p = \pi =$
 1983 0.5.

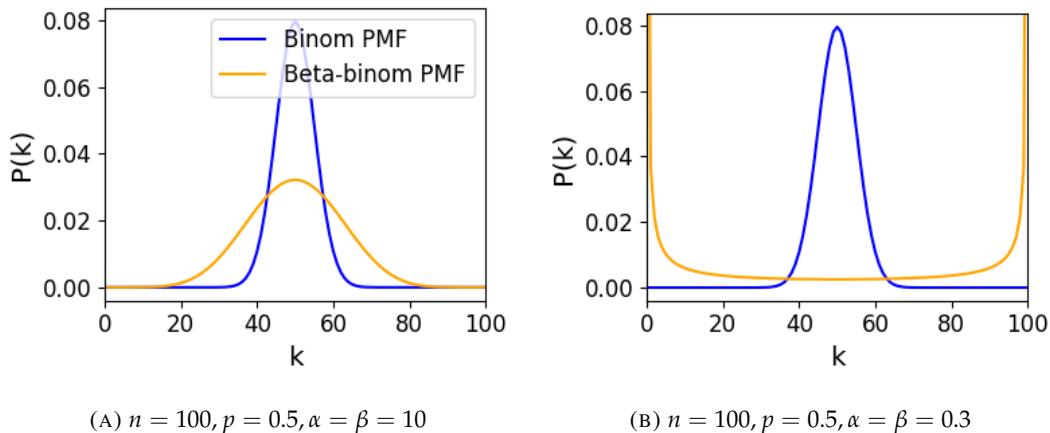


FIGURE 4.1: Figures showing the over-dispersion possible for a beta-binomial distribution relative to a binomial distribution. Parameters are shown in the captions.

1984 Conway-Maxwell-binomial distribution

1985 The Conway-Maxwell-binomial distribution (COMb distribution) is a three parameter generalisation of the binomial distribution that allows for over dispersion and under dispersion

relative to the binomial distribution. The parameters of the distribution are n the number of Bernoulli trials, and two shape parameters $0 \leq p \leq 1$, and $\nu \in \mathbb{R}$.

The probability mass function of the COMb distribution is

$$P(k; n, p, \nu) = \frac{1}{S(n, p, \nu)} \binom{n}{k}^{\nu} p^k (1-p)^{n-k} \quad (4.6)$$

where

$$S(n, p, \nu) = \sum_{j=0}^n \binom{n}{j}^{\nu} p^j (1-p)^{n-j} \quad (4.7)$$

The only difference between this PMF and the PMF for the standard binomial is the introduction of ν and the consequent introduction of the normalising function $S(n, p, \nu)$.

Indeed, if $\nu = 1$ the COMb distribution is identical to the binomial distribution with the same values for n and p . We can see in figure 4.2d that the KL-divergence $D_{KL}(P_{COMb}(n, p, \nu) || P_{Bin}(n, p)) = 0$ along the line where $\nu = 1$. The analytical expression for the divergence is

$$D_{KL}(P_{COMb}(k; n, p, \nu) || P_{Bin}(k; n, p)) = (\nu - 1) E_{P_{COMb}(k; n, p, \nu)} \left[\log \binom{n}{k} \right] \quad (4.8)$$

$$- \log S(n, p, \nu) \quad (4.9)$$

At $\nu = 1$, we have $S(n, p, 1)$ which is just the sum over the binomial PMF, so $S(n, p, 1) = 1$ and therefore $D_{KL}(P_{COMb}(n, p, \nu) || P_{Bin}(n, p)) = 0$.

If $\nu < 1$ the COMb distribution will exhibit over-dispersion relative to the binomial distribution. If $p = 0.5$ and $\nu = 0$ the COMb distribution is the discrete uniform distribution, and if $\nu < 0$ the mass of the COMb distribution will tend to build up near $k = 0$ and $k = n$. This over-dispersion represents positive association in the Bernoulli variables. An example of this over-dispersion can be seen in figure 4.2b.

If $\nu > 1$ the COMb distribution will exhibit under-dispersion relative to the binomial distribution. The larger the value of ν the more probability mass will build up at $n/2$ for even n , or at $\lfloor n/2 \rfloor$ and $\lceil n/2 \rceil$ for odd n . This under-dispersion represents negative association in the Bernoulli variables. An example of this under-dispersion can be seen in figure 4.2a.

It should be noted that the p parameter of the COMb distribution does not correspond to the mean of the distribution, as is the case for the binomial p parameter, and beta-binomial π parameter. That is, the COMb p parameter is not a location parameter. An illustration of this can be seen in figure 4.2c. This is because an interaction between the p and ν parameters skews the mean. There is no analytical expression for the mean of the COMb distribution.

ν	Relative dispersion	Association between neurons/variables
< 1	over	positive
1	none	none
> 1	under	negative

TABLE 4.2: Relative dispersion of the COMb distribution, and association between Bernoulli variables as represented by the value of the ν parameter.

2007 Since the COMb distribution has the potential to capture positive and negative associa-
 2008 tions between the neurons/Bernoulli variables, it should be an excellent candidate for mod-
 2009 elling the number of active neurons in a neuronal ensemble.

2010 We wrote a dedicated Python package to enable easy creation and fitting of COMb dis-
 2011 tribution objects. The format of the package imitates the format of other distribution objects
 2012 from the `scipy.stats` Python package. The COMb package can be found here:
 2013 https://github.com/thomasjdelaney/Conway_Maxwell_Binomial_Distribution

2014 4.3.6 Fitting

2015 We fitted binomial, beta-binomial, and Conway-Maxwell-binomial (COMb) distributions to
 2016 the neural activity in each of the overlapping windows covering each trial. To fit the distribu-
 2017 tions we minimised the appropriate negative log likelihood function using the data from the
 2018 window.

There is an analytical solution for maximum likelihood estimate of the binomial distribution's p parameter.

$$\hat{p} = \frac{1}{n} \sum_{i=1}^N k_i \quad (4.10)$$

2019 We minimised the negative log likelihood function of the beta-binomial distribution nu-
 2020 merically. We calculated the negative log likelihood for a sample directly, by taking the sum
 2021 of the log of the probability mass function for each value in the sample. We minimised the
 2022 negation of that function using the `minimise` function of the `scipy.optimize` Python
 2023 package.

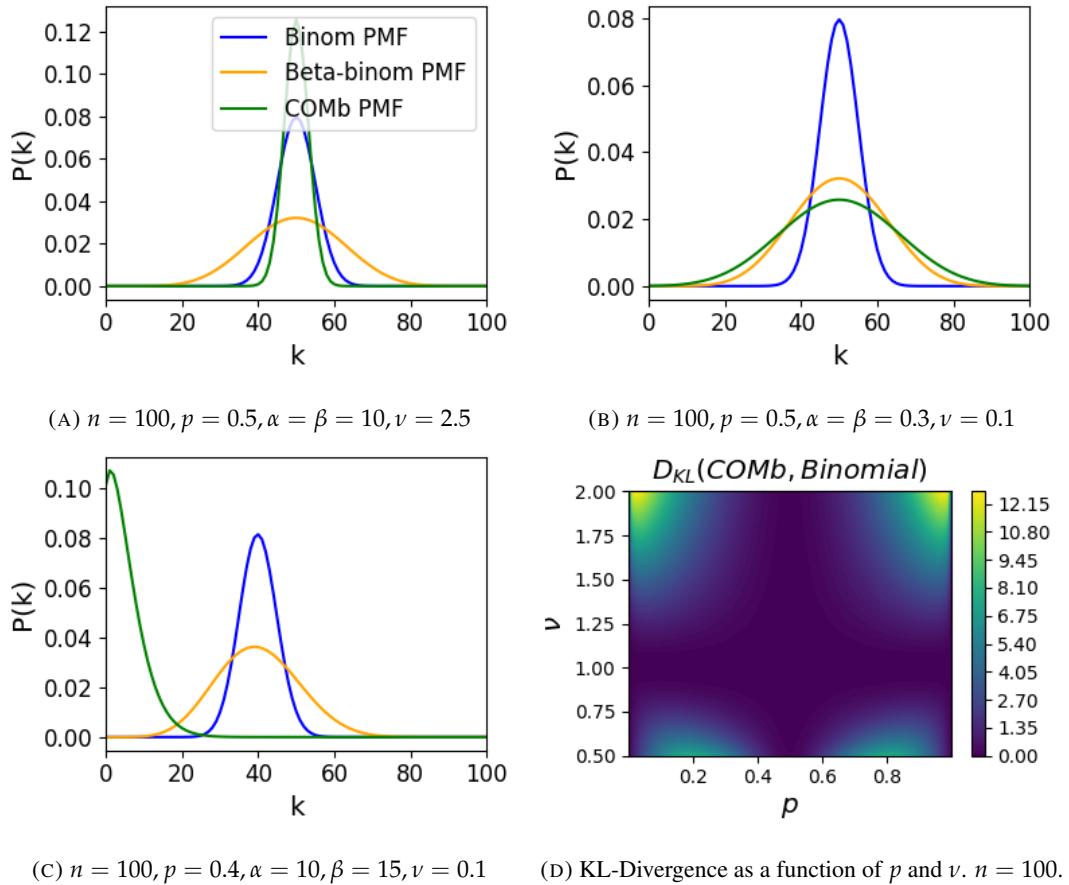


FIGURE 4.2: Figures showing (A) the under-dispersion and (B) over-dispersion permitted by the COMb distribution relative to a binomial distribution. (C) illustrates that the p parameter of the COMb distribution does not correspond to the mean of the distribution, as it does for the binomial and beta-binomial distributions. (D) shows a heatmap for the value of the Kullback-Liebler divergence between the COMb distribution and the standard binomial distribution with same value for n , as a function of p and ν . Parameters are shown in the captions.

The log likelihood function of the COMb distribution given some sample $\{k_1, \dots, k_N\}$ is

$$\ell(p, \nu | k_1, \dots, k_N) = N [n \log(1 - p) - \log S(n, p, \nu)] \quad (4.11)$$

$$+ \log \frac{p}{1 - p} \sum_{i=1}^N k_i \quad (4.12)$$

$$+ \nu \sum_{i=1}^N \log \binom{n}{k_i} \quad (4.13)$$

2024 We minimised the negation of this function using numerical methods. More specifically, we
2025 used the `minimise` function of the `scipy.optimize` Python package.

2026 **4.3.7 Goodness-of-fit**

2027 After fitting, we measured the goodness-of-fit of each model/distribution with their log like-
2028 lihood. We calculated this directly using the `logpmf` functions of the distribution objects in
2029 Python.

2030 **4.4 Results**

2031 We defined a neuron as *active* in a time bin if it fires at least one spike during the time interval
2032 covered by that bin. We measured the number of active neurons in the primary visual cortex
2033 of a mouse in 1ms bins across 160 trials of a moving bar visual stimulus. We then slid a
2034 100ms window across these 1ms bins taking measurements, and fitting distributions along
2035 the way. We did the same for neurons in the thalamus, hippocampus, striatum, and motor
2036 cortex. We repeated the analysis for 5ms time bins with 40 bin windows, and 10ms time bins
2037 with 40 bin windows.

2038 **4.4.1 Increases in mean number of active neurons and variance in number of
2039 active neurons at stimulus onset in some regions**

2040 We measured the average number of active neurons, and the variance of the number of active
2041 neurons in a 100ms sliding window starting 500ms before stimulus onset until 1000ms after
2042 stimulus onset. We found differences in the response across regions. There were no observed
2043 changes in response to the stimulus in the motor cortex or the striatum. The changes in the
2044 other regions are detailed below.

2045 **Primary visual cortex**

2046 We found a transient increase in both the average and variance of the number of active neu-
2047 rons at stimulus onset, followed by a fall to pre-stimulus levels, followed by another transient
2048 increase (see figure 4.3). The oscillation in both of these measurements appear to reflect the
2049 frequency of the stimulus (see Data section 4.2.1), and it is known that stimulus structure can
2050 influence response structure(Litwin-Kumar, Chacron, and Doiron, 2012). We see a similar
2051 but lower amplitude oscillation at the end of the stimulus presentation.

2052 **Hippocampus**

2053 In the hippocampus we observed a transient increase in the average number of active neurons
2054 and in the variance of the number of active neurons at stimulus onset (see figure 4.4). The
2055 increase lasted about 125ms, and the subsequent fall to baseline took the a similar amount of
2056 time.

2057 **Thalamus**

2058 In the thalamus we observed a transient increase in the both the average and variance of
2059 the number of active neurons on stimulus onset, followed by a fall to pre-stimulus levels,
2060 followed by a sustained increase until the stimulus presentation ends.

2061 As one you might expect for a visual stimulus, the change in the average number of active
2062 neurons was greatest in the primary visual cortex. In this region, this quantity doubled on
2063 stimulus onset. In contrast, in the hippocampus and the thalamus, the average number of
2064 active neurons only increased by a fraction of the unstimulated baseline value. The duration
2065 of the response in V1 and the hippocampus at stimulus onset was 300 – 400ms, but the
2066 response in the thalamus appeared to last for the duration of stimulus presentation. The V1
2067 also showed a change in the average number of active neurons at stimulus end. The change
2068 was similar to that observed at stimulus onset, but smaller in magnitude (see figures 4.3, 4.4,
2069 and 4.5)

2070 **4.4.2 Conway-Maxwell-binomial distribution is usually a better fit than bino-
2071 mial or beta-binomial**

2072 Since the Conway-Maxwell-binomial distribution has not been fitted to neuronal data before,
2073 it is not clear that it would be a better fit than the binomial or beta-binomial distributions.
2074 In order to find out which parametric distribution was the best fit for the largest proportion

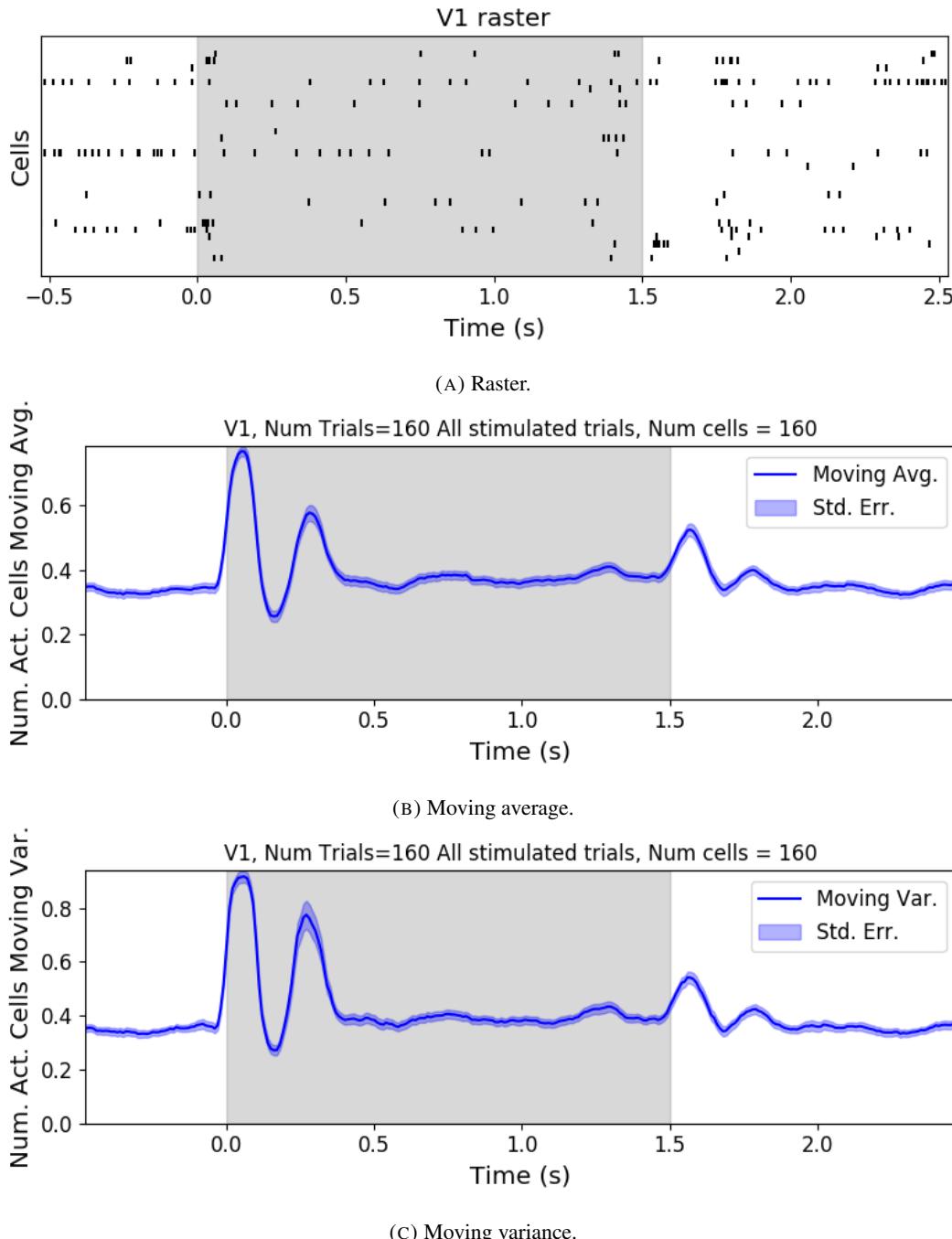


FIGURE 4.3: (A) Raster plot showing the spikes fired by 33 randomly chosen neurons in the primary visual cortex. (B-C) (B) average and (C) variance of the number of active neurons, measured using a sliding window 100ms wide, split into 100 bins. The midpoint of the time interval for each window is used as the timepoint (x-axis point) for the measurements using that window. The grey shaded area indicates the presence of a visual stimulus. The opaque line is an average across the 160 trials that included a visual stimulus of any kind. We can see a transient increase in the average number of active neurons and the variance of this number, followed by a fluctuation and another increase.

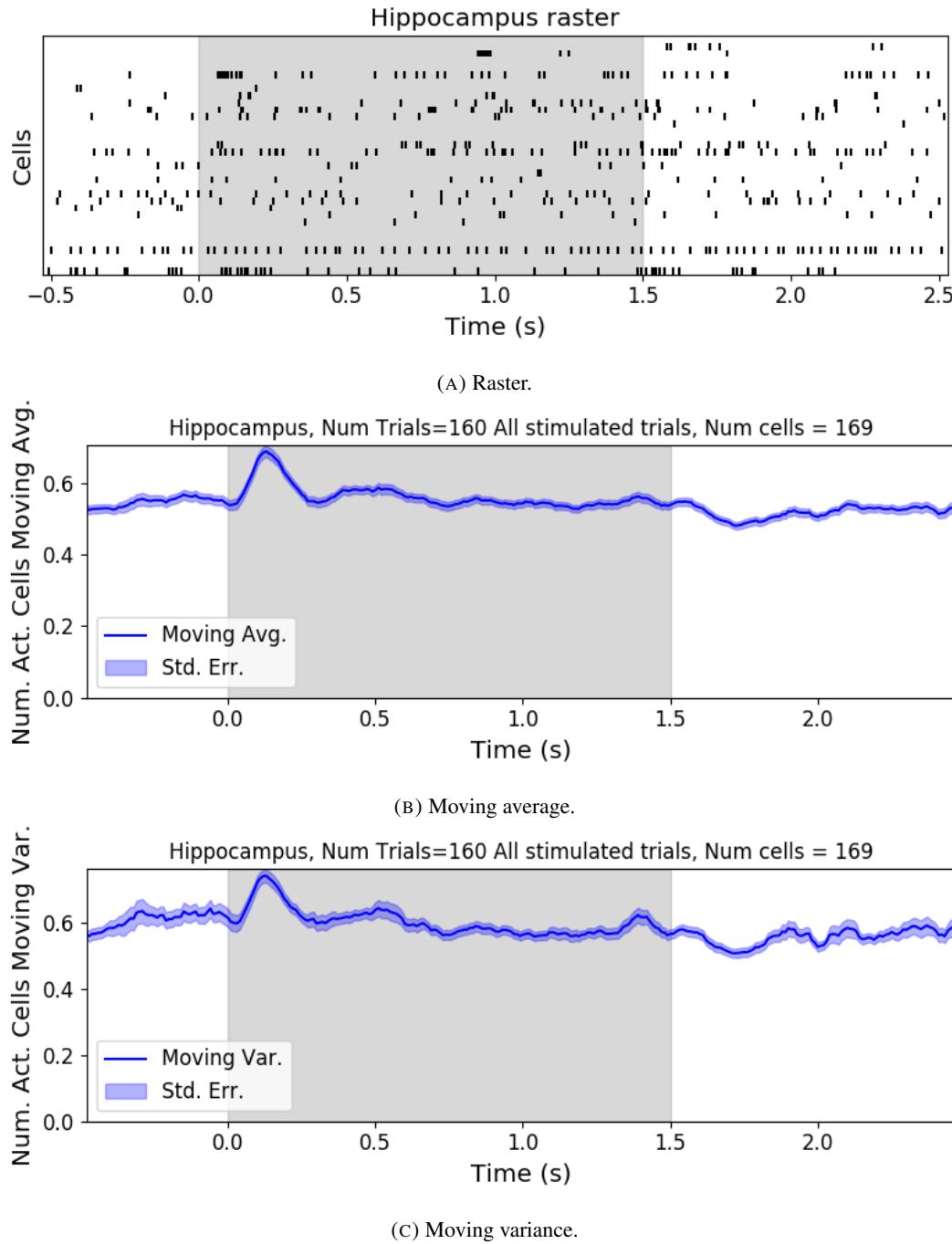


FIGURE 4.4: (A) Raster plot showing the spikes fired by 33 randomly chosen neurons in the hippocampus. (B-C) (B) average and (C) variance of the number of active neurons, measured using a sliding window 100ms wide, split into 100 bins. The midpoint of the time interval for each window is used as the timepoint (x-axis point) for the measurements using that window. The grey shaded area indicates the presence of a visual stimulus. The opaque line is an average across the 160 trials that included a visual stimulus of any kind. We can see a transient increase in the average number of active neurons and the variance of this number at stimulus onset.

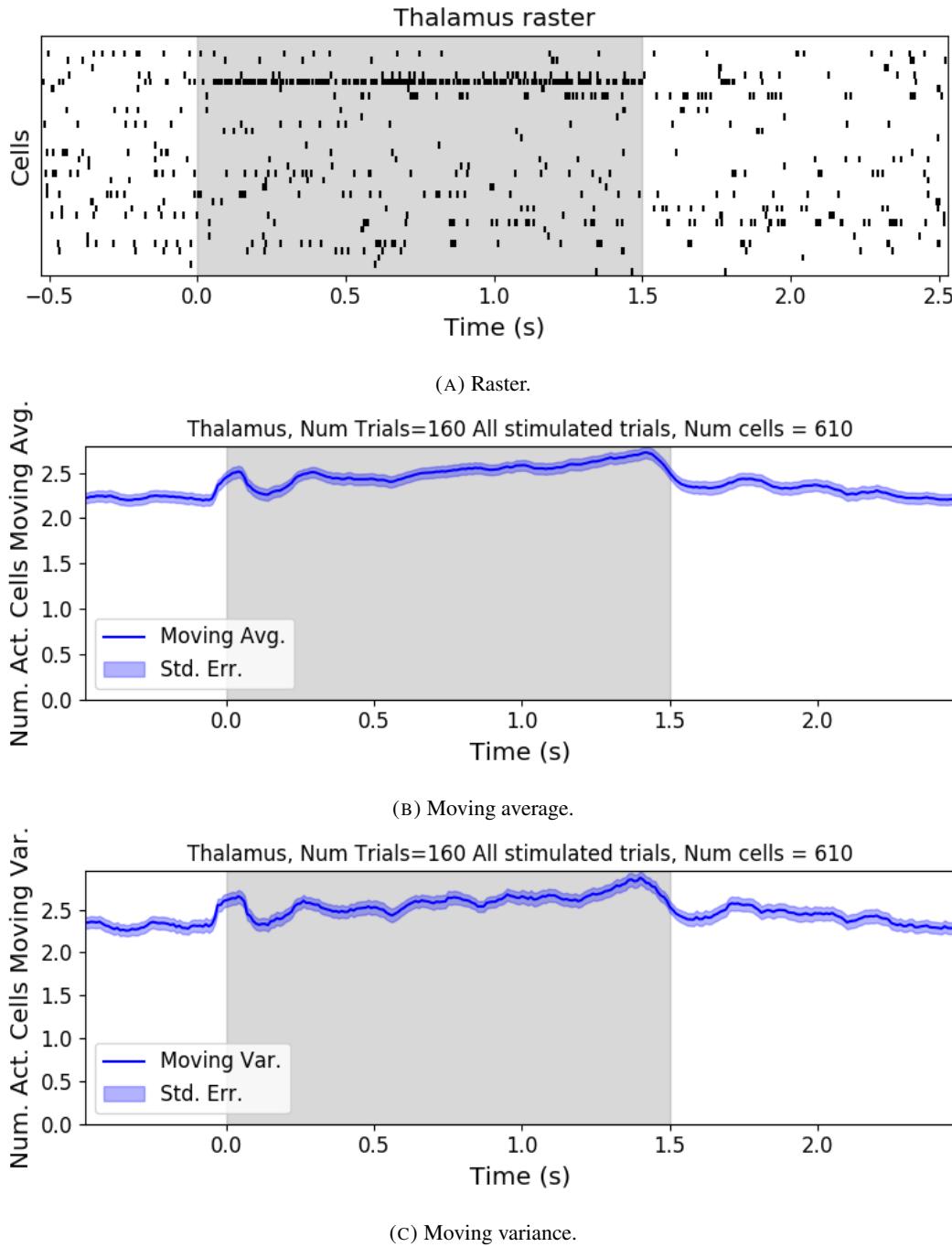
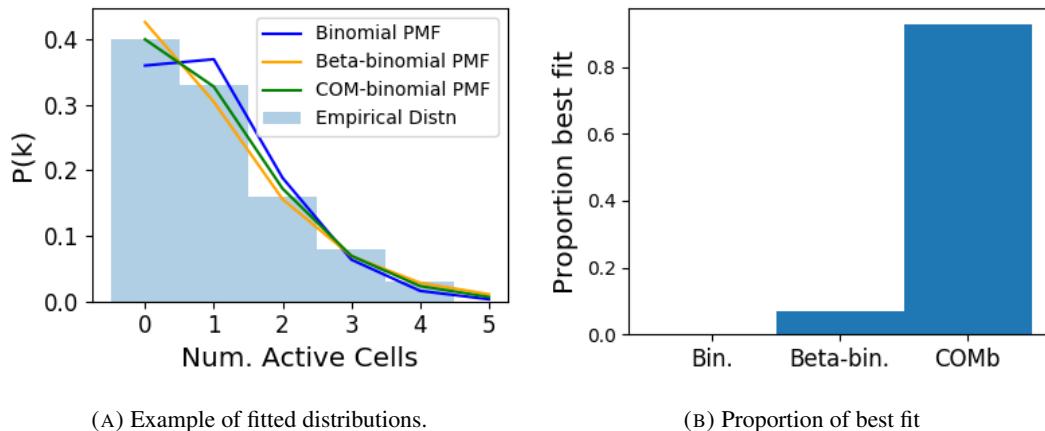


FIGURE 4.5: (A) Raster plot showing the spikes fired by 33 randomly chosen neurons in the thalamus. (B-C) (B) average and (C) variance of the number of active neurons, measured using a sliding window 100ms wide, split into 100 bins. The midpoint of the time interval for each window is used as the timepoint (x-axis point) for the measurements using that window. The grey shaded area indicates the presence of a visual stimulus. The opaque line is an average across the 160 trials that included a visual stimulus of any kind. We can see an immediate increase at stimulus onset, a subsequent fall, and another sustained increase until the stimulus presentation ends.

of our data, we fit a binomial, a beta-binomial, and a Conway-Maxwell-binomial (COMb) distribution to each window for each bin width, and each region. Then we assessed the goodness-of-fit of each distribution by calculating the log-likelihood of each fitted distribution using the associated sample. We measured the proportion of samples for which each distribution was the best fit, for each bin width value and each region.

We found that the COMb distribution was the best fit for most of the samples regardless of bin width or region. The bin width had an effect on the number of samples for which the COMb distribution was the best fit. The results are summarised in table 4.3. For a bin width of 1ms, the COMb distribution was the best fit for over 90% of samples, the beta-binomial distribution was the best fit for less than 10% of samples, and the binomial distribution was the best fit for less than 1% of samples, across regions. For 5ms bins, the COMb distribution was the best fit for 70 – 80% of samples, the beta-binomial distribution was the best fit for 20 – 30% of the samples, and again the binomial distribution was the best fit for less than 1% of samples, across regions. Finally, for 10ms bins, the COMb distribution was the best fit for 53 – 80% of samples, the beta-binomial distribution was the best fit for 20 – 47% of the samples, and the binomial distribution was the best fit for less than 0.1% of samples, across regions.



(A) Example of fitted distributions. (B) Proportion of best fit

FIGURE 4.6: (A) An example of the binomial, beta-binomial, and Conway-Maxwell-binomial distributions fitted to a sample of neural activity. The Conway-Maxwell-binomial distribution is the best fit in this case. The histogram shows the empirical distribution of the sample. The probability mass function of each distribution is indicated by a different coloured line. (B) Across all samples in all trials, the proportion of samples for which each fitted distribution was the best fit. The Conway-Maxwell-binomial distribution was the best fit for 93% of the samples taken from V1 using a bin width of 1ms.

Bin Width (ms)	Binomial	Beta-binomial	COMb
1ms	< 1%	< 10%	> 90%
5ms	< 0.1%	20 – 30%	70 – 80%
10ms	< 0.1%	20 – 47%	53 – 80%

TABLE 4.3: Proportion of samples for which each distribution was the best fit, grouped by bin width. The COMb distribution is the best fit most of the time.

2092 **4.4.3 Conway-Maxwell-binomial distribution captures changes in association
2093 at stimulus onset**

2094 We fit a Conway-Maxwell-binomial (COMb) distribution to the number of active neurons in
2095 the 1ms time bins in a 100ms sliding window. We also measured the correlation coefficient
2096 between the spike counts of all possible pairs of neurons, and took the average of these
2097 coefficients. We did this for all the trials with a visual stimulus. We observed a reduction in
2098 the COMb distribution's ν parameter at stimulus onset from around 1 to between 0 and 1 (see
2099 figure 4.7a). A value of ν less than 1 indicates positive association between the neurons (see
2100 section 4.3.5). We might expect to see this positive association reflected in the correlation
2101 coefficients, but this is not the case. We see no change in the time series of average correlation
2102 measures at stimulus onset.

2103 This may be due to the very short time bin we used in this case. We know that using small
2104 time bins can artificially reduce correlation measurements (Cohen and Kohn, 2011). In this
2105 case, fitting the COMb distribution may be a useful way to measure association in a neuronal
2106 ensemble over very short timescales (< 10ms).

2107 **4.4.4 Replicating stimulus related quenching of neural variability**

2108 Churchland et al. (2010) inspected the effect of a stimulus on neural variability. One of the
2109 measures of neural variability that they employed was the Fano factor of the spike counts of
2110 individual cells (see section 4.3.4). They found a reduction in neural variability as measured
2111 by the Fano factor in various cortical areas in a macaque at the onset of various visual stimuli,
2112 or a juice reward (**churchland**).

2113 We measured the Fano factor of the spike count of each cell in each brain region, during
2114 each trial. We measured the mean and standard error of these Fano factors from 500ms
2115 before stimulus onset until 1000ms after stimulus end. For the primary visual cortex, we
2116 found a transient reduction in the Fano factor immediately after stimulus onset. We used
2117 a Mann-Whitney U test to check that the Fano factors measured in a window starting at

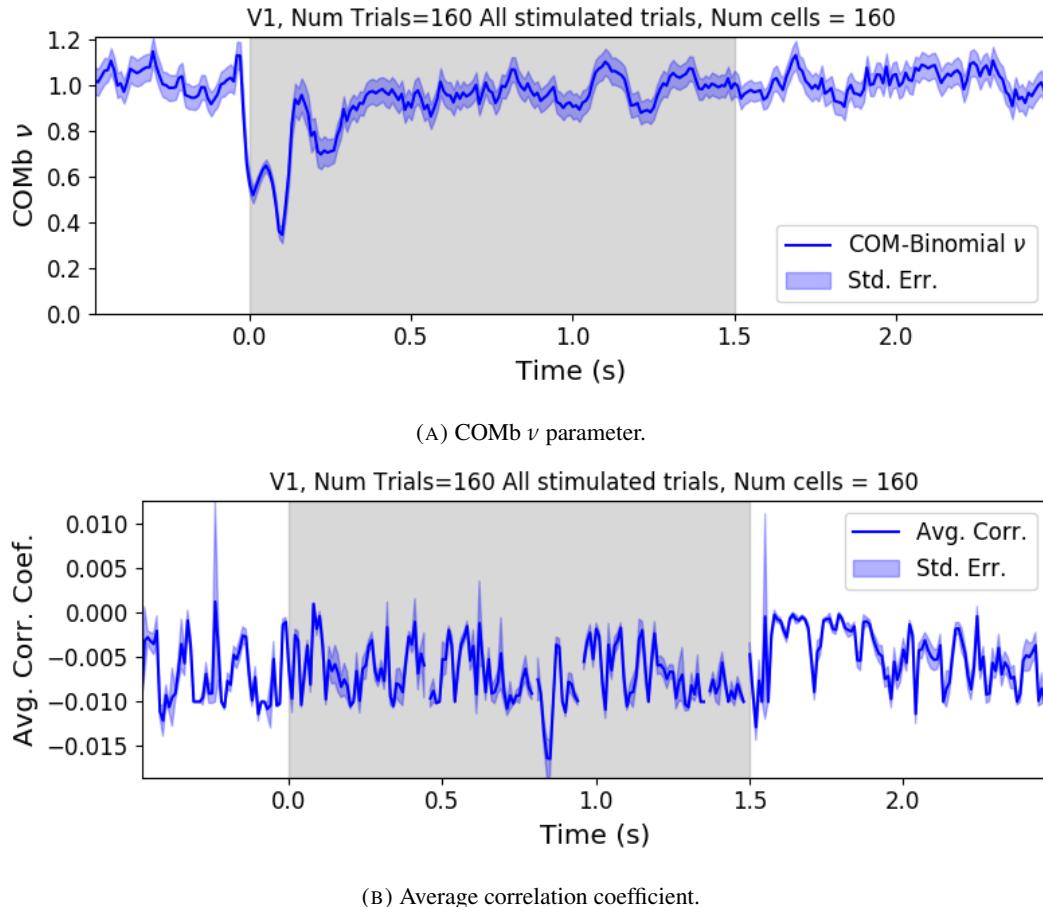
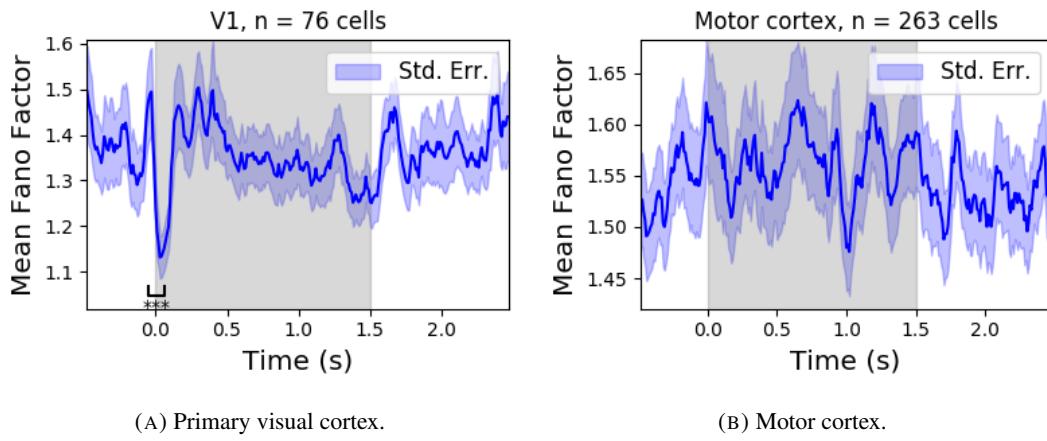


FIGURE 4.7: (A) We fit a Conway-Maxwell-binomial distribution to the number of active neurons in 1ms time bins of a 100ms sliding window. We did this for all trials with a visual stimulus and took the average across those trials. We see a transient drop in value for the distribution's ν parameter at stimulus onset. This shows an increase in association between the neurons. (B) We measured the correlation coefficient between the spike counts of all possible pairs of neurons in the same sliding window. We took the average of those coefficients. We also did this for every visually stimulated trial, and took the average across trials. The increase in association is not reflected with an increase in average correlation.

stimulus onset and ending 100ms later were significantly lower than the factors measured in a window ending at stimulus onset ($p < 0.001$, see figure 4.8a). We did not get this statistically significant result in any other region.

Our findings agree with those of Churchland et al. for the primary visual cortex. However Churchland also found a reduction in the Fano factor in the dorsal premotor cortex (PMd) at stimulus onset. Our measurements from the mouse motor cortex show no change at stimulus onset (see figure 4.8b). This could indicate some difference in the functionality of the motor cortex in a macaque and the motor cortex of a mouse.

Similar to these findings in the Fano factor, we found a reduction in the ν parameter of the COMB distribution on stimulus onset in V1 (figure 4.7a) and in no other region from



(A) Primary visual cortex.

(B) Motor cortex.

FIGURE 4.8: (A) The mean Fano factor of the spike counts of the cells in the primary visual cortex. Means were taken across cells first, then across trials. There was a significant decrease in the Fano factors immediately after stimulus onset. (B) The mean Fano factor of the spike counts of the cells in the motor cortex. No significant change in measurements at any point.

which we had data. Specifically, the ν parameter reduced from around 1, to between 1 and 0. This represents a change from no association between the neurons, to a positive association. It is possible that this positive association may be responsible for the reduction in the Fano factor.

4.5 Discussion

Our aim in this research was to develop a new statistical method for analysing the activity of a neuronal ensemble at very short timescales. We wanted our method to use information taken from the whole ensemble, but we also wanted the method to be quick and easy to implement. It is likely that analysis methods with these characteristics will become valuable as electrophysiological datasets include readings from more cells over longer time periods. In this case, we used the number of active, or spiking, neurons in a very short time bin ($< 10\text{ms}$) as a measure of ensemble activity.

First of all, we showed that there were changes in response that we could model at these very short time scales in some of the brain regions from which we had recordings. We observed changes in the average number of active neurons, and the variance of the number of active neurons in three different brain regions in response to visual stimuli. Since we know that correlated behaviour is associated with sensory perception (deCharms and Merzenich, 1996), we might hope to measure the pairwise correlations within the neuronal population in order to further investigate these responses. But, using such short time bins can produce

artificially small spike count correlation measurements (Cohen and Maunsell, 2009). Overcoming this limitation was one of our objectives for our new method. In order to do this, we abandoned the idea of measuring the correlations directly and embraced the concept of *association*. In order to quantify the association between neurons, we used the Conway-Maxwell-binomial distribution to model the number of active (spiking) neurons in an ensemble as a sum of possibly associated Bernoulli random variables.

We showed that the Conway-Maxwell-binomial distribution performed better than the more common options of the binomial and beta-binomial distributions. Furthermore, we showed that the positively associated behaviour between neurons in the primary visual cortex could be captured by fitting a Conway-Maxwell-binomial distribution, but was not captured by the more standard approach of measuring the spike count correlation. The associated behaviour could not be measured using spike count correlations, because of the very short bins required to capture short timescale behaviour.

We replicated a famous result from Churchland et al (2010) relating to the quenching of neural variability in cortical areas at stimulus onset, and in doing so, we established a correspondence between the association quantifying parameter of the Conway-Maxwell-binomial (COMb) distribution and the neural variability as measured by the Fano factor. We found a reduction in the ν parameter of the COMB distribution at stimulus onset, indicating a change from no association to positive association between neurons in V1. We found a corresponding reduction in the Fano factor of the individual cells in V1. The positive association between neurons induced by the stimulus would constrain the neurons to fire at the same time. The stimulus also induced a larger number of neurons to spike. These two actions combined could cause an increase in the firing rate of individual cells that is greater in magnitude than the increase in firing rate variability. If this is indeed the case, then the association as captured by the COMB distribution could be regarded as one of the ‘natural parameters’ of the ensemble response for short timescales. That is, a quantity that directly measures some aspect of the behaviour of the ensemble. In this case, it the correlated behaviour of the individual neurons is captured.

This work could be just a first step in creating analysis methods based on the Conway-Maxwell-binomial distribution, or similar statistical models. One way to extend the method would be to pair it up with the ‘Population Tracking model’ (O’Donnell et al., 2017). This model attempts to characterise the interaction between an ensemble and each member of the ensemble by quantifying the probability of spiking for a given a cell, given the number of active cells in the whole population. Combining this model with the COMB distribution

2181 would give us a model that could accurately fit the number of active neurons at any moment,
2182 and that gives a probability of firing for each cell, and therefore probabilities for full spiking
2183 patterns, without adding a huge number of parameters to fit.

2184 A more complex way to extend the model would be to fit a Conway-Maxwell-binomial
2185 distribution to data recorded from multiple brain regions simultaneously, with a different fit
2186 for each region, then to analyse the temporal relationship between the fitted parameters of
2187 each region. If we analysed the time series of the COMB distribution parameters from the
2188 different regions, looking at cross-correlations between regions, this may give some results
2189 relating to the timescales in which information is processed in different brain regions.

2190 **Chapter 5**

2191 **Studies with practical limitations &**
2192 **negative results**

2193 *Abstract*

2194 Here I will present some details on research topics that I started, but that unfortunately did not
2195 lead anywhere useful. There are two pieces of research, based on two papers. Each paper is
2196 related to the overall theme of my PhD of analysing and modelling behaviours of populations
2197 of neurons. The first part is based on a model of parallel spike trains including higher order
2198 interactions by Shimazaki et al (2012). The second part is based on a multiscale model for
2199 making inferences on hierarchical data.

2200 **5.1 Dynamic state space model of pairwise and higher order neu-**
2201 **ronal correlations**

2202 In their paper Shimazaki et al (2012) aimed to model spike trains from populations of neurons
2203 in parallel, with pairwise and higher order dynamic interactions between the trains. They
2204 modelled the spike trains as multi-variate binary processes using a log-linear model, and they
2205 extracted spike interaction parameters using a Bayesian filter/EM-algorithm. They developed
2206 a goodness-of-fit measure for the model to test if including these higher order correlations
2207 is necessary for an accurate model. Their measure was based on the Bayes factor but they
2208 also assessed the suitability of higher order models using the AIC and BIC. So the increase
2209 in the number of parameters associated with fitting higher order interactions was taken into
2210 account. They tested the performance of the model on synthetic data with known higher
2211 order correlations. They used the model to look for higher order correlations in data from
2212 awake behaving animals. They use the model to demonstrate dynamic appearance of higher
2213 order correlations in the monkey motor cortex (Shimazaki et al., 2012).

2214 We used the available Python repository to implement the model, and we successfully
2215 worked through the tutorial provided. But we found that the model did not scale well to
2216 larger populations. We attempted to fit the model to a population of 10 neurons and found we
2217 didn't manage to finish the process. Since, our goal was to find a model to scale to hundreds
2218 or thousands of neurons, we decided that this model was no longer worth pursuing.

2219 **5.2 A multiscale model for hierarchical data applied to**
2220 **neuronal data**

2221 In their paper Kolaczyk et al (2001) developed a framework for a modelling hierarchically
2222 aggregated data, and making inferences based on a model arising from this framework. They
2223 assumed that a hierarchical aggregation existed on the data in question, where each element at
2224 each level of the hierarchy had some associated measurements, an associated mean process,
2225 which was the expected value of these measurements. They also assumed that the measure-
2226 ments of each parent were equal to the sum of the measurements from all of its children.
2227 They showed that these assumptions gave rise to a relationship between parent and child
2228 measurements across all levels of the hierarchy, where the product of the likelihood of the
2229 parameters of the lowest level of the hierarchy can be expressed as products of conditional
2230 likelihoods of the elements of higher levels of the hierarchy (Kolaczyk and Huang, 2010).

2231 They gave examples of these expressions for measurements sampled from Gaussian dis-
2232 tributions, and Poisson distributions, and showed the definitions of the hierarchical param-
2233 eters which reparametrise the distribution of these data taking the hierarchy into account.
2234 They go on to suggest prior distributions for this multiscale model, and integrate these priors
2235 to give posterior distributions for the measurements from each element at each level in the hi-
2236 erarchy, and expressions for the MAP estimated parameters of each the associated processes
2237 (Kolaczyk and Huang, 2010).

2238 We implemented their model in Python by creating some synthetic data from Poisson
2239 distributions, and defining a hierarchy by agglomerating these data. We calculated the MAP
2240 estimates using our knowledge of the hierarchy, and using the expressions given in the paper.
2241 We found that the MAP estimates were far less accurate than would be achieved by simply
2242 ignoring the hierarchy during estimation, and using a maximum likelihood approach. After
2243 that, we decided to move on.

2244 **Chapter 6**

2245 **Discussion**

2246 Ideas (not in order):

2247 • We built the model, it's biophysical, Judging by Greenberg biophysical modelling is
2248 the way to go.

2249 • how we fitted (amplitude and power spectrum), resulted in similar spike inference perf

2250 • we investigated the effects of buffers, indicator properties

2251 • investigated the modelled fluorescence trace of various firing rates including high ones

2252 • our fluorescence model could be useful in a number of situations.

2253 • voltage imaging

2254 • Applied new network science to new electrophysiological dataset.

2255 • applied clustering comparison measurements to assess similarity to anatomy

2256 • time bin width has an effect on correlation size, and correlation distribution

2257 • further differences in means, different when we take regions into account

2258 • used new method to find dimensions of additional structure

2259 • we detected communities, they resemble anatomical division at short timescales

2260 • conditioned on video (mention and explain)

2261 • different types of correlations

2262 • Results might be intuitive, but are new nonetheless (as far as I know)

2263 • Potential for more network science applications?

- 2264 ● Applied the COMB distribution to neuronal data for the first time.
- 2265 ● investigated changes in response to stimulus, different for different regions
- 2266 ● best fit
- 2267 ● captures changes better than correlations, offers a good alternative to correlation for
- 2268 quantifying association
- 2269 ● replicated stimulus related quenching
- 2270 ● captures correlated behaviour by quantifying *association*.
- 2271 ● coupling with existing models could yield some nice models.
- 2272 ● More statistical invention could be useful. Conway-Maxwell-Poisson distribution

2273 In this project, we attempted to address some of the challenges in data collection from
2274 large neuronal ensembles (specifically with calcium imaging) and some of the problems in
2275 analysing the data collected from large neuronal ensembles.

2276 Firstly, we investigated the relationship between cell biochemistry, action potentials and
2277 the fluorescence traces produced by fluorescent calcium indicators. We did this by building
2278 a biophysical model that takes in a spike train and returns the fluorescence trace that that
2279 spike would induce. The model included mechanics for the binding of calcium to fluorescent
2280 and endogenous mobile and immobile buffers, and the consequent changes in concentration
2281 of free and bounded calcium. The model consisted of 17 parameters, 13 of which were
2282 set according to data from the literature, and 4 of which were free parameters. We trained
2283 the model using simultaneously collected spiking and calcium imaging data (Berens et al.,
2284 2018). We fitted the model by matching the $\Delta F/F_0$ in response to an action potential, and
2285 by matching the power spectrum of the actual fluorescence trace. This meant that our model
2286 would include the correct amount of noise as well as return the correct change in amplitude
2287 in response to an action potential.

2288 Since our model produced fluorescence traces, we could apply spike inference algorithms
2289 to the modelled fluorescence traces that our model produced after training, and compare the
2290 performance of the algorithms on the modelled traces to their performance on the real traces.
2291 We used three spike inference algorithms, two of which were based on modelling the cal-
2292 cium trace as an autoregression (Friedrich and Paninski, 2016; Pnevmatikakis et al., 2016),
2293 and another inference algorithm that was a little more biologically inspired, but amounted to

2294 a very similar algorithm (Deneux et al., 2016). We compared the performance of the algo-
2295 rithms by using them to infer spikes from 20 real and modelled fluorescence traces induced
2296 by 20 corresponding real spike trains. We then used several binary classification measures
2297 (true positive rate, accuracy etc.) to asses the quality of the spike inference for the real and
2298 modelled fluorescence traces. We found that ...

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