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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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Author:

Thomas J. DELANEY

Supervisors:

Dr. Cian O'DONNELL

Dr. Michael C. ASHBY

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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

12

July 6, 2020

¹⁵ Declaration of Authorship

¹⁶ I, Thomas J. DELANEY, declare that this thesis titled, "Investigating, implementing, and
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

42

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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For/Dedicated to/To my...

398 Introduction

399 Ideas (not in order):

- 400 • From small to big datasets (in terms of number of neurons)
- 401 • Big datasets mean statistical methods are more necessary (curse of dimensionality)
- 402 • Big datasets mean higher order correlations are more meaningful (schneidman)
- 403 • Exploit pairwise correlations in different way (eight probe)
- 404 • abandon correlations embrace association (COMB)
- 405 • electrophysiology drawbacks vs calcium benefits
- 406 • calcium drawbacks (fluorescence modelling) (mention nuclear filling and cell pathology)
- 407

408 Since Hodgkin and Huxley's squid experiments featuring a single axon (Hodgkin and
409 Huxley, 1939), to more recent research with spike sorted data from ~ 24000 neurons from
410 34 brain regions from 21 mice (Allen et al., 2019), the number of neurons contributing to
411 electrophysiological datasets has been growing. Recording methods using two-photon cal-
412 cium imaging have also been used to extract data from populations containing over 10000
413 neurons (Peron et al., 2015). This dramatic growth in the number of neurons to analyse
414 required a dramatic change in analysis methods.

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

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

428 weeks for longitudinal studies (Chen et al., 2013). Because the fluorescent indicator is deliv-
429 ered to the cell by adeno-associated viruses, there can be problems with indicator gradients
430 around the infection site, and expression levels will change in individual cells over weeks
431 (Tian et al., 2009; Chen et al., 2013), but these problems can be solved by using lines of
432 transgenic mice (Dana et al., 2014).

433 If the imaging data is collected at a high enough frequency, and the signal-to-noise ratio
434 of the fluorescence trace is high enough, it should be possible to infer the spike times to some
435 level of accuracy. For example, the calmodulin based indicator GCaMP6s has a sufficiently
436 high signal-to-noise ratio that isolated action potentials can be detected and inferred (Chen
437 et al., 2013). Many spike inference algorithms exist (Vogelstein et al., 2010; Pnevmatikakis
438 et al., 2016; Friedrich and Paninski, 2016; Pnevmatikakis et al., 2013; Pnevmatikakis et al.,
439 2014; Deneux et al., 2016; Greenberg et al., 2018), but the relationship between spiking and
440 fluorescence change is not fully understood

⁴⁴¹ **Chapter 1**

⁴⁴² **Sensitivity of the**
⁴⁴³ **spikes-to-fluorescence transform to**
⁴⁴⁴ **calcium indicator and neuron**
⁴⁴⁵ **properties**

446

Abstract

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

460

1.1 Introduction

461 Although fluorescent calcium indicators such as GCaMP are widely used to monitor neuronal
462 activity, the relationship between the fluorescence signal and the underlying action potential
463 firing is imperfect. The fluorescence signal has a low signal-to-noise ratio, and most indi-
464 cators' kinetics are slow relative to the millisecond-timescale dynamics of the membrane
465 voltage (example in Figure 1). This makes spike inference difficult. Furthermore, the effects
466 of the indicator and cell properties on the fluorescence signal are unknown. For example,
467 genetically encoded indicators can accumulate within neurons over weeks and months (Chen
468 et al., 2013). Studies using calcium-sensitive fluorescent dyes have shown that indicator con-
469 centration has substantial effects on the spike-to-fluorescence relationship (Maravall et al.,
470 2000). Therefore spike rates inferred from GCaMP fluorescence signals may give mislead-
471 ing results if comparing across imaging sessions. More generally, the poor understanding of
472 the spike-to-fluorescence transform means experimenters may not know whether to trust the
473 outputs of spike train inference methods in any given application.

474 Spike trains are usually inferred from the time series of intensity values of one pixel of the
475 fluorescence image, where the pixel is located at the cell's soma. The problems of identifying
476 these pixels, and inferring spikes from their time series can solved separately or together.
477 When attempting to infer spikes, the fluorescence trace is modelled as a linear combination of
478 calcium concentration dynamics, a baseline calcium concentration, and some Gaussian noise.
479 The calcium concentration dynamics are modelled as an autoregressive process of degree 1
480 or 2 with a pulse input corresponding to the spike train, or the number of spikes fired in a
481 time step. The model includes no dynamics for the fluorescent indicator itself. Furthermore,
482 in order to make this model into an easily solvable linear programming problem the number
483 of spikes fired in a timestep is not restricted to non-negative integers but to arbitrary non-
484 negative values (Vogelstein et al., 2010; Pnevmatikakis et al., 2016; Friedrich and Paninski,
485 2016; Pnevmatikakis et al., 2013; Pnevmatikakis et al., 2014). More biologically inspired
486 spike inference models do exist (Deneux et al., 2016), but their fundamentals are very similar.
487 In this work, we investigated the effect of changing dynamics and buffer concentrations on
488 the accuracy of the inference algorithms based on these models.

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

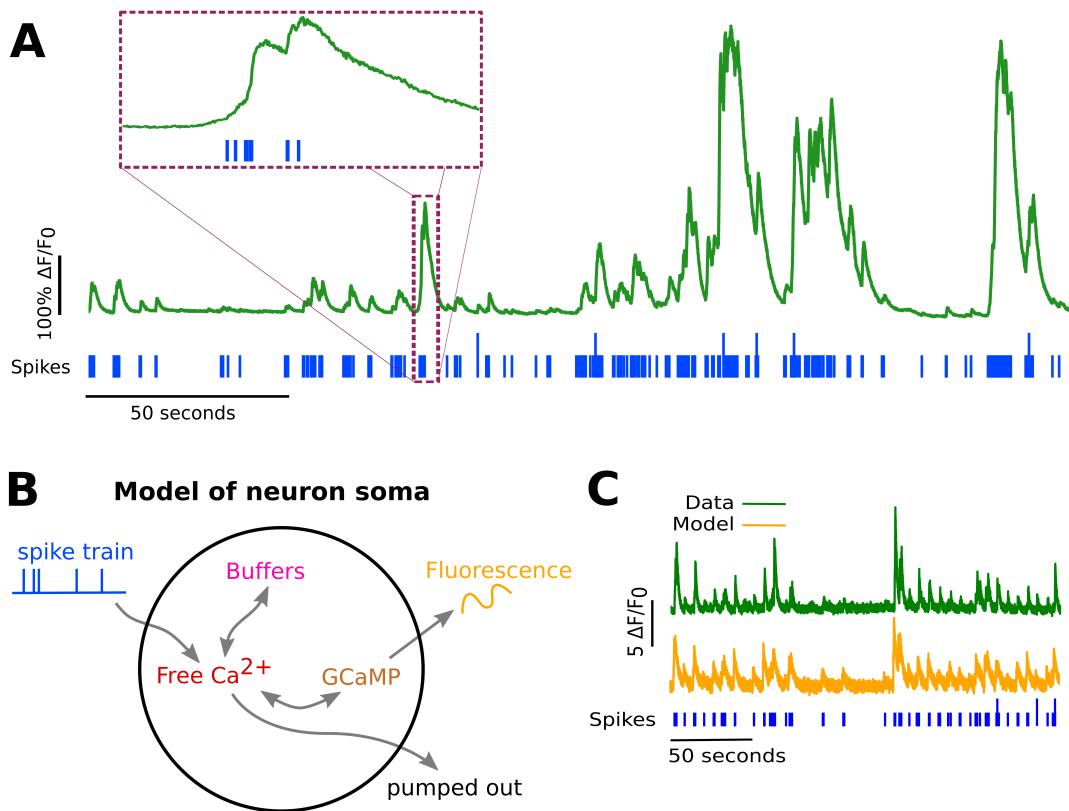


FIGURE 1.1:

- 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).

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

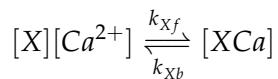
494 The model we developed consisted of free calcium, fluorescent indicator molecules, and
495 mobile and immobile endogenous calcium buffers. The indicator molecules which were
496 bound to a calcium molecule could be either excited, i.e. able to release a photon, or relaxed.
497 In order to reproduce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

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

504 **1.2 Results**

505 **1.2.1 A biophysical computational model can generate accurate fluorescence
506 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



507 where X is the buffer concentration and Ca^{2+} is the calcium concentration. Each species
508 could therefore exist in two states: either bound with calcium or unbound. To model the
509 imaging process, we also added a third, excited state to the indicator. When in the calcium-
510 bound state, the indicator could be converted to an excited state, corresponding to the absorp-
511 tion of a photon. The rate of this excitation process could be interpreted as the intensity of
512 the light illuminating the sample. Once excited, the species decayed back to the unexcited
513 state at a fixed rate, corresponding to the spontaneous emission of photons. The total emitted
514 fluorescence signal was interpreted as proportional to this de-excitation flux. To represent
515 experimental noise in the photon capture process, we drew a random number of captured

516 photons at each time step from a binomial distribution, parameterised by a number p that
517 corresponds to the mean fraction of released photons that are captured.

518 The model had 17 parameters in total describing the molecules' concentrations and re-
519 action rates (Methods). We set 13 of these parameters to values from the literature. The
520 remaining 4 parameter values we fit to publicly-available data (Berens et al., 2018), briefly
521 explained as follows (see Methods for full details). Single neurons from acute rat cortical
522 slices expressing GCaMP6f were imaged with two-photon microscopy while the membrane
523 potentials of the somata of the same neurons were simultaneously recorded via whole-cell
524 patch clamp electrophysiology. In this dataset, the electrical recordings give unambiguous
525 information about neurons' spike times. To do the parameter fitting, we feed these spike
526 trains as inputs to the computational model. After running, the model returns a simulated
527 fluorescence trace. We aimed to find the model parameter values that give the best match
528 between this simulated fluorescence trace and the real fluorescence time series recorded in
529 the corresponding neuron. To do this we used a suite of optimisation procedures to jointly
530 fit both the real neuron's fluorescence time series and power spectrum, which capture com-
531 plementary information about the spikes-to-fluorescence mapping (Methods). We performed
532 the fitting procedure independently for each of the 20 neurons in the spikefinder dataset
533 (<http://spikefinder.org>). After fitting, the model produced realistic-looking fluorescence time
534 series (Figure 1.1).

535 **1.2.2 Spike inference algorithms perform similarly on real data compared with**
536 **time series simulated from the model**

537 Researchers often pass the fluorescence time series through a spike inference tool before per-
538 forming further statistical analyses. These spike inference algorithms take the fluorescence
539 trace as input and attempt to estimate the neuronal spike train that triggered them (Vogelstein
540 et al., 2010; Pnevmatikakis et al., 2016; Friedrich and Paninski, 2016; Pnevmatikakis et al.,
541 2013; Pnevmatikakis et al., 2014; Deneux et al., 2016). Part of our motivation for building
542 this model was to allow us to ask the question: how do the properties of the cell and the
543 calcium indicator affect the quality of spike inference? In order to trust the conclusions from
544 our model, we should first be confident that spike inference from our simulated fluorescence
545 traces is similar to that from the real data. To test this we passed each of the simulated fluores-
546 cence traces through three previously published spike inference algorithms, quantified their
547 performance against the ground-truth electrophysiology data, repeated the procedure for the
548 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 true spikes, the OASIS algorithm (Friedrich and Paninski, 2016) correctly detected approximately 35% of the true spikes, and the ML spike algorithm (Deneux et al., 2016) correctly detected approximately 15% of the true spikes (see figure 1.2). Notably, for two of the three inference algorithms, the quality of inference was also fairly consistent for individual spike trains, not just the group means ($p > 0.05$, paired t-test). This demonstrates that the models were generating fluorescence time series that were similarly difficult to decode as the real 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.

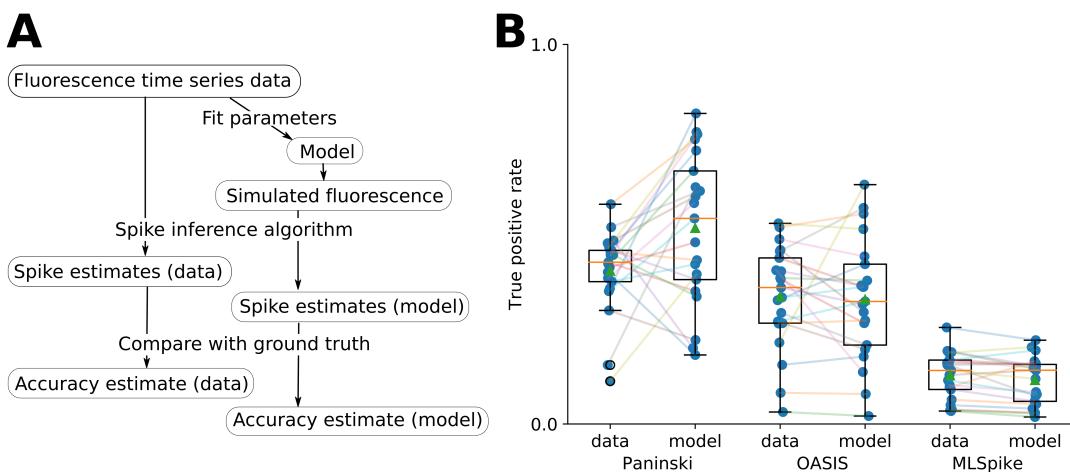


FIGURE 1.2:
A: Workflow to compare spike inference for real versus simulated fluorescence data.
B: True positive rates achieved by three different spike inference algorithms when applied to observed spike trains, and simulated spike trains. Data points overlaid as blue circles. The performance is similar from real and simulated data for each of the algorithms.

560

561 1.2.3 Relative effects of various buffers to the fluorescence signal

562 One of the benefits of computational models over laboratory experiments is that we can
563 observe all the variables in the simulation to gain insight into the system's dynamics, which
564 can be difficult to do in the lab. We plotted the concentrations of the various species over
565 time for a version of the model fit to one data set, in response to the same train of spikes used
566 for fitting (figure 1.3). Figure 1.3a shows the absolute values of the species concentrations,

567 summed. Consistent with experimental estimates (Maravall et al., 2000), only a small fraction
568 ($\sim 0.1\%$) of calcium is free and unbound to any buffer. Of the bound calcium, the vast
569 majority, ($\sim 96\%$) is bound to the GCaMP indicator. The two types of endogenous buffer
570 are bound to the remaining calcium ($\sim 4\%$). An influx of calcium from a single spike adds
571 very little to the total calcium, in relative terms (red line in Figure 3a).

572 When calcium entered the model neuron it was rapidly buffered (Bartol et al., 2015).
573 However the relative fractions of which buffer molecules bound to the influxed calcium was
574 dynamic, and changed over time . Figure 1.3 (b-f) shows the time course of the various
575 species over time in response to a calcium influx event from a single action potential. Cru-
576 cially, the indicator $[BCa]$ competed with the endogenous buffers $[ImCa]$ and $[ECa]$ – all
577 three bind calcium on similar timescales. This implies that the timecourse and amplitude of
578 the $[BCa]$ variable will also depend on the binding rates and availabilities of the endogenous
579 buffers. For example if we decreased the concentration of an endogenous buffer, we might
580 expect both a faster rise time and greater peak amplitude of the $[BCa]$ signal in response to
581 a calcium influx event. The slowest component of the decay had a similar time constant for
582 $[BCa]$, $[ImCa]$ and $[ECa]$, which in turn matched the $[Ca]$ extrusion time constant in our
583 model ($\sim 6.29 \times 10^{-22} \text{Ms}^{-1}$). This implies that the buffers and the indicator had reached
584 a dynamic equilibrium and were jointly tracking the free calcium concentration as calcium
585 was slowly extruded from the cell.

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

594 **1.2.4 Spike inference accuracy is sensitive to indicator properties, and likely 595 varies within and between cells**

596 The above results imply that the fluorescence signal depends on the relative properties of
597 both GCaMP and the endogenous buffers. We next used the model to directly ask how
598 sensitive spike inference was to these components. We focused on three key parameters that

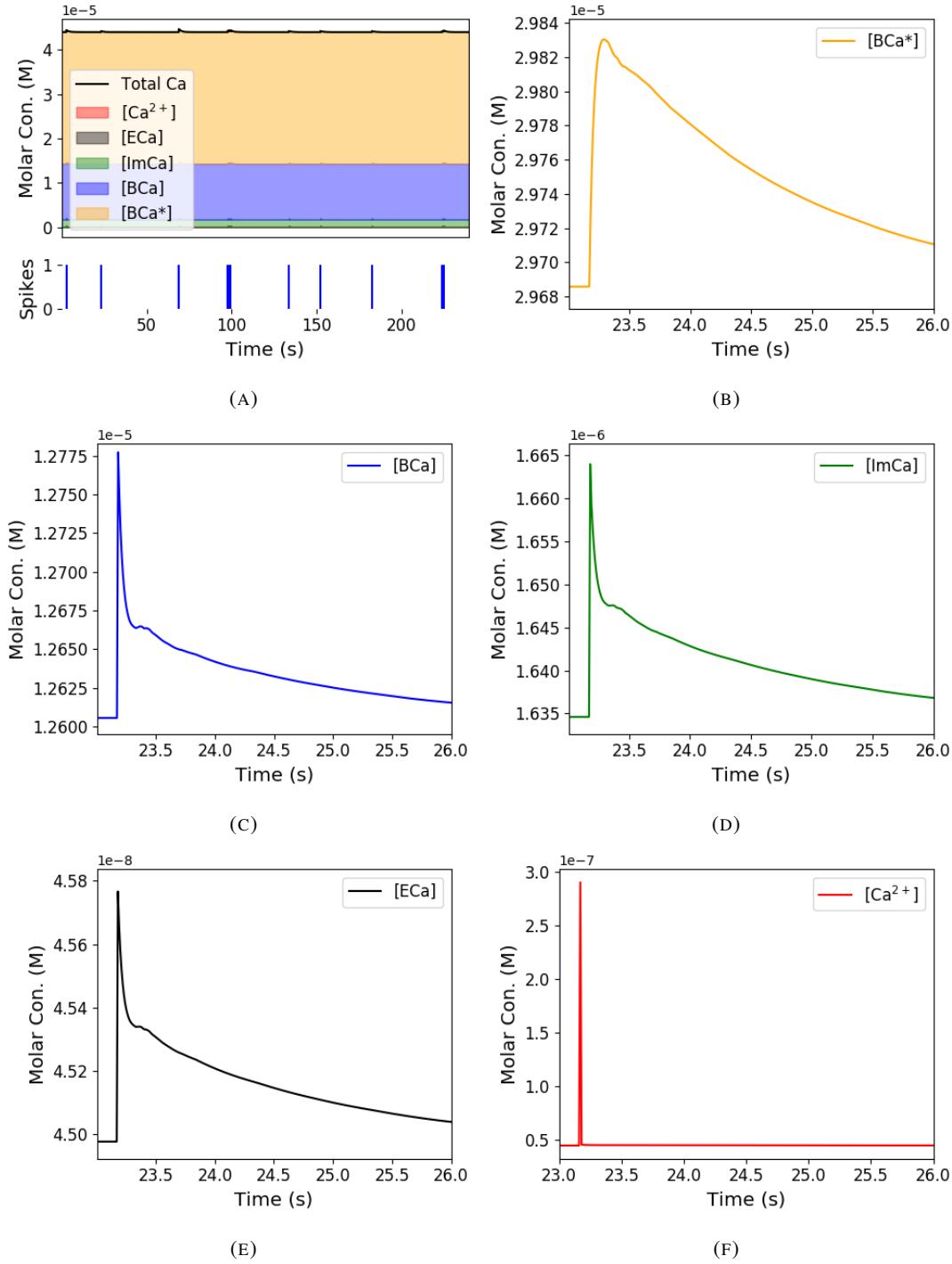


FIGURE 1.3: **Calcium Buffering Dynamics** (a) The proportions of bound and free calcium concentrations within a cell, with the associated spike train. (b)-(f) The dynamics of the concentration of (b) excited indicator bound calcium, (c) indicator bound calcium, (d) immobile endogenous buffer bound calcium, (e) mobile endogenous buffer bound calcium, and (f) free calcium in response to an action potential at ~ 23.2 s.

599 likely vary from cell to cell and experiment to experiment: GCaMP binding kinetics, GCaMP
600 concentration, and endogenous buffer concentration.

601 Several variants of GCaMP itself have been made that differ in calcium binding kinetics,
602 baseline fluorescence, fluorescence efficiency, and other factors. For example, GCaMP6f has
603 a decay time constant of $\sim 1\text{s}$, while GCaMP6s has a decay time constant of $\sim 2\text{s}$ (Chen
604 et al., 2013). Here we asked how these differences in binding kinetics affect spike inference.
605 We jointly varied the calcium binding and unbinding rates of the indicator by the same factor
606 over a range from 100-fold slower to 100-fold faster from the fitted values, and simulated the
607 fluorescence response for each of the parameter settings in response to the same spike trains
608 as before (figure 1.4). Notably this manipulation does not affect the indicators affinity, and
609 therefore would not affect steady-state responses to prolonged changes in calcium. Instead
610 it is likely to affect its sensitivity to the spike train dynamics. We computed two summary
611 measures from the simulated fluorescence traces: the signal-to-noise ratio for a single spike
612 (Methods, section 1.4.6), and the accuracy of spike inference for each of the spike trains. We
613 observed a reduction in the signal-to-noise ratio and the spike inference quality when we set
614 the binding and unbinding rates were set to one hundredth of their fitted values, and to one
615 tenth of their fitted values. When we increased the value of both binding rates, we observed
616 no change in these measurements. The reduction in both rates lead to smaller increases in
617 fluorescence in response to an action potential and a longer decay time (figure 1.4a), this
618 caused the reduction in signal-to-noise ratio. As both rates were increased, the change in
619 $\Delta F/F_0$ in response to an action potential increased and the decay time decreased slightly,
620 but the fluorescence trace created by these values was very similar to the trace created by the
621 fitted values.

622 Second, the overall concentrations of GCaMP often varies from cell to cell. For exam-
623 ple different cells, even of the same type in the same tissue, can express different levels of
624 GCaMP, due to proximity to the infection site, or the cell becoming ‘nuclear-filled’ (Tian et
625 al., 2009; Chen et al., 2013). Also, GCaMP is often used for longitudinal experiments where
626 the same cells are re-imaged across multiple days or weeks. However since GCaMP expres-
627 sion typically ramps up over time (Chen et al., 2013), the accuracy of spike inference may
628 differ across multiple longitudinal recordings in the same cell. We addressed this by varying
629 the concentration of calcium indicator in the model, simulating spike trains and measuring
630 signal-to-noise ratio and spike inference accuracy on the resulting fluorescence traces. Both
631 increasing and decreasing the concentration of the indicator had effects on the fluorescence

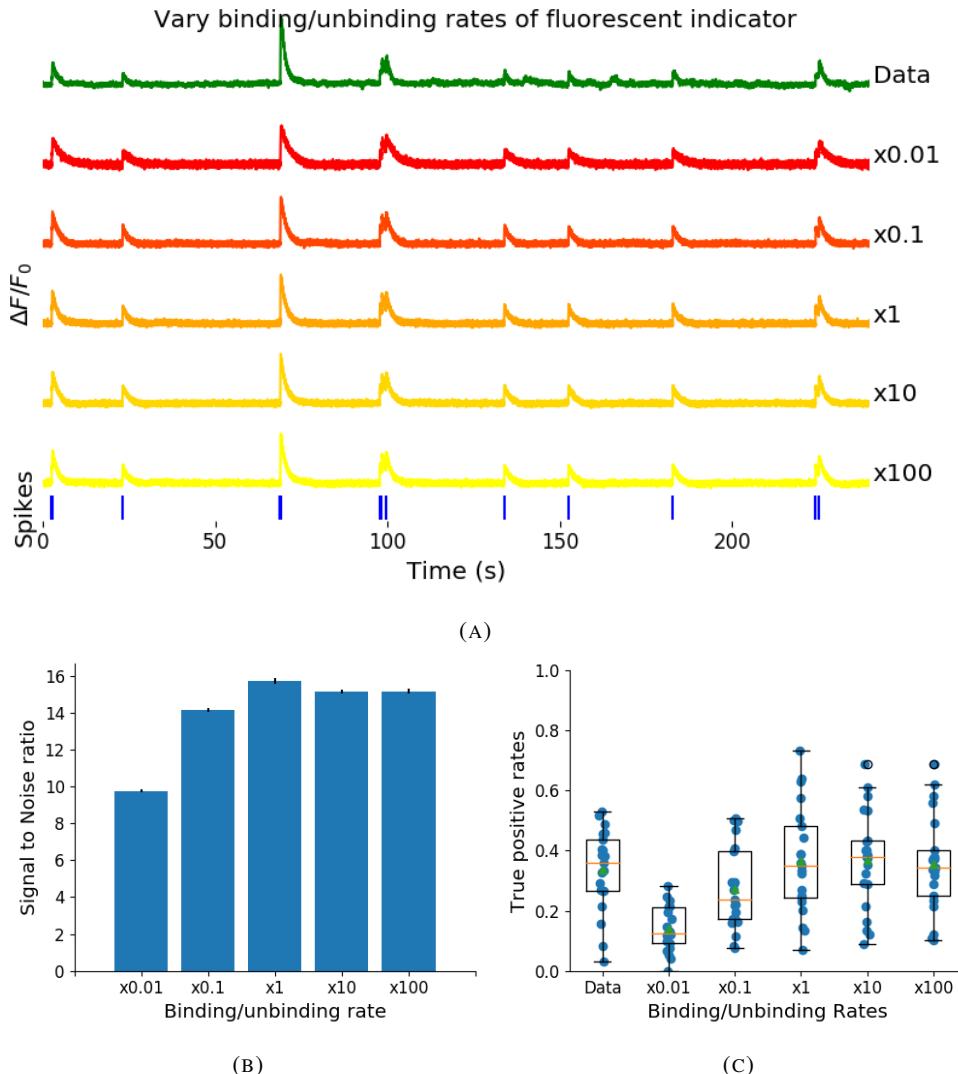


FIGURE 1.4: (a) An example trace for each of the five pairs of values used for the binding and unbinding rates of the fluorescent calcium indicator. (b) The signal-to-noise ratio of the modelled fluorescence traces using each of the four perturbed values, and the experimental value. The SNRs for the two pairs with values lower than the experimental value are lower than the experimental 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.

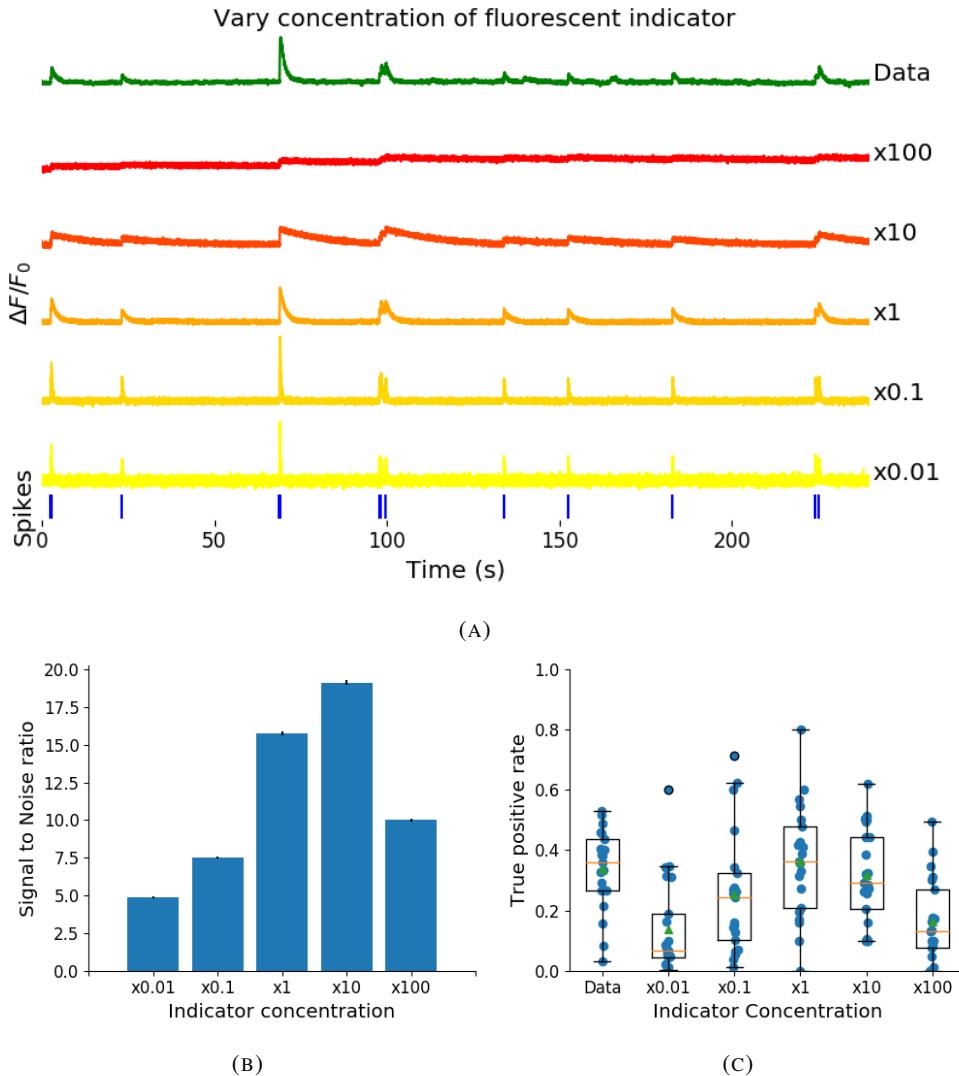


FIGURE 1.5: (a) An example trace for each of the five perturbed values for the concentration of fluorescent calcium indicator. The top two traces are produced by the lower perturbed values, the middle trace is produced by the experimental value, and the lowest two traces are produced when using the higher 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.

trace, signal-to-noise ratio, and spike inference. The signal-to-noise ratio and spike inference quality decreased with decreased indicator concentration, and both showed a decrease when the indicator concentration was increased to 100 times its fitted value (figure 1.5). The signal-to-noise ratio showed an increase when the indicator concentration was increased to 10 times its fitted value, but there was no corresponding change in the spike inference quality. The decrease in indicator concentration caused a reduction in the increase in $\Delta F / F_0$ in response to an action potential, and an increase in the decay time of this increase (figure 1.5a). The increase in indicator concentration had the opposite effect, it caused an increase in the change in $\Delta F / F_0$ in response to an action potential, and a decrease in the decay time.

Third, the concentration and types of endogenous calcium buffers also vary from neuron to neuron, both within and between cell types (Bartol et al., 2015; Maravall et al., 2000; Neher and Augustine, 1992). Since the calcium buffer capacity of neurons is high, around 50-70 (Lee et al., 2000) in excitatory hippocampal pyramidal cells, around 100-250 (Lee et al., 2000) in inhibitory hippocampal pyramidal cells, and 900-200 in Purkinje cells (depending on the age of the subject), these endogenous buffers compete with GCaMP for binding to calcium, and variations in endogenous buffer concentration may affect GCaMP signal and therefore spike inference. To address this we varied the concentration of the endogenous buffer in the model neuron over five orders of magnitude from 0.8 to 8000 μM , simulated calcium fluorescence traces in response to the same set of spike trains, and performed spike inference on the resulting fluorescence time series. Increasing the endogenous buffer concentration had a substantial effect on the GCaMP fluorescence signal, both decreasing its amplitude and slowing its kinetics (figure 1.6(a)). This corresponded with a decrease in both single-spike signal-to-noise ratio (figure 1.6(b)) and spike inference accuracy (figure 1.6(c)). In contrast, decreasing endogenous buffer capacity from the fitted value had little effect on either the GCaMP signal or spike inference (figure 1.6).

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

The fluorescence signal recorded from neurons using calcium indicators is typically much slower than changes in membrane potential for two reasons: first, because the calcium and the indicator have slow binding and unbinding kinetics, the signal is a low-pass filtered version of the membrane potential. Second, neuronal two-photon imaging experiments are often performed in scanning mode, which limits their frame rate to $\sim 10\text{Hz}$ or slower. This implies that multiple spike events that occur close in time might be difficult to resolve from a

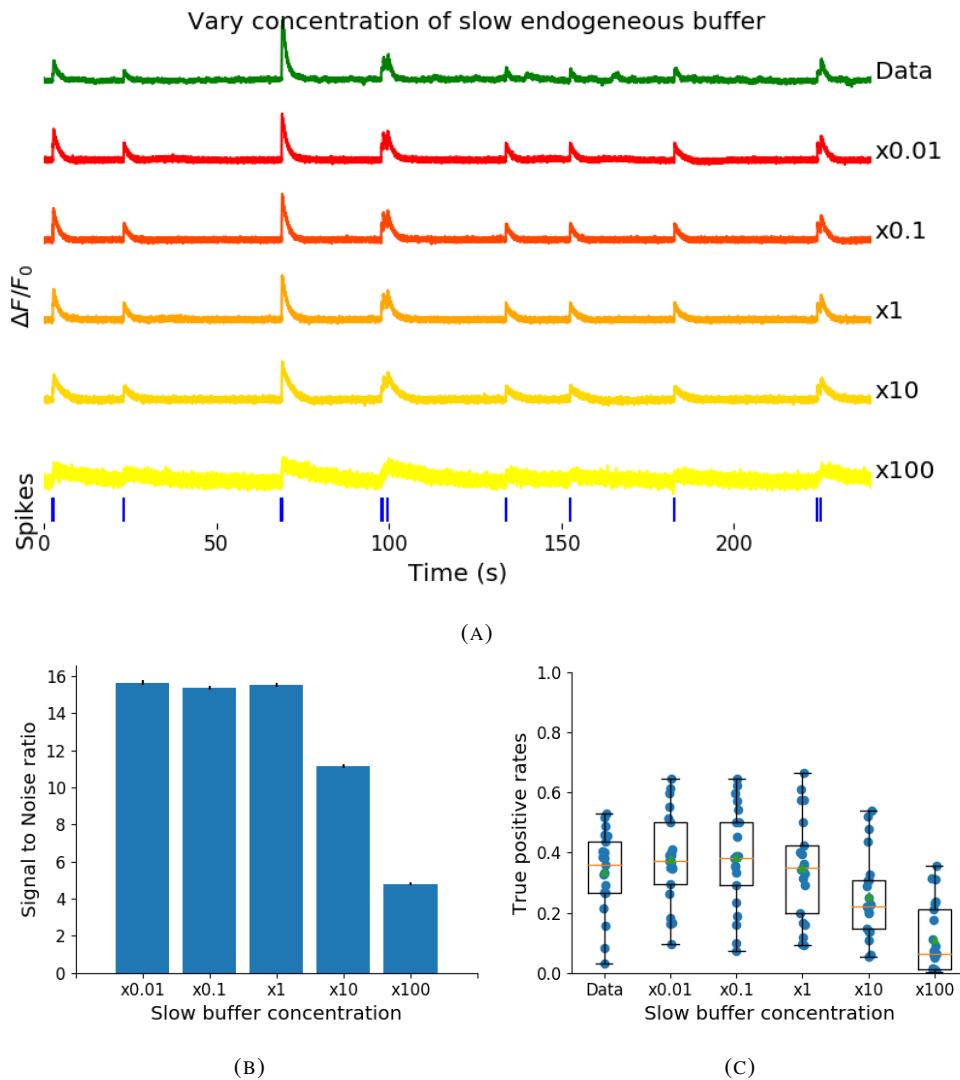


FIGURE 1.6: (a) An example trace for each of the five perturbed values for the concentration of immobile endogenous buffer. (b) The signal-to-noise ratio of the modelled fluorescence traces using each of the four perturbed values, and the experimental value. The lower values for the immobile buffer produce 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.

calcium indicator time series. Many cells, especially several types of inhibitory interneurons, fire tonically at rates higher than 10Hz. We used the model to test whether spike inference accuracy depended on the neuron's firing frequency by driving the cell with spike trains sampled from a Poisson processes of varying frequency. We simulated a variable firing rate using an Ornstein-Uhlenbeck process, and simulated the spike trains using a Poisson distribution 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 1.8(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 1.8(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.

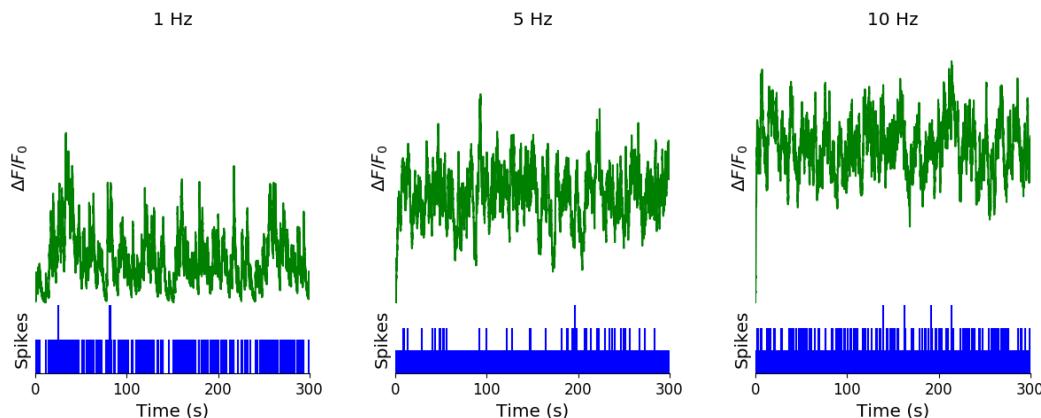


FIGURE 1.7: Simulating fluorescence traces at different firing rates Example modelled traces created using simulated spike trains with a mean firing rate of 1Hz (left column), 5Hz (middle column), and 10Hz (right column). Note the difference in amplitude with different mean firing rates.

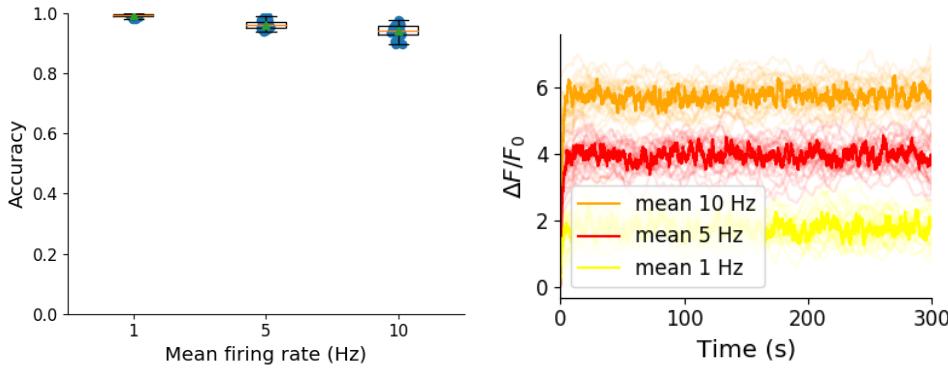


FIGURE 1.8: **Inference quality and $\Delta F/F_0$ vs Firing rate** (left) The spike inference accuracy when applied to 30 traces created using simulated spike trains with mean firing rates of 1, 5, and 10 Hz. (right) The mean $\Delta F/F_0$ across those 30 traces for each frequency.

683 1.3 Discussion

684 We designed a biophysical model for the changes in free calcium and bound calcium con-
 685 centrations within a cell soma with a fluorescent calcium indicator. We used this model to
 686 model the fluorescence trace resulting from a spike train in this cell. We fit the free parame-
 687 ters of the model by matching the power spectrum and amplitude of fluorescence traces with
 688 simultaneously measured spike trains. We inferred spikes from real fluorescence traces and
 689 modelled fluorescence traces, and measured the quality of the spike inference in both cases.
 690 We found that the spike inference quality was similar in both cases. We perturbed the concen-
 691 tration of the calcium buffers in the model, and the binding/unbinding rates of those buffers
 692 in the model, and measured the effect on the signal-to-noise ratio (SNR) of the modelled
 693 fluorescence traces and the spike inference quality.

694 For the fluorescent calcium indicator, we found that any large perturbation away from
 695 the experimental value led to a reduction in SNR, and spike inference quality. For the bind-
 696 ing/unbinding rates, we kept the ratio of these rates constant, but altered their values in paral-
 697 lel. The lower values caused a reduction in SNR, and a reduction in spike inference quality.
 698 For the endogenous buffer concentration, an increase above the experimental value caused a
 699 reduction in SNR and spike inference quality.

700 Although the model produced visually similar time series to the real data, there were a
 701 few aspects it did not capture. First, the real data featured some low-frequency components
 702 that did not appear related to the spike events. These were not captured by the models we
 703 used in this study, but could be added in future by adding a suitable low-frequency term to the
 704 resulting time series. Second, the real data seemed to have some nonlinearities not captured in

705 the model, for example the response to two nearby spikes was greater than expected from the
706 linear sum of two single spikes. This may be due to the co-operative binding of Calmodulin
707 to calcium, which gives calmodulin a supra-linear sensitivity to calcium concentration. The
708 model, in contrast behaved much more linearly, but could be extended in future to include
709 such nonlinearities. Third, in the real data the fluorescence peak amplitude seemed to vary
710 from spike to spike, even for well-isolated spike events. However in our model we assumed
711 each spike lead to the same fixed-amplitude injection of calcium to the cell, leading to much
712 greater regularity in fluorescence peak amplitudes. This variability could be added in future
713 versions of the model by making the injected calcium peak a random variable. Fourth, we
714 modelled the soma as a single compartment, but in reality there is likely a non-uniform spatial
715 profile of calcium concentration. This may matter because some endogenous buffers might
716 access calcium right as it influxes from the extracellular space, whereas the majority of the
717 fluorescence signal is more likely coming from the bulk of the cytoplasm. Future models
718 could attempt to model these spatial dependencies to assess whether they affect the overall
719 spike inference procedure.

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

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

739 A recent paper by Greenberg et al (2018) described a biophysical model for spike train
740 inference called the ‘Sequential binding model’. Similar to our model, this model included
741 parameters for two types of endogenous buffer. But this model also included dynamics for
742 calcium binding to and unbinding from these endogenous buffers. Furthermore, this model
743 included dynamics for calcium binding to and unbinding from the four binding sites present
744 on a GCaMPs6 molecule. In the accuracy measurements specified in that paper, this model
745 performed better than the MLspike algorithm, which is also partially a biophysically model,
746 and it performed better than the constrained non-negative deconvolution algorithm. The se-
747 quential binding model also biophysically interpretable parameters, and its fitted parameters
748 for quantites such as buffering capacity and calcium influx upon action potential firing fall in
749 line with experimental values (Greenberg et al., 2018). Biophysical models like this appear
750 to be the way forward for spike inference algorithms.

751 1.4 Methods

752 1.4.1 Calcium dynamics model

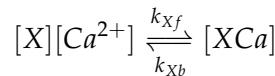
753 We wrote a biophysical model of the calcium dynamics within a cell soma. When a neu-
754 ron fires an action potential, voltage-dependent calcium ion-channels open up that allow a
755 current of Ca^{2+} to flow into the neuron (Koch, 1999). The increase in the free calcium ion
756 concentration inside of the cell, along with changes in the concentration of potassium and
757 sodium, causes the change in cell membrane potential, which must be depolarised. The de-
758 polarising process consists of free calcium ions leaving the cell through open ion channels,
759 or binding to molecules within the cell called buffers, or calcium storage by organelles such
760 as the endoplasmic reticulum. A diagram illustrating the cell, its channels, and its buffers
761 can be seen in figure 1.1A. There are several different types of calcium buffer, each with
762 different dynamics and different concentrations within different types of excitable cell. The
763 fluorescent calcium indicator is another calcium buffer, with the useful property that when it
764 is bound to a calcium ion, the bound molecule may become excited by a photon and release
765 a photon in return. This is what creates the fluorescence. After the action potential has taken
766 place, the free calcium concentration within the cell will return to a baseline level (Maravall
767 et al., 2000).

768 We modelled the the dynamics of five molecular concentrations,

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

- 770 • Fluorescent indicator bound calcium, $[BCa]$
- 771 • Endogenous mobile buffer bound calcium, $[ECa]$
- 772 • Endogenous immobile buffer bound calcium, $[ImCa]$
- 773 • Excited buffered calcium, $[BCa^*]$

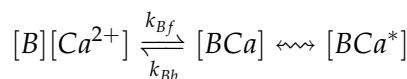
The term ‘buffering’ refers to free calcium ions coming into contact with buffer molecules and binding together. Diagrammatically:



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

778 There are a number different endogenous buffers in any neuron. Which buffers are
779 present, and the buffers’ concentrations vary from cell to cell. In order to capture the ef-
780 fects of mobile and immobile endogenous buffers without introducing several parameters,
781 they were modelled as two buffers. One representing mobile buffers and the other represent-
782 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.



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

$$\begin{aligned} \frac{d[Ca^{2+}]}{dt} = & k_{Bb}[BCa] + k_{Eb}[ECa] + k_{Imb}[ImCa] \\ & - k_{Bf}[B][Ca^{2+}] - k_{Ef}[E][Ca^{2+}] - k_{Imf}[Im][Ca^{2+}] \\ & + \beta([Ca_0^{2+}] - [Ca^{2+}]) \end{aligned} \quad (1.1)$$

$$\frac{d[BCa]}{dt} = k_{Bf}[B][Ca^{2+}] - k_{Bb}[BCa] + r[BCa^*] - e[BCa] \quad (1.2)$$

$$\frac{d[ECa]}{dt} = k_{Ef}[E][Ca^{2+}] - k_{Eb}[ECa] \quad (1.3)$$

$$\frac{d[ImCa]}{dt} = k_{Imf}[Im][Ca^{2+}] - k_{Imb}[ImCa] \quad (1.4)$$

$$\frac{d[BCa^*]}{dt} = \eta[BCa] - r[BCa^*] \quad (1.5)$$

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

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

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

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

Photon release & capture

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

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

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

816 **1.4.2 Parameter optimisation**

817 The free parameters of the model are as follows:

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

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

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

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

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

- 827 1. The frequency power spectrum of the trace was measured.
- 828 2. The power spectrum was smoothed using a boxcar smoother (aka. sliding average, box
829 smoother).
- 830 3. The log of the smoothed power spectrum was measured.
- 831 4. Use the model to create a modelled fluorescence trace.
- 832 5. Apply steps 1, 2, and 3 to the modelled fluorescence trace.
- 833 6. Calculate the root mean squared difference between the log power of the actual fluo-
834 rescence trace, and the log power of the modelled fluorescence trace.
- 835 7. Calculate the root mean squared difference between the actual fluorescence trace and
836 the modelled fluorescence trace.
- 837 8. Use an optimisation algorithm to reapply this process, attempting to minimize the sum
838 of the two root mean squared differences at each iteration.

839 Using the root mean squared difference of the log power spectra as part of the objective
840 function forces the model to match the noise frequency of the actual fluorescence. Using
841 the root mean squared difference of the traces themselves forces the model to match the
842 amplitude of the fluorescence trace more accurately.

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

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

862 **Fixed parameters**

863 As well as the optimised parameters mentioned in section 1.4.2, the model also has thirteen
864 fixed parameters. Please see table 1.1 for details of these parameters and their values. In
865 an application of the model, these parameters can be changed in order to model any given
866 fluorescent calcium indicator.

867 **1.4.3 Julia**

868 The programming language used to write and execute the model was 'Julia'. Julia is a dy-
869 namic programming language designed for technical computing. Julia was designed specif-
870 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 1.1: **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.

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

875 **1.4.4 Spike inference**

876 We used spike inference algorithms to compare the quality of spike inference using the mod-
877 elled traces to the quality of spike inference using the observed traces. We also used the
878 spike inference algorithms to assess the effect of parameter perturbation on the spike infer-
879 ence. Three algorithms were used:

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

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

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

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

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

- 909 ● Accuracy
- 910 ● True positive rate (aka recall, sensitivity, hit rate)
- 911 ● True negative rate (aka specificity)
- 912 ● Precision
- 913 ● Negative predicted value
- 914 ● False negative rate (aka miss rate)
- 915 ● False positive rate (aka fall-out)
- 916 ● False discovery rate
- 917 ● False omission rate

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

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

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

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

938 **Comparing spike inference quality**

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

943 **1.4.5 Perturbation analysis**

944 In order to measure the sensitivity of spike inference to changes in a given model parameter,
945 we perturbed the parameter and compared the quality of spike inference with the perturbed
946 parameters to the quality of spike inference with the experimental or optimised parameters.
947 In order to maximise the possibility of observing a difference due to the perturbation, we
948 perturbed the chosen parameter by a relatively large amount. For example, the experimen-
949 tal value for the molar concentration of the fluorescent indicator within the cell was 10^{-4}M
950 (Maravall et al., 2000). The perturbed values used for this parameter were 10^{-2}M , 10^{-3}M ,
951 10^{-5}M , and 10^{-6}M . The quality of the inference was compared by measuring the true posi-
952 tive rate for each perturbed value and using a t-test to compare the distributions of the results.

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

956 **1.4.6 Signal-to-noise ratio**

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

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

965 **1.4.7 Data sources**

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

⁹⁶⁹ **Chapter 2**

⁹⁷⁰ **Functional networks expand across
anatomical boundaries as correlation
time-scale increases**

⁹⁷¹

⁹⁷²

973 *Abstract*

974 Decades of research has established that correlated spiking plays a crucial role in represent-
975 ing sensory information. One drawback associated with the recent improvement in recording
976 technology and consequent large datasets is the difficulty in analysing higher order correla-
977 tions in large neuronal ensembles. One benefit of these datasets that has not yet been explored
978 is the opportunity to compare correlations within anatomical regions to correlations across
979 anatomical regions. In this work, we measured correlations between neurons residing in
980 nine different brains regions in three awake and behaving mice. Using the these correlation
981 measurements, we created weighted undirected graph networks and applied network science
982 methods to detect functional communities in our neural ensembles. We compared these func-
983 tional communities to their anatomical distribution. We repeated the analysis, using different
984 timescales for our correlation measurements, and found that functional communities were
985 more likely to be dominated by neurons from a single brain region at shorter timescales
986 (< 100ms).

987 2.1 Introduction

988 Decades of research has established that correlations play a crucial role in representing sen-
989 sory information. For example, the onset of visual attention has been shown to have a greater
990 affect on the correlations in the macaque V4 region than on the firing rates in that region
991 (Cohen and Maunsell, 2009). Recent findings show that spontaneous behaviours explain cor-
992 relations in parts of the brain not associated with motor control (Stringer et al., 2019), that
993 satiety state appears to have a brain wide representation (Allen et al., 2019), and that subject
994 exploratory and non-exploratory states are represented in the amygdala (Gründemann et al.,
995 2019). So, behavioural states are likely represented across many regions of the brain, not just
996 motor related areas. In order to understand the brain, we must understand the interactions
997 between neurons and regions.

998 Because of limitations in recording technology almost all research has explored corre-
999 lations between neurons within a given brain region, or within only two regions at most
1000 (Wierzynski et al., "2009"; Patterson et al., 2014; Girard, Hupé, and Bullier, 2001). Rel-
1001 atively little is known about correlations between neurons in many different brain regions.
1002 However, the recent development of ‘Neuropixels’ probes (Jun et al., 2017) has allowed
1003 extracellular voltage measurements to be collected from multiple brain regions simultane-
1004 ously routinely, and in much larger numbers than traditional methods. In this project we
1005 used a publicly-available Neuropixels dataset to analyse correlations between different brain
1006 regions (Stringer et al., 2019).

1007 A drawback associated with the improvement in recording technology is an increase in
1008 the difficulty in analysing these data. For example, analysing the i th order interactions of
1009 N neurons generally requires estimation of N^i parameters. A number that becomes astro-
1010 nomical for large N . New methods are required for analysing these new large datasets. We
1011 attempted to address this requirement in this piece of research by applying a cutting-edge
1012 network science community detection method to neural data.

1013 Another unexplored area of research is the changes in cell interactions at different timescales.
1014 Studies have shown different timescales for fluctuations in spiking activity (Murray et al.,
1015 2014), and different time scales for event representation (Baldassano et al., 2017) across dif-
1016 ferent brain regions. Still most studies focus on quantifying interactions at a given timescale.
1017 But neurons may interact differently, or may interact with different neurons at different
1018 timescales. Here we explore correlated communities of neurons at different timescales.

1019 In this work, we measured correlations between binned spike counts from neurons from

1020 nine different regions of the mouse brain. These measurements induced a weighted undirected graph or network where each neuron is represented by a node, and the strength of
1021 the connection between these nodes/neurons is the strength of the correlation between their
1022 spike counts. We then applied newly invented network methods (Humphries et al., 2019)
1023 to this network to find any community structure, and place the neurons in these correlation
1024 based communities. Finally, we compared these functional communities to the anatomical
1025 membership of the neurons.

1027 To investigate the functional communities and their relationship with anatomy at different
1028 time scales, we repeated these analyses using different length bin widths when binning spike
1029 times.

1030 To find and analyse functional networks while controlling for the subject's behaviour, we
1031 conditioned the binned spike counts on data from a video of the subject's face, and repeated
1032 our analysis for spike count correlations (or noise correlations) and signal correlations.

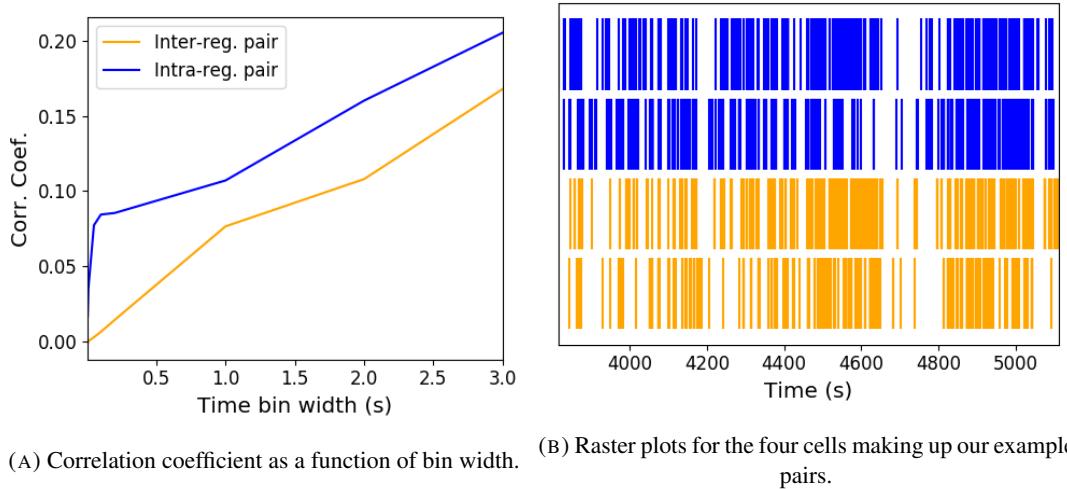
1033 **2.2 Results**

1034 Note that in the following text, we refer to the correlation coefficient between two sequences
1035 of spike counts from two different cells as the *total correlation*. We refer to the correlation
1036 between spike counts in response to a certain stimulus as the *spike count correlation* aka
1037 *noise correlation*, and we refer to the correlation between mean or expected responses to
1038 different stimuli as the *signal correlation* (Cohen and Kohn, 2011).

1039 The nine different brain regions from which we had data were the caudate putamen (CP),
1040 frontal motor cortex (FrMoCtx), hippocampus (HPF), lateral septum (LS), midbrain (MB),
1041 primary visual cortex (V1), superior colliculus (SC), somatomotor cortex (SomMoCtx), and
1042 thalamus (TH).

1043 **2.2.1 Average correlation size increases with increasing time bin width**

1044 First we inspected the affect of time bin width on total correlations. We know that using short
1045 time bins results in artificially small correlation measurements (Cohen and Kohn, 2011), so
1046 we expected to see an increase in correlation amplitude with increasing time bin width. That
1047 is exactly what we observed. Taking 50 cells at random, we calculated the total correla-
1048 tion between every possible pair of these cells, using different time bin widths ranging from
1049 0.005s to 3s. We found that the longer the time bin width, the greater the correlations (see
1050 figure 2.2a).



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

FIGURE 2.1: (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.

1051 We also separated the positively correlated pairs from the negatively correlated pairs
 1052 using the mean correlation of each pair across all bin widths (see section 2.5.2). We found
 1053 that the positively correlated pairs become more positively correlated with increasing time bin
 1054 width, and the negatively correlated pairs become more negatively correlated with increasing
 1055 time bin width (see figures 2.2b and 2.2c).

1056 In figure 2.1a we plot correlations from two example pairs, one pair from within a region,
 1057 and one pair between regions. It can be seen that the correlation coefficient increases with
 1058 bin width. The correlations can be observed by eye in the raster plot for these cells in figure
 1059 2.1b.

1060 When taking the mean across all pairs, the positively correlated pairs dominate in terms
 1061 of both number of pairs, and amplitude of correlations. Therefore the mean across all pairs
 1062 is positive.

1063 These results were observed in each of the three mouse subjects from which we had data.

1064 2.2.2 Goodness-of-fit for Poisson and Gaussian distributions across increasing 1065 time bin widths

1066 We wanted to investigate if the width of the time bin used to bin spike times into spike counts
 1067 had an effect on the distribution of spike counts. We used the χ^2 statistic as a goodness-of-fit
 1068 measure for Poisson and Gaussian (normal) distributions to the spike count of 100 randomly

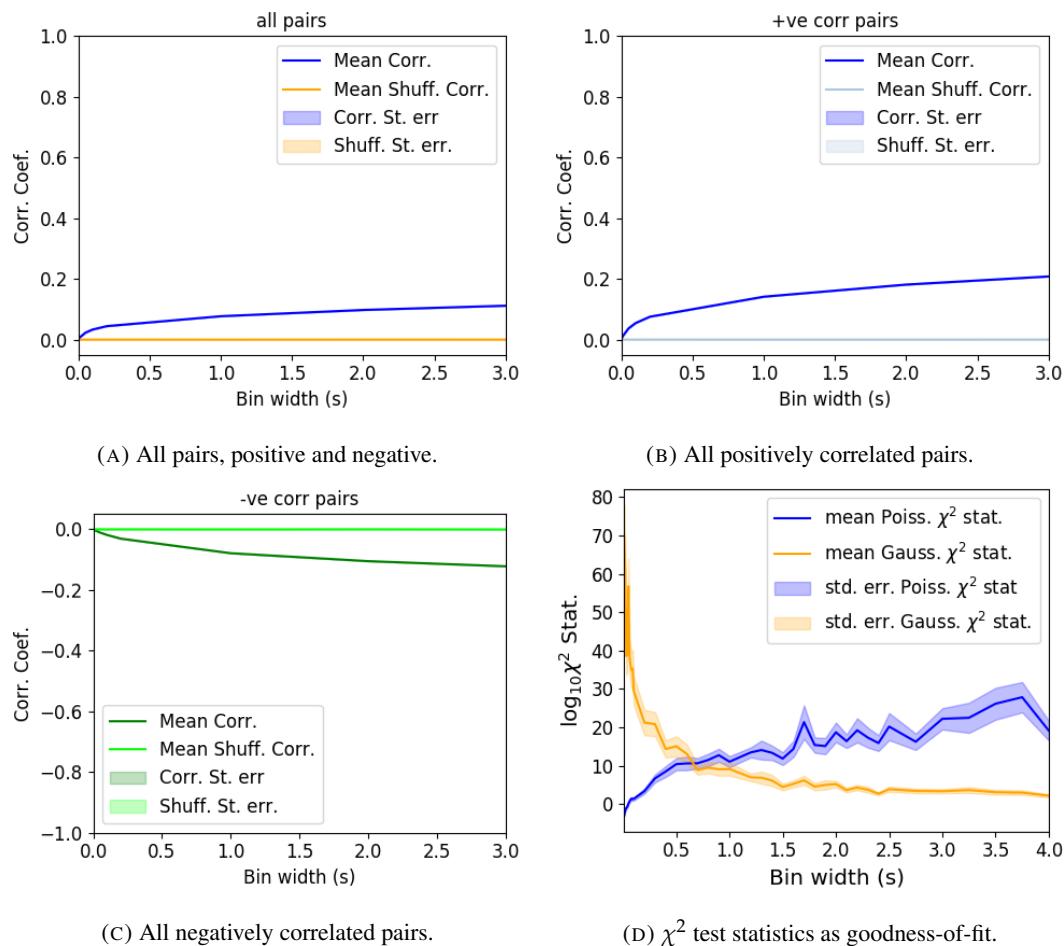


FIGURE 2.2: Mean correlation coefficients measured from pairs of 50 randomly chosen neurons. (A) All possible pairs, (B) positively correlated pairs, and (C) negatively correlated pairs. (D) Mean and standard error of χ^2 test statistics for Poisson and Gaussian distributions fitted to neuron spike counts.

1069 chosen neurons for a number of bin widths ranging from 0.01s to 4s. For the χ^2 statistic, the
1070 higher the value, the worse the fit.

1071 We expected a Poisson distribution to be a better fit for shorter time bin widths because
1072 spike counts must be non-negative, therefore any distribution of spike counts with mass dis-
1073 tributed at or close to 0 will be skewed. The distribution of spike counts is more likely to be
1074 distributed close to 0 when the time bin widths used to bin spike times into spike counts are
1075 small relative to the amount of time it takes for a neuron to fire an action potential ($\sim 1\text{ms}$ in
1076 the case of non-burst firing neurons).

1077 We expected a Gaussian distribution to be a better fit for longer time bin widths, because
1078 a Poisson distribution with a large rate is well approximated by a Gaussian distribution with
1079 mean and variance equal to the Poisson rate. Therefore, a Gaussian distribution would ap-
1080 proximate the mean of a collection of large spike counts, and have more flexibility than a
1081 Poisson distribution to fit the variance.

1082 We found that that a Poisson distribution is the best fit for shorter time bins less than 0.7s
1083 in length. Then a Gaussian distribution is a better fit for time bins greater than 0.7s in length
1084 (see figure 2.2d).

1085 2.2.3 Differences between and inter- and intra- regional correlations decrease 1086 with increasing bin width

1087 We investigated the differences in distribution between inter-regional correlations, i.e. corre-
1088 lations between neurons in different brain regions, and intra-regional correlations, i.e. corre-
1089 lations between neurons in the same brain region.

1090 Firstly, we investigated these quantities for all possible pairs of ~ 500 neurons taken
1091 from across all the 9 brain regions from which we had data. We distributed these neurons as
1092 evenly as possible across all of the regions, so that cells from one region would not dominate
1093 our data. We observed that the mean intra-regional correlations were always higher than the
1094 mean inter-regional correlations for every value of time bin width used. We also observed
1095 that as the time bin width increased these mean correlations increased and the difference
1096 between the mean inter-regional and intra-regional correlations grew (see figure 2.3 (Left)).

1097 Stringer et al. (2019) had a similar finding using the same data. They used only one value
1098 for the time bin width, 1.2s. Using this time bin width to bin spike times and measure total
1099 correlations, they found that the mean ‘within-region’ correlations were always greater than
1100 the ‘out-of-region’ correlations (Stringer et al., 2019). The figure from their paper showing
1101 this result can be seen in figure 2.3 (Right).

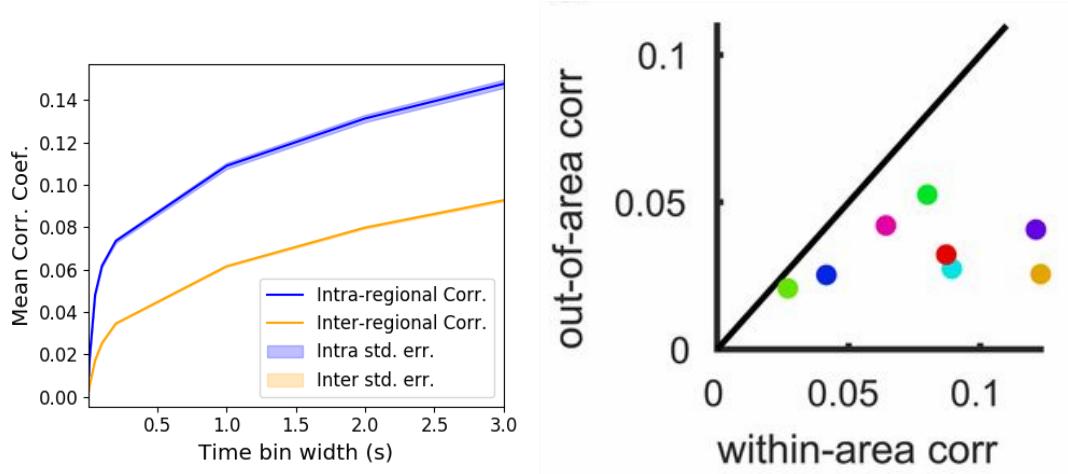


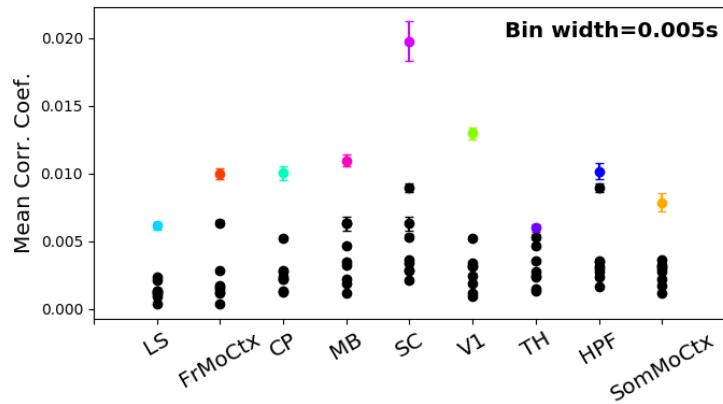
FIGURE 2.3: (Left) The mean intra-region and inter-region correlations using all possible pairs of ~ 500 neurons, spread across 9 different brain regions. (Right) Courtesy of Stringer et al. (2019), mean inter-regional (out-of-area) correlation coefficients vs mean intra-regional (within-area) correlation coefficients for a bin width of 1.2s. Note that the intra-regional coefficients are higher in each case.

1102 Examples of the correlations of one intra-regional pair and one inter-regional pair can be
1103 seen in figure 2.1.

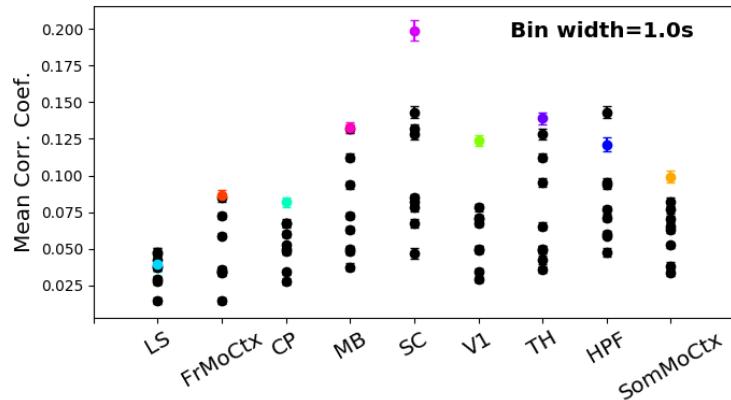
1104 Secondly, we separated those pairs into intra-regional and inter-regional groups. We
1105 noted that the mean intra-regional correlations (coloured dots in figures 2.4a and 2.4b) for
1106 a given region tended to be higher than the mean inter-regional correlations (black dots in
1107 figures 2.4a and 2.4b) involving cells from that region. However, in contrast with our previous
1108 result, we noted that the difference between the mean intra-regional correlations and most
1109 highly correlated inter-regional correlations reduced as we increased the time bin width (see
1110 figures 2.4a and 2.4b). This shows that the mean correlations shown in figure 2.3 are not
1111 distributed evenly across all region pair combinations.

1112 Finally, to see these regional mean correlations in a bit more detail, to examine the indi-
1113 vidual pair combinations in particular, we displayed these data in a matrix of mean corre-
1114 lations (see figure 2.5), showing the mean intra-regional correlations on the main diagonal, and
1115 the mean inter-regional correlations off diagonal. Comparing a version of this figure created
1116 using a short time bin width of 5ms (figure 2.5a) and a version using a longer time bin width
1117 of 1s (figure 2.5b) we observed that the mean intra-regional correlations are always relatively
1118 high in comparison to the mean inter-regional correlations, but the mean correlations in some
1119 inter-regional pairs are relatively much higher when using the longer time bin width.

1120 This could indicate information being processed quickly at a local or within-region level,
1121 and the local representations of this information spreading between regions at longer timescales.

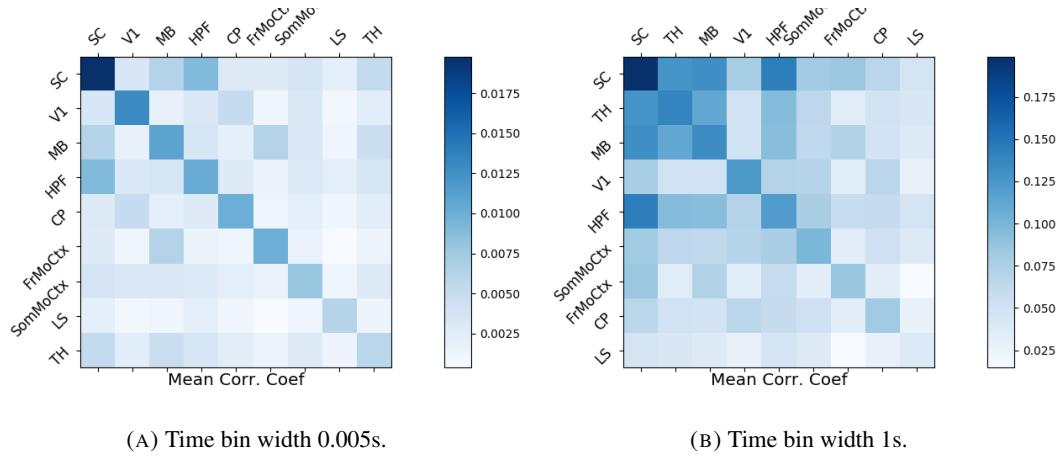


(A) Mean inter-regional and intra-regional correlations using a time bin width of 5ms.



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

FIGURE 2.4: 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.



(A) Time bin width 0.005s.

(B) Time bin width 1s.

FIGURE 2.5: 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.

1122 These results were consistent across the three mouse subjects. But, the relative magni-
 1123 tudes of the mean intra-regional and inter-regional correlations were not consistent. For ex-
 1124 ample, the region with the highest mean intra-regional correlations when using 1s bin widths
 1125 for subject one is the superior colliculus (SC), but for subject two it is the midbrain (MB).

1126 **2.2.4 Connected and divided structure in correlation based networks reduces
 1127 in dimension with increasing bin width**

1128 We used the correlation measurements to create weighted undirected graphs/networks where
 1129 each node represents a neuron, and the weight of each edge is the pairwise correlation be-
 1130 tween those neurons represented by the nodes at either end of that edge. We aimed to find
 1131 communities of neurons within these networks, and compare the structure of these commu-
 1132 nities to the anatomical division of those neurons. The first step of this process involved
 1133 applying the ‘spectral rejection’ technique developed by Humphries et al (2019) (Humphries
 1134 et al., 2019). This technique compares our data network to a chosen null network model, and
 1135 finds any additional structure in the data network beyond that which is captured in the null
 1136 network model (if there is any such structure).

1137 By comparing the eigenspectrum of the data network to the eigenspectrum of many sam-
 1138 ples from the null network model, this technique allows us to estimate the dimensionality of
 1139 the additional structure in the data network, and gives us a basis for that vector space. It also
 1140 divides the additional structure into connected structure, and k -partite (or divided) structure.
 1141 For example, if our algorithm found two dimensions of additional connected structure, and

1142 one dimension of additional divided structure. We might expect to find three communities,
1143 that is groups more strongly connected within group than without, and we might expect to
1144 find bi-partite structure, that is two sets that are more strongly connected between groups
1145 than within groups.

1146 The technique also finds which nodes contribute to this additional structure, and divides
1147 our data network into signal and noise networks. The details of spectral rejection and node
1148 rejection can be found in sections 2.5.5 and 2.5.5 respectively, and a full overview can be
1149 found in (Humphries et al., 2019).

1150 We chose the sparse weighted configuration model (see section 2.5.5) as our null network
1151 model. This model matches the sparsity and the total weight of the original network but
1152 distributes the weight at random across the sparse network.

1153 We applied the spectral rejection method to our networks based on total correlations using
1154 different values for the time bin width. We observed that for smaller time bin widths, our data
1155 networks had both k -partite structure, and community structure. As the width of the time bin
1156 increased, we found that the k -partite structure disappeared from our data networks, and the
1157 dimension of the community structure reduced in two of the three mice from which we had
1158 data (see figure 2.6).

1159 2.2.5 Detecting communities in correlation based networks

1160 We applied the community detection procedure described in section 2.5.5 to our signal net-
1161 works for our various time bin widths. We detected a greater number of smaller communities
1162 at shorter time bin widths, and a smaller number of larger communities for longer time bin
1163 widths (see figure 2.7). This was expected after the results found in section 2.2.4. We found
1164 more dimensions of additional structure at shorter time bin widths, therefore we found more
1165 communities at shorter time bin widths.

1166 We also noticed that at short time bin widths the communities detected tended to be
1167 dominated by cells from one region. Whereas communities existing in networks created
1168 using wider time bin widths tended to contain cells from many different brain regions. More
1169 on this in the next section.

1170 2.2.6 Functional communities resemble anatomical division at short timescales

1171 In order to quantify the similarity of the communities detected to the anatomical division of
1172 the cells. We treated both the anatomical division and the communities as clusterings of these
1173 cells. We then used measures for quantifying the difference or similarity between clusterings

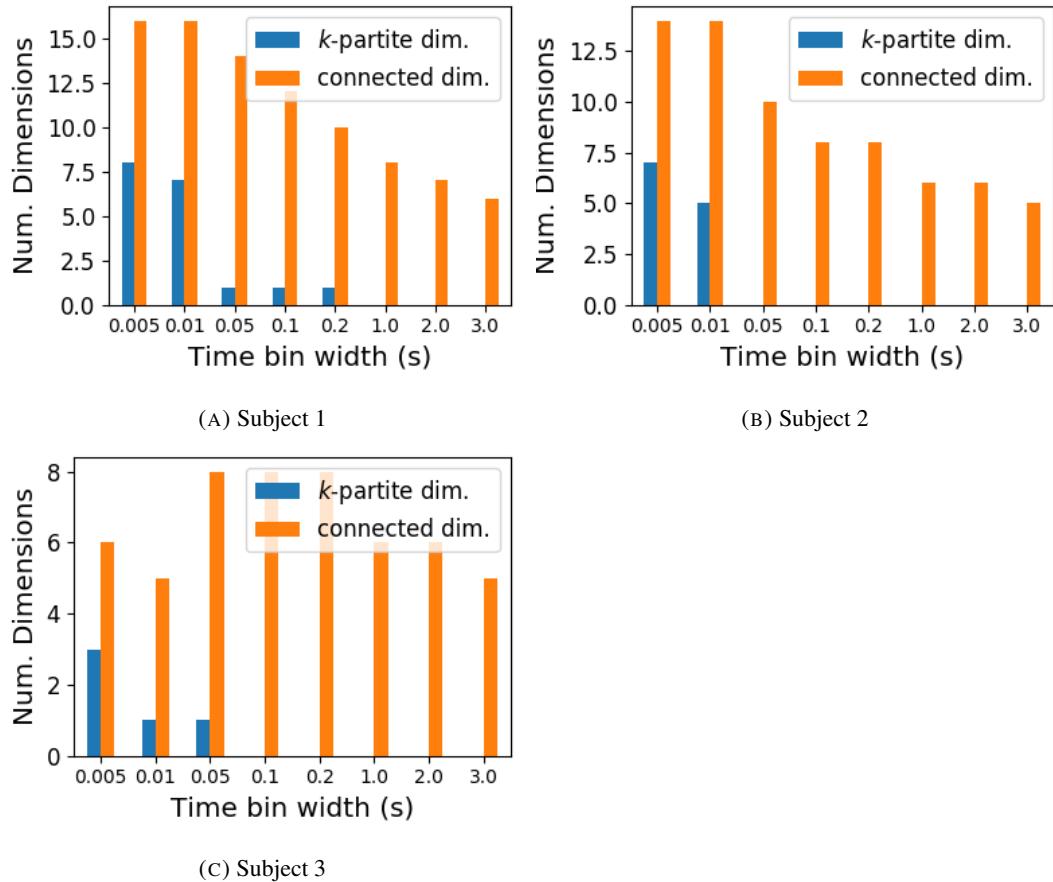


FIGURE 2.6: The number of dimensions in the *k*-partite and connected structure in the correlation based networks beyond the structure captured by a sparse weighted configuration null network model (see section 2.5.5), shown for different time bin widths. Note that the *k*-partite structure disappears 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).

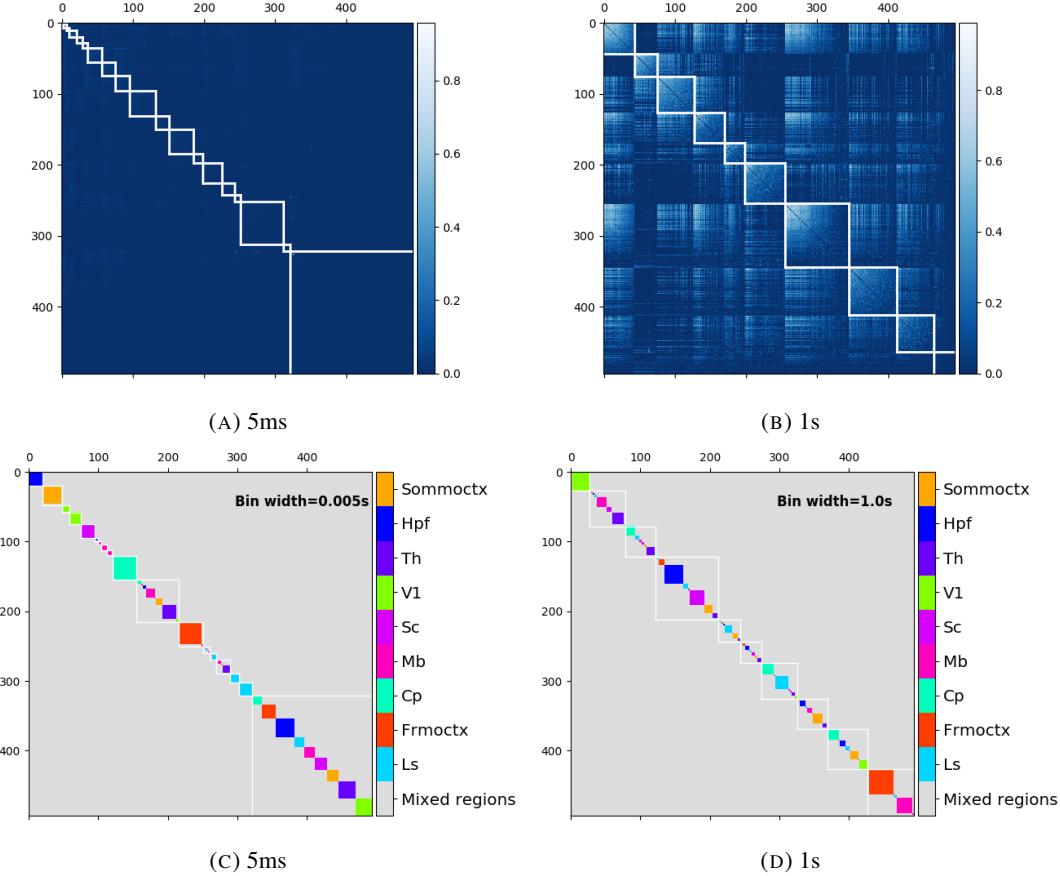


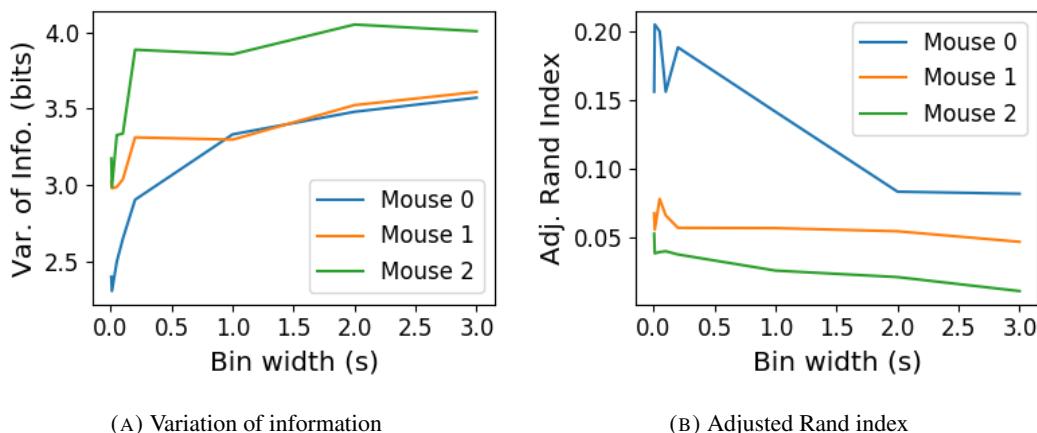
FIGURE 2.7: (A-B) Correlation matrices with detected communities indicated by white lines. Each off main diagonal entry in the matrix represents a pair of neurons. Those entries within a white square indicate that both of those neurons are in the same community as detected by our community detection 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.

to quantify the difference or similarity between the detected communities and the anatomical division. Details of these measures can be found in section 2.5.6 or in (Vinh, Epps, and Bailey, 2010).

We used two different types of measures for clustering comparison; information based measures (see section 2.5.6) and pair counting based measures (see section 2.5.6). We include one example of each in figure 2.8.

The variation of information is the information based measure included in figure 2.8a. This measure forms a metric on the space of clusterings. The larger the value for the variation of information, the more different the clusterings.

The adjusted Rand index is the pair counting based measure included in figure 2.8b. In contrast with the variation of information, the adjusted Rand index is a normalised similarity measure. The adjusted Rand index takes value 1 when the clusterings are identical, and takes value 0 when the clusterings are no more similar than chance.



(A) Variation of information

(B) Adjusted Rand index

FIGURE 2.8: (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.

Both measures indicated that the detected communities and the anatomical division of the cells were more similar when we used shorter time bins widths (see figure 2.8). This indicates that correlated behaviour in neuronal ensembles is more restricted to individual brain regions at short timescales (< 250ms), and the correlated activity spreads out across brain regions over longer time scales.

1192 **2.2.7 Conditional correlations & signal correlations**

1193 In light of the excellent research of Stringer et al (2019) showing that spontaneous behaviours
 1194 can drive activity in neuronal ensembles across the visual cortex and midbrain (Stringer et
 1195 al., 2019), we decided to control for the mouse’s behaviour when performing our analyses.
 1196 It is possible that our community detection process may be detecting communities across
 1197 multiple brain regions at longer time scales due to aggregating neuronal activity driven by
 1198 several spontaneous behaviours occurring during the time interval covered by a given time
 1199 bin. A time bin of 1s, for example, could contain a spike count where those spikes were driven
 1200 by different spontaneous behaviours. We aimed to investigate this possibility by applying our
 1201 community detection analysis to conditional correlation measures.

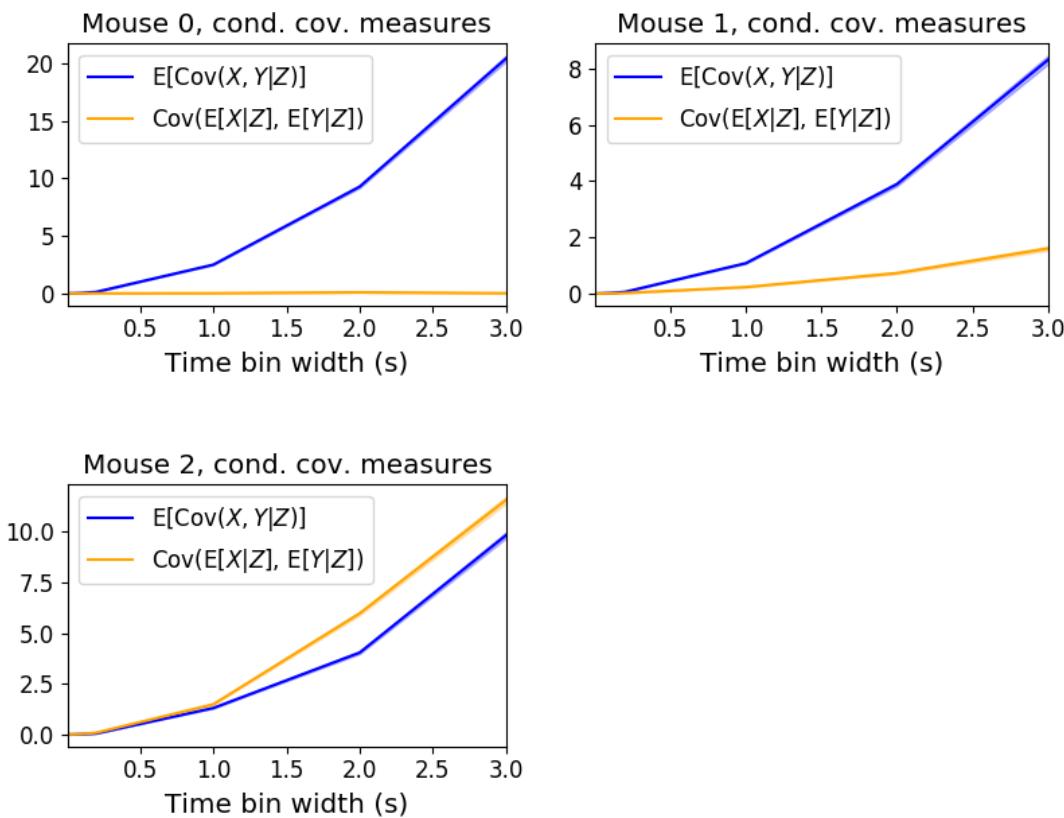


FIGURE 2.9: Comparing the components of the total covariance across different values for the time bin width. We observed a consistent increase in $E[\text{cov}(X, Y|Z)]$ as the time bin width increased. But we saw different trends for $\text{cov}(E[X|Z], E[Y|Z])$ for each mouse.

1202 We used the top 500 principal components of a video of the mouse’s face as a measure of
 1203 the mouse’s behaviour (see section 2.4.2). We modelled the spike counts as a linear combi-
 1204 nation of the principal components using linear regression with ElasticNet regularisation (see

section 2.5.3). Using this model, we quantified the expected spike count given the mouse’s behaviour $E[X|Z_1, \dots, Z_{500}]$.

We used these expected values to measure $\text{cov}(E[X|Z], E[Y|Z])$, and we used that value, the covariance $\text{cov}(X, Y)$, and the *law of total covariance* (see section 2.5.3) to measure $E[\text{cov}(X, Y|Z)]$. Here X and Y represent spike counts from individual cells, and Z is shorthand for the 500 principal components mentioned above. The two components of the covariance, $\text{cov}(E[X|Z], E[Y|Z])$ and $E[\text{cov}(X, Y|Z)]$, represent a ‘signal covariance’ and expected value of a ‘spike count covariance’ respectively, analogous to the signal correlation and spike count correlation (Cohen and Kohn, 2011).

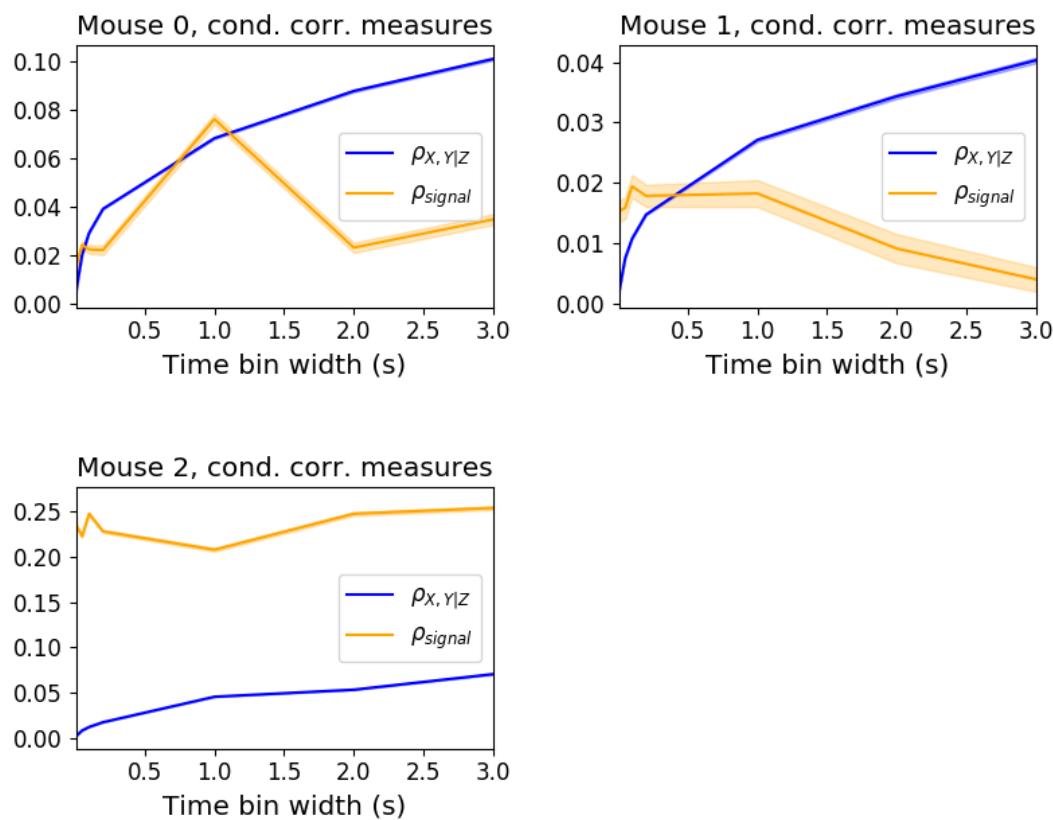


FIGURE 2.10: Comparing the components of the total covariance across different values for the time bin width. We saw a consistent increase in $\rho_{X,Y|Z}$ as the time bin width increased in all three subjects. But we saw different trends in ρ_{signal} for each of the subjects.

We examined the means of these components for different values of the time bin width (see figure 2.9). 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.

Using $\text{cov}(E[X|Z], E[Y|Z])$ we measured the signal correlation, ρ_{signal} , and using $E[\text{cov}(X, Y|Z)]$ we measured the event conditional correlation, $\rho_{X,Y|Z}$ (see section 2.5.3 for more details).

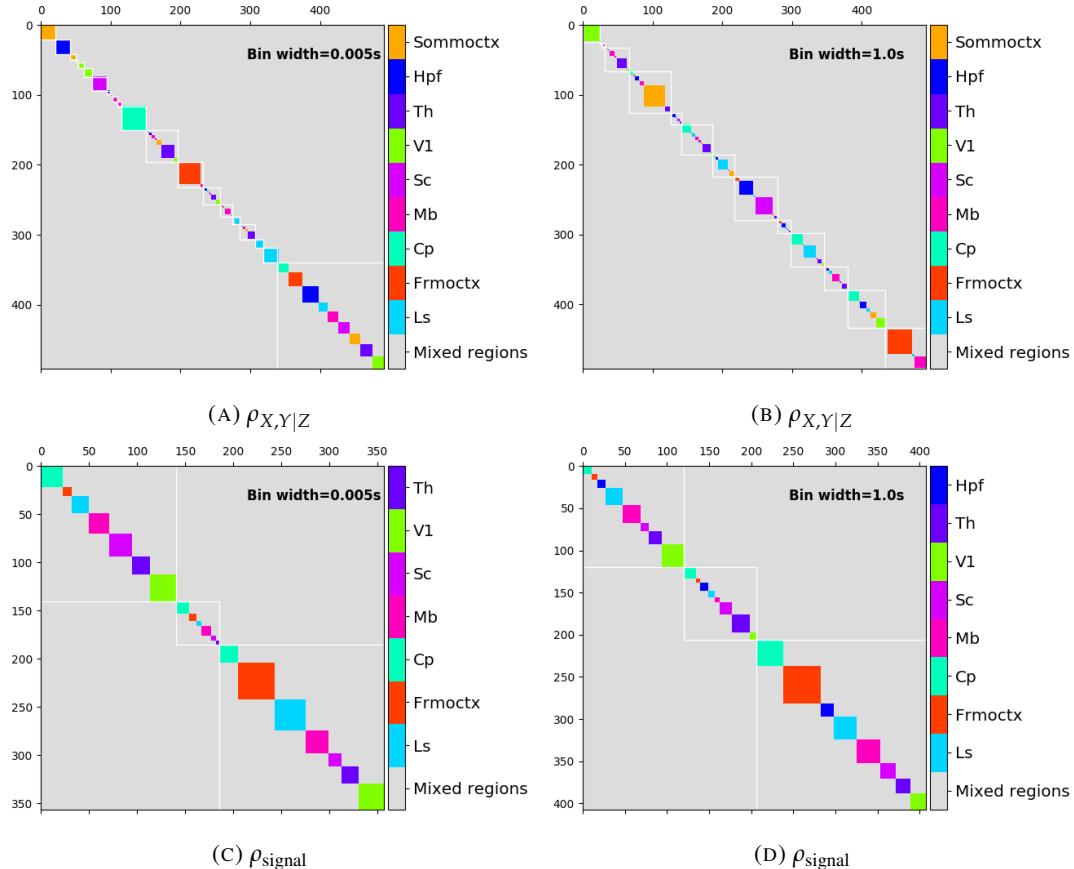


FIGURE 2.11: Matrices showing the regional membership of pairs by colour, and the communities in which those pairs lie. (A-B) Detected communities and regional membership matrix for network based on rectified spike count correlation $\rho_{X,Y|Z}$, using time bin widths of 0.005s and 1s respectively. (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.

1219 We saw a consistent increase in $\rho_{X,Y|Z}$ as the time bin width increased, this corresponds to
 1220 the result for $E[\text{cov}(X, Y|Z)]$. We observed different trends for ρ_{signal} for each mouse, this
 1221 corresponds to the result for $\text{cov}(E[X|Z], E[Y|Z])$.

1222 We applied our network noise rejection and community detection process to networks
 1223 based on the spike count correlations $\rho_{X,Y|Z}$ and the signal correlations ρ_{signal} . We noted that
 1224 the community detection on $\rho_{X,Y|Z}$ behaved similarly to the community detection on the total
 1225 correlation. We can see this in figures 2.11a and 2.11b. At very short time bin widths, we
 1226 detect more communities, and those communities often contain cells from one brain region
 1227 only. At longer time bin widths, we detect fewer communities, and those communities tend
 1228 to contain cells from multiple brain regions. When we examine the distance between (or
 1229 similarity between) the anatomical division of the cells, and the detected communities we
 1230 notice that the two clusterings are more similar at shorter time bin widths (see figure 2.12).

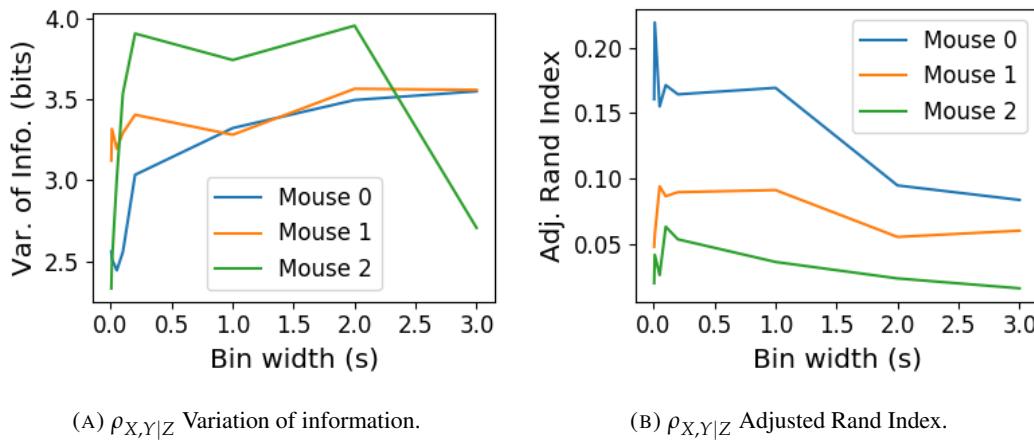
(A) $\rho_{X,Y|Z}$ Variation of information.(B) $\rho_{X,Y|Z}$ Adjusted Rand Index.

FIGURE 2.12: 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.

1231 When we applied the network noise rejection and community detection process to the
 1232 networks based on the signal correlations ρ_{signal} we found the number of communities we
 1233 detected reduced with increasing time bin width. But the number of communities detected
 1234 was less than that for the total correlations or the spike count correlations. The communi-
 1235 ties detected always tended to contain cells from multiple regions at both short and long
 1236 timescales (see figures 2.11c and 2.11d). The communities detected bore very little relation
 1237 to the anatomical division of the cells. The adjusted Rand index between the community

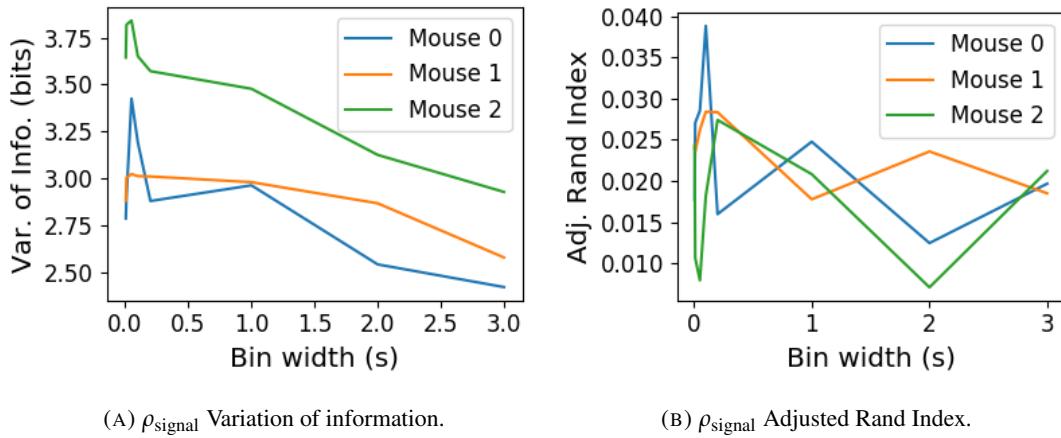


FIGURE 2.13: Distance and similarity measures between the anatomical division of the neurons, and the communities detected in the network based on the signal correlations ρ_{signal} . (A) The variation of information is a ‘distance’ measure between clusterings. The distance between the anatomical ‘clustering’ 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 time bin width increases.

1238 clustering and the anatomical ‘clustering’ is close to zero for every time bin width (see figure
 1239 2.13b). This indicates that the similarity between the clusterings is close to chance. We did
 1240 observe a slight downward trend in the variation of information with increasing bin width
 1241 (see figure 2.13a), but this is more likely due to a decrease in the number of communities
 1242 detected rather than any relationship with anatomy.

1243 We also observed that the network noise rejection process rejected some of the cells
 1244 when applied to the network based on the signal correlations. This means that those cells
 1245 did not contribute to the additional structure of the network beyond that captured by the
 1246 sparse weighted configuration model. This is why the matrices in figures 2.11c and 2.11d are
 1247 smaller than their analogues in figures 2.11a and 2.11b.

1248 2.2.8 Absolute correlations and negative rectified correlations

1249 At the moment, the network noise rejection protocol can only be applied to weighted undi-
 1250 rected graphs with non-negative weights. This meant that we had to rectify our correlated
 1251 networks before applying the network noise rejection and community detection process. We
 1252 wanted to investigate what would happen if instead of rectifying the correlations, we used the
 1253 absolute value, or reversed the signs of the correlations and then rectified.

1254 When we used the absolute value of the correlations, we found very similar results to

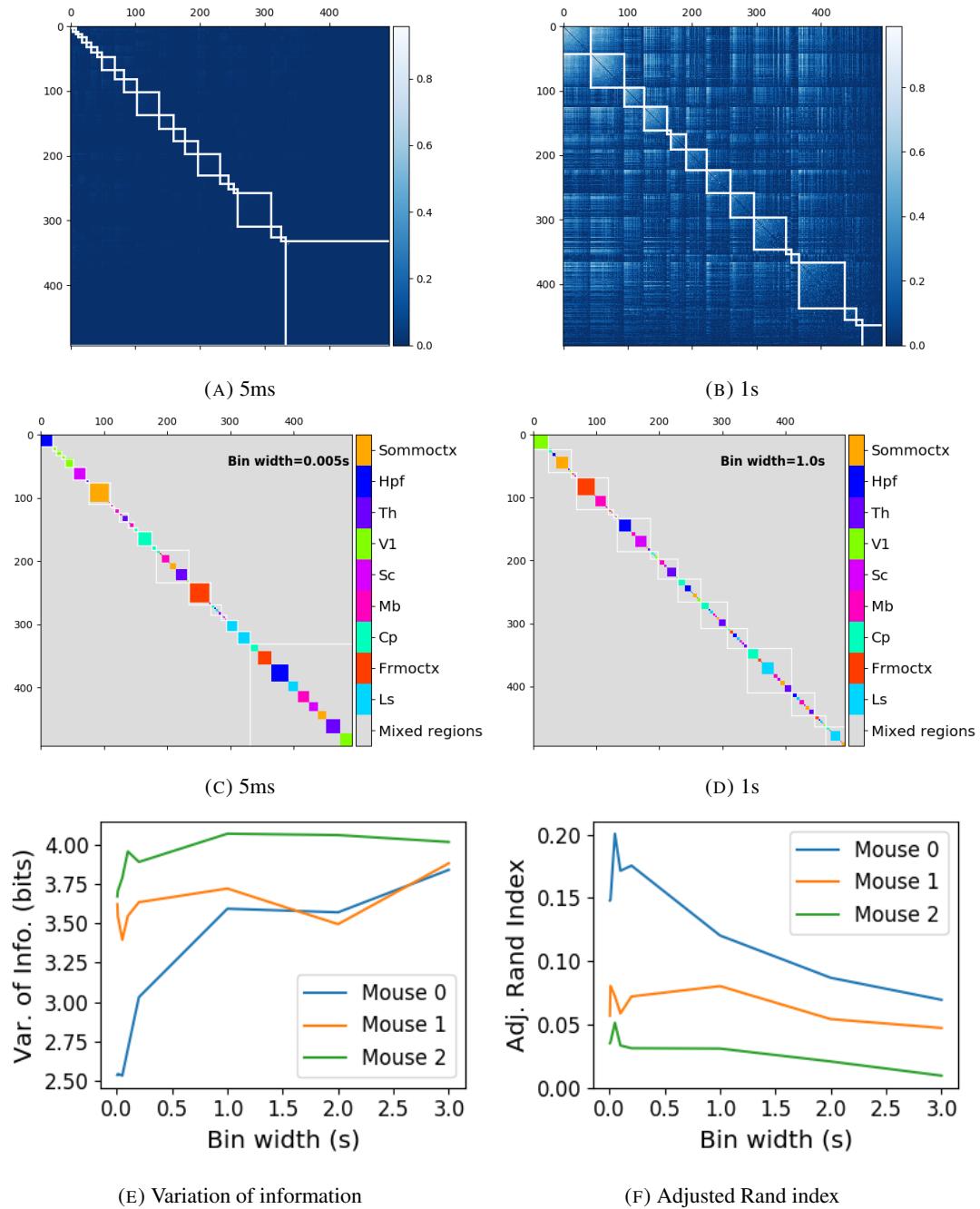


FIGURE 2.14: (A-B) Absolute correlation matrices with detected communities indicated by white lines. These communities are based on the absolute value of the total correlation between each pair of cells. Those entries within a white square indicate that both of those neurons are in the same community. 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.

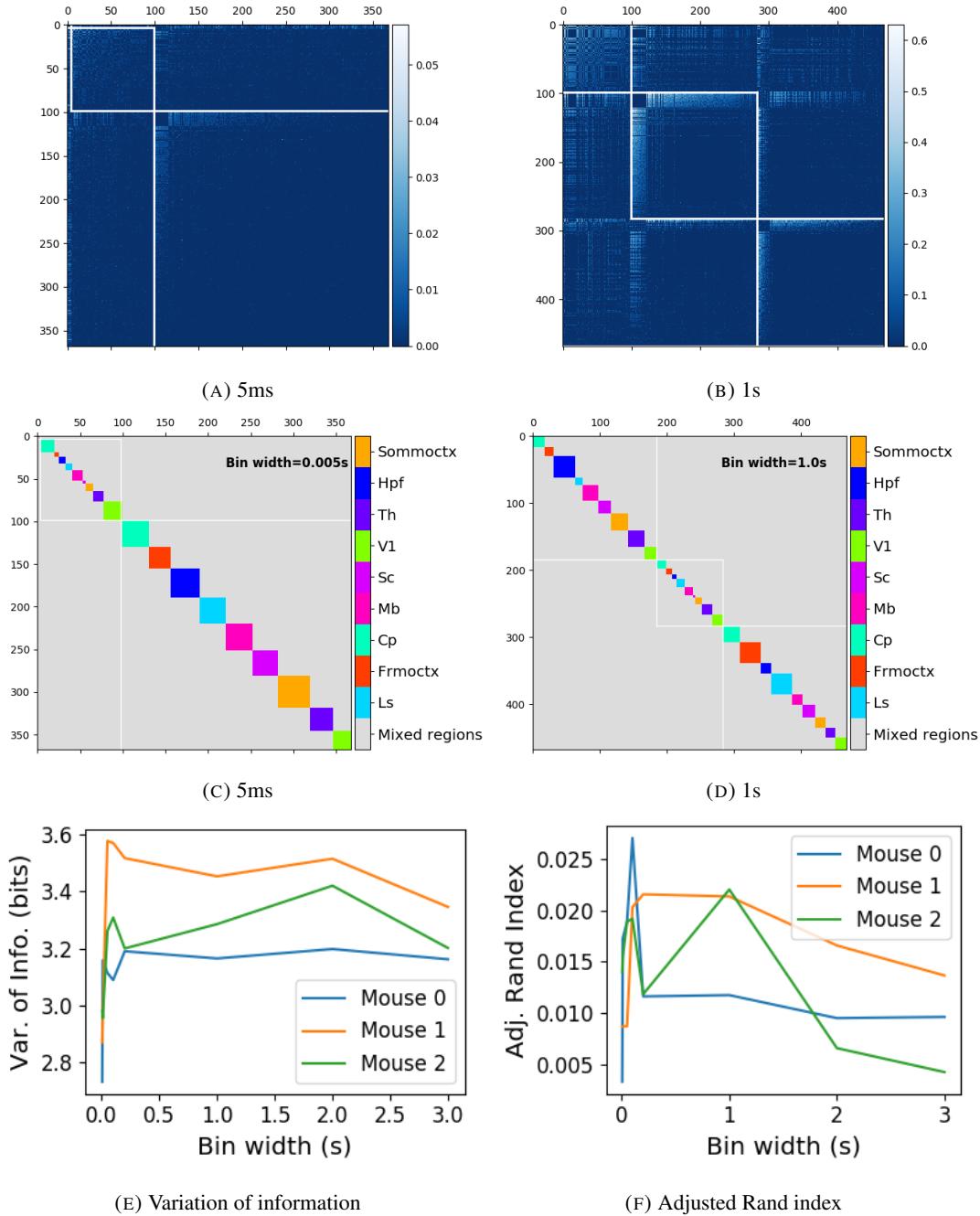


FIGURE 2.15: (A-B) Sign reversed rectified correlation matrices with detected communities indicated by white lines. Those entries within a white square indicate that both of those neurons are in the same community. Matrices shown are for 5ms and 1s time bin widths respectively. Main diagonal entries 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.

1255 those shown above for the rectified total correlations and the rectified spike count corre-
1256 lations. We detected more communities using shorter bin widths, and these communities
1257 were more similar to the brain's anatomy than those communities detected using a longer bin
1258 width (see figure 2.14). The only exception being that we detected more communities. This
1259 could indicate that we detected both positively and negatively correlated communities, but
1260 we haven't done any further investigation so we cannot say for sure.

1261 When we used the sign reversed rectified correlated networks, we tended to find fewer
1262 communities. Each community contained cells from many different anatomical regions, at
1263 both long and short bin widths (see figures 2.15a, 2.15b, 2.15c, 2.15d). The communities
1264 bore little relation to the anatomical distribution of the cells, this can be seen in figure 2.15f,
1265 the values close to zero indicate that the similarity between the two clusterings are around
1266 chance level. This indicates that there was not much structure in the negatively correlated
1267 networks beyond that captured by the sparse weighted configuration model.

1268 2.3 Discussion

1269 It is well established that the brain uses correlated behaviour in neuronal ensembles to repre-
1270 sent the information taken in through sensation (Cohen and Maunsell, 2009; Litwin-Kumar,
1271 Chacron, and Doiron, 2012; deCharms and Merzenich, 1996). However, most studies that
1272 examine the nature of these correlations in-vivo, study an ensemble of cells from only one or
1273 two brain regions (Cohen and Kohn, 2011; Wierzynski et al., "2009"; Patterson et al., 2014;
1274 Girard, Hupé, and Bullier, 2001). Furthermore, recent results have shown that behaviour can
1275 drive correlated activity in multiple brain regions, including those not normally associated
1276 with motor control (Stringer et al., 2019; Gründemann et al., 2019; Allen et al., 2019). In this
1277 study, we utilised one of the newly recorded large datasets containing electrophysiological
1278 recordings from multiple brain regions simultaneously. We investigated correlated behaviour
1279 in these different brain regions and we investigated correlated behaviour between neurons in
1280 different regions, during spontaneous behaviour.

1281 A number of studies have found that the timescale of correlated behaviour induced by a
1282 stimulus can be modulated by the stimulus structure and behavioural context. For example,
1283 the spike train correlations between cells in weakly electric fish are modulated by the spa-
1284 tial extent of the stimulus (Litwin-Kumar, Chacron, and Doiron, 2012), and neurons in the
1285 marmoset primary auditory cortex modulate their spike timing (and therefore correlation) in
1286 response to stimulus features without modulating their firing rate (deCharms and Merzenich,

1287 1996). Furthermore, the width of the time bins over which spike counts are measured has
1288 been shown to have an effect on the magnitude of those correlations (Cohen and Kohn, 2011).
1289 Despite this, very little research has been done comparing correlation measures from the same
1290 dataset at different timescales. We investigated this by varying the time bin width used to bin
1291 spike times into spike counts from as short as 5ms up to 3s.

1292 In order to further investigate the effect of these correlations at different timescales, we
1293 regarded our neuronal ensemble as a weighted undirected graph, where each neuron is rep-
1294 resented by a node, and the weight on each edge is the correlation between the neurons
1295 connected by that edge. We then applied a novel clustering method from network science
1296 (Humphries et al., 2019) to identify communities in these networks. Communities in a net-
1297 work graph refer to sets of nodes that are more strongly connected to each other than the
1298 nodes outside of their set. Another way to put this is to say that the nodes in a community
1299 are more strongly connected than *expected*. What connection strength might be expected is
1300 defined by a null network model. We chose a null network model that matched the sparsity
1301 and total strength of our correlation based data networks. So, if two cells were in the same
1302 community, those cells were more correlated than would be expected given the correlation
1303 strength of their ensemble.

1304 These networks, and the community detection process, were completely agnostic of the
1305 anatomical division of the cells in our ensemble. When we compared the detected commu-
1306 nities with the anatomical division of the cells using distance and similarity measures for
1307 clusterings, we found that the detected communities were more similar to the anatomical
1308 division at shorter timescales. That is, when we used a wider time bin to count spikes, and
1309 computed pairwise correlations with these spike counts, the correlated communities tended to
1310 exist within anatomical regions at shorter timescales, and tended to span anatomical regions
1311 at longer timescales. This could reflect localised functional correlations at short time scales
1312 rippling outwards across brain regions at longer timescales. The brain may be processing
1313 some information quickly locally, and carrying out further, perhaps more detailed, represen-
1314 tation over a longer timescale across many regions using the representations that were just
1315 built locally.

1316 These changes in communities across timescales could also be driven by the anatomy
1317 of the individual cells. For example, it may simply take longer to transmit action potentials
1318 over longer distances, hence correlated activity over longer timescales will exist between
1319 anatomical regions, rather than within. However, the switch to almost exclusively multi-
1320 regional functional networks at 1s bin widths, rather than a mixture of multi-region, and

single-region suggests that the inter-regional correlations either overpower, or inhibit the local correlations. So there may be more at play than just timescales.

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

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

Another space for further investigation is the community detection. The algorithm that we used here never detects overlapping communities. But functional communities could indeed have overlaps. Clustering methods that detect overlapping clusters do exist (Baadel, Thabtah, and Lu, 2016). Applying one of those algorithms could yield some interesting results. Also, the community detection algorithm that we used here cannot process graphs with negative weights, this forced us to separate positive and negative correlations before applying our network noise rejection and community detections process, or use the absolute value of our

1355 correlations. A community detection algorithm that can work on weighted undirected graphs
1356 with negative weights could yield some interesting results here.

1357 2.4 Data

1358 The data that we used in this project were collected by Nick Steinmetz and his lab members
1359 (Stringer et al., 2019).

1360 2.4.1 Brain regions

1361 Neuropixels probes were used to collect extracellular recordings (Jun et al., 2017) from three
1362 different mice. The mice were awake, headfixed, and engaging in spontaneous behaviour.
1363 The mice were of different sexes and different ages. One mouse was ‘wild-type’, the others
1364 were mutants. Details as follows:

- 1365 1. male, wild type, P73.
- 1366 2. female, TetO-GCaMP6s, Camk2a-tTa, P113
- 1367 3. male, Ai32, Pvalb-Cre, P99

1368 Eight probes were used to collect readings from 2296, 2668, and 1462 cells respectively.

1369 Data were collected from nine brain regions in each mouse:

- 1370 • Caudate Putamen (CP)
- 1371 • Frontal Motor Cortex (Frmoctx)
- 1372 • Hippocampal formation (Hpf)
- 1373 • Lateral Septum (Ls)
- 1374 • Midbrain (Mb)
- 1375 • Superior Colliculus (Sc)
- 1376 • Somatomotor cortex (Sommocortex)
- 1377 • Thalamus (Th)
- 1378 • Primary visual cortex (V1)

1379 Readings were continuous and lasted for about 1 hour (Stringer et al., 2019). Locations of
1380 each of the probes can be seen in figure 2.16.

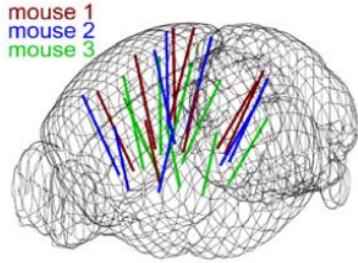


FIGURE 2.16: **Probe Locations:** The locations of the probes in each of the three mouse brains (Stringer et al., 2019).

1381 2.4.2 Video recordings

1382 Video recordings of the mouse's face were taken during the spontaneous behaviour. We had
 1383 access to the top 500 principle components and top 500 eigenvectors of the processed videos.
 1384 The frequency of recording was slightly less than 40Hz. Each frame contained 327×561
 1385 pixels. These principal components were used as behavioural data. We controlled for these
 1386 components when taking measurements conditioned on behaviour.

1387 2.5 Methods

1388 2.5.1 Binning data

1389 We transformed the spike timing data into binned spike count data by dividing the exper-
 1390 imental period into time bins and counting the spikes fired by each cell within the time period
 1391 covered by each of those bins. The data were divided into time bins of various widths ranging
 1392 from 0.01s to 4s.

1393 If the total length of the recording period was not an integer multiple of the time bin
 1394 width, we cut off the remaining time at the end of the recording period. This period was at
 1395 most 3.99s. This is far less than the total recording time of around 1 hour. So, this detail
 1396 would not affect our results.

1397 2.5.2 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:

$$\rho_{XY} = \frac{\text{cov}(X, Y)}{\sigma_X \sigma_Y} \quad (2.1)$$

$$= \frac{E[(X - \mu_X)(Y - \mu_Y)]}{\sigma_X \sigma_Y} \quad (2.2)$$

1398 where E denotes the expected value, μ denotes the mean, and σ denotes the standard deviation.
 1399 The correlation coefficient is a normalised measure of the covariance. It can take values
 1400 between 1 (completely correlated) and -1 (completely anti-correlated). Two independent
 1401 variables will have a correlation coefficient of 0. But, having 0 correlation does not imply
 1402 independence.

If we do not know the means and standard deviations required for equation 2.1, but we have samples from X and Y , Pearson's sample correlation coefficient is defined as:

$$r_{XY} = \frac{\sum_{i=1}^n (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_{i=1}^n (x_i - \bar{x})^2} \sqrt{\sum_{i=1}^n (y_i - \bar{y})^2}} \quad (2.3)$$

1403 where $\{(x_i, y_i)\}$ for $i \in \{1, \dots, n\}$ are the paired samples from X and Y , and $\bar{x} = \frac{1}{n} \sum_{i=1}^n x_i$,
 1404 and $\bar{y} = \frac{1}{n} \sum_{i=1}^n y_i$ are the sample means.

1405 In practice we used the python function `scipy.stats.pearsonr` to calculate the
 1406 correlation coefficients.

1407 **Total correlations, r_{SC}**

1408 The total correlation (r_{SC}) of two cells is the correlation between the spike counts of those
 1409 cells in response to a given stimulus condition.

1410 **Shuffled total correlations**

1411 We measured the shuffled total correlations between two neurons by randomly permuting one
 1412 of the neuron's spike counts and measuring the total correlations. These shuffled correlations
 1413 were useful when measuring the effect of time bin width on correlations, and when decid-
 1414 ing which correlations should be preserved when creating correlation networks (see section
 1415 2.5.5).

1416 **Separating Correlations & Anti-correlations**

1417 In order to compare the effect of bin width on measures of negative r_{SC} (anti-correlation) and
 1418 positive r_{SC} separately, we had to separate correlated and anti-correlated pairs. To do this, we

simply measured the mean r_{SC} , taking the mean across all the bin widths. If this quantity was positive or zero we regarded the pair as positively correlated. If this quantity was negative we regarded the pair as anti-correlated.

2.5.3 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 2.4.2). 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

$$E[X|Z_1, \dots, Z_{500}] = \int_{x \in X} x P(X = x|Z_1, \dots, Z_{500}) dx \quad (2.4)$$

$$= \int_{x \in X} x \frac{P(X = x, Z_1, \dots, Z_{500})}{P(Z_1, \dots, Z_{500})} dx \quad (2.5)$$

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

Linear regression

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

• $L1$ or 'Lasso'

• $L2$ or 'Ridge regression'

• 'Elastic net' regularisation (a linear combination of both $L1$ and $L2$ regularisation penalties)

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

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

$$L(\lambda_1, \lambda_2, \boldsymbol{\beta}) = |\mathbf{x} - \mathbf{Z}\boldsymbol{\beta}|^2 + \lambda_2|\boldsymbol{\beta}|_2 + \lambda_1|\boldsymbol{\beta}|_1 \quad (2.6)$$

where

$$|\boldsymbol{\beta}|_2 = \sum_{j=1}^m \beta_j^2 \quad (2.7)$$

$$|\boldsymbol{\beta}|_1 = \sum_{j=1}^m |\beta_j| \quad (2.8)$$

The naïve elastic net estimator $\hat{\boldsymbol{\beta}}$ is the minimiser of the system of equations 2.6 (Zou and Hastie, 2005)

$$\hat{\boldsymbol{\beta}} = \arg \min_{\boldsymbol{\beta}} L(\lambda_1, \lambda_2, \boldsymbol{\beta}) \quad (2.9)$$

1435 We implemented the model using the `ElasticNetCV` method of Python's
1436 `sklearn.linear_models` package.

1437 As well as using the PCs, we also tried fitting the models using the raw video data recon-
1438 structed from the PCs and eigenvectors. These models performed worse than those using the
1439 PCs. We expected this because each representation contains the same amount of information,
1440 but the raw video representation spreads this information across many more components.
1441 This requires more parameter fitting, but given the same information.

1442 **Conditional covariance**

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

$$\text{cov}(X, Y) = E[\text{cov}(X, Y|Z)] + \text{cov}(E[X|Z], E[Y|Z]) \quad (2.10)$$

1443 where these expected values are calculated with respect to the distribution of Z as a random
1444 variable.

1445 The law of total covariance breaks the covariance into two components. The first com-
1446 ponent $E[\text{cov}(X, Y|Z)]$ is the expected value, under the distribution of Z , of the conditional
1447 covariance $\text{cov}(X, Y|Z)$. This covariance could be interpreted as the unnormalised version
1448 of what Cohen et al. (2011) call the spike count correlation (Cohen and Kohn, 2011), aka.
1449 the noise correlation. In particular, this is the covariance of the spike counts in response to
1450 repeated presentation of identical stimuli.

1451 The second component is analogous to what Cohn et al. (2011) call the *signal correlation*
1452 (Cohen and Kohn, 2011). In particular, $\text{cov}(E[X|Z], E[Y|Z])$ is the covariance between

1453 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 proceeded to calculate

$$E[\text{cov}(X, Y|Z_1, \dots, Z_{500})] = \text{cov}(X, Y) - \quad (2.11)$$

$$\text{cov}(E[X|Z_1, \dots, Z_{500}], E[Y|Z_1, \dots, Z_{500}]) \quad (2.12)$$

1454 Measures of conditional correlation

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

$$\rho_{XY|Z} = \frac{E[\text{cov}(X, Y|Z)]}{\sqrt{E[\text{var}(X|Z)]}E[\text{var}(Y|Z)]} \quad (2.13)$$

1455 Although this is not an actual correlation, it is an intuitive analogue to the correlation as a
1456 normalised version of the covariance.

For comparison, we also measured the ‘signal correlation’

$$\rho_{\text{signal}} = \frac{\text{cov}(E[X|Z], E[Y|Z])}{\sqrt{\text{var}(E[X|Z])}\sqrt{\text{var}(E[Y|Z])}} \quad (2.14)$$

1457 this is an actual correlation.

1458 2.5.4 Information Theory

1459 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

$$H(X) = - \sum_{n=1}^N p_n \log_2 p_n \quad (2.15)$$

1460 This quantity is also known as the information entropy or the ‘surprise’. It measures the
1461 amount of uncertainty in a random variable. For example, a variable with a probability of 1
1462 for one outcome, and 0 for all other outcomes will have 0 bits entropy, because it contains no
1463 uncertainty. But a variable with a uniform distribution will have maximal entropy as it is the
1464 least predictable. This quantity is analogous to the entropy of a physical system (Shannon,

1465 1948). Note that any base may be used for the logarithm in equation 2.15, but using base 2
 1466 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

$$H(X, Y) = - \sum_{n=1}^N \sum_{m=1}^M P(X = x_n, Y = y_m) \log_2 P(X = x_n, Y = y_m) \quad (2.16)$$

1467 If X and Y are independent then $H(X, Y) = H(X) + H(Y)$. Otherwise $H(X, Y) <$
 1468 $H(X) + H(Y)$. 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

$$H(Y|X) = - \sum_{n=1}^N \sum_{m=1}^M P(X = x_n, Y = y_m) \log_2 \frac{P(X = x_n, Y = y_m)}{P(X = x_n)} \quad (2.17)$$

1469 When X and Y are independent $H(Y|X) = H(Y)$. Intuitively, we learn nothing of Y by
 1470 knowing X , so Y is equally uncertain whether we know X or not. If Y is totally dependent
 1471 on X , then the fraction in the logarithm is 1, which gives $H(Y|X) = 0$.

1472 These entropy measures are the basis of the mutual information measure.

1473 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

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

1474 Therefore, the maximum entropy of the binned spike counts of a neuron is $\log_2(n_{\max} + 1)$.
 1475 Of course, it would be very unusual for a neuron to fire in accordance with the discrete
 1476 uniform distribution. Most measurements of entropy taken on binned spiking data will be
 1477 much lower than the maximum. See figure 2.17 to see the maximum entropy as a function of
 1478 the maximum observed spike count.

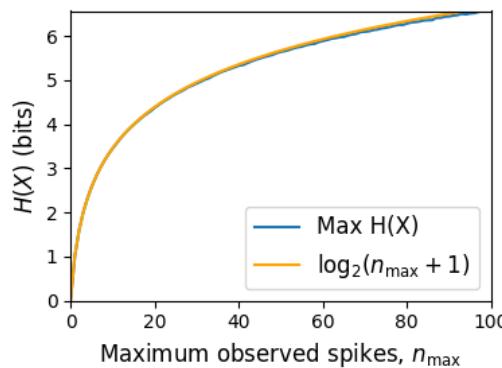


FIGURE 2.17: **Entropy Limit:** The upper limit on entropy of binned spike count data as a function of the maximum observed spike count. The orange line is the analytical maximum. The blue line is the entropy of samples with $N = 1000$ data points taken from the discrete uniform distribution.

1479 **Mutual Information $I(X; Y)$**

1480 The mutual information can be defined mathematically in a number of ways, all of which are
 1481 equivalent. These definitions illustrate the different ways of interpreting the mutual informa-
 1482 tion.

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

$$I(X; Y) = H(Y) - H(Y|X) \quad (2.18)$$

$$= H(X) - H(X|Y) \quad (2.19)$$

1483 Equation 2.18 fits with the following intuition: The mutual information between X and Y is
 1484 the reduction in uncertainty about X gained by knowing Y , or vice versa. We could also say
 1485 the mutual information is the amount of information gained about X by knowing Y , or vice
 1486 versa.

Another useful entropy based definition for the mutual information is

$$I(X; Y) = H(X) + H(Y) - H(X, Y) \quad (2.20)$$

1487 This definition is useful because it does not require the calculation of conditional probabilities.
 1488

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

$$I(X;Y) = - \sum_{n=1}^N \sum_{m=1}^M P(X = x_n, Y = y_m) \log_2 \frac{P(X = x_n, Y = y_m)}{P(X = x_n)P(Y = y_m)} \quad (2.21)$$

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

$$I(X;Y) = D_{KL}(P(X,Y) || P(X)P(Y)) \quad (2.22)$$

1489 So, we can also think of the mutual information as a measure of the difference between
 1490 the joint distribution of X and Y , and the product of their marginal distributions. Since the
 1491 product of the marginal distributions is the joint distribution for independent variables, we
 1492 can think of the mutual information as a measure of the variables' dependence on one another.

1493 The minimum value that $I(X;Y)$ can take is 0. This occurs when the random variables
 1494 X and Y are independent. Then we have $H(X|Y) = H(X)$, and $H(Y|X) = H(Y)$, which
 1495 according to equation 2.18, gives $I(X;Y) = 0$. We also have that $H(X,Y) = H(X) +$
 1496 $H(Y)$ in this case, which according equation 2.20, gives $I(X;Y) = 0$. Finally, we also have
 1497 $P(X,Y) = P(X)P(Y)$, which leaves us with 1 in the argument for the logarithm in equation
 1498 2.21, which again gives $I(X;Y) = 0$.

1499 The mutual information reaches its maximum value when one of the variables X and
 1500 Y is completely determined by knowing the value of the other. In that case $I(X;Y) =$
 1501 $\min\{H(X), H(Y)\}$.

1502 Variation of Information $VI(X,Y)$

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

$$VI(X;Y) = H(X) + H(Y) - 2I(X;Y) \quad (2.23)$$

We can rewrite this as the summation of two positive quantities

$$VI(X;Y) = [H(X) - I(X;Y)] + [H(Y) - I(X;Y)] \quad (2.24)$$

1503 In English, the variation of information is the summation of the uncertainty in the random
1504 variables X and Y excluding the uncertainty shared by those variables.

1505 This measure will become more relevant when we go on to talk about clusterings because
1506 $VI(X; Y)$ forms a metric on the space of clusterings.

1507 **Measuring entropies & mutual information**

1508 In practice, we measured the mutual information between spike counts using Python and the
1509 python package `pyitlib`. We used the PT-bias correction technique to estimate the bias of
1510 our measurements when measuring the mutual information between the spike counts of two
1511 cells (Treves and Panzeri, 1995).

1512 When measuring the mutual information between clusterings we used Python, but we
1513 used the `mutual_info_score`, `adjusted_mutual_info_score`, and
1514 `normalized_mutual_info_score` functions from the `sklearn.metrics` part of
1515 the `sklearn` package.

1516 **2.5.5 Network analysis**

1517 **Correlation networks**

1518 In order to analyse functional networks created by the neurons in our ensemble, we mea-
1519 sured the total correlation between each pair of neurons. These measurements induced an
1520 undirected weighted graph/network between the neurons. The weight of each connection
1521 was equal to the total correlation between each pair of neurons.

1522 We followed the same procedure for total correlations 2.5.2, spike count correlations, and
1523 signal correlations 2.5.3.

1524 **Rectified correlations**

1525 At the time of writing, the community detection method outlined in (Humphries et al., 2019)
1526 could only be applied to networks with positively weighted connections. But many neuron
1527 pairs were negatively correlated. To apply the community detection method, we *rectified* the
1528 network, by setting all the negative weights to zero.

1529 We also looked for structure in the network created by negative correlations by reversing
1530 the signs of the correlations, and rectifying these correlations before applying our network
1531 analysis.

1532 Finally, we used the absolute value of the correlations as the weights for the graph/network.
1533 By doing this, we hoped to identify both correlated and anti-correlated functional communi-
1534 ties of neurons.

1535 **Sparsifying data networks**

1536 When creating our correlation networks, we wanted to exclude any correlations that could
1537 be judged to exist ‘by chance’. To do this, we measured the 5th and 95th percentile of
1538 the shuffled correlations (see section 2.5.2) for the given mouse and time bin width. We
1539 then set all the data correlations between these two values to 0. This excluded any ‘chance’
1540 correlations from our network, and created a sparser network. This allowed us to make use
1541 of the ‘sparse weighted configuration model’ as described in section 2.5.5.

1542 **Communities**

1543 Given some network represented by an adjacency matrix \mathbf{A} , a community within that net-
1544 work is defined as a collection of nodes where the number of connections within these nodes
1545 is higher than the expected number of connections between these nodes. In order to quan-
1546 tify the ‘expected’ number of connections, we need a model of expected networks. This is
1547 analogous to a ‘null model’ in traditional hypothesis testing. We test the hypothesis that our
1548 data network departs from the null network model to a statistically significant degree. For
1549 undirected unweighted networks, the canonical model of a null network is the configuration
1550 model (Fosdick et al., 2016). Since we are working with weighted sparse networks, we used
1551 more suitable null models, described below.

1552 **Weighted configuration model**

1553 The *weighted configuration model* is a canonical null network model for weighted networks.
1554 Given some data network, the weighted configuration model null network will preserve the
1555 degree sequence and weight sequence of each node in the data network. But the edges will
1556 be distributed randomly (Fosdick et al., 2016). Any structure in the data network beyond
1557 its degree sequence and weight sequence will not be captured in the weighted configuration
1558 model. So, this model can be used in testing the hypothesis that this extra structure exists.

1559 **Sparse weighted configuration model**

1560 The *sparse weighted configuration model* is another null network model. Similar in nature to
1561 the weighted configuration model (see section 2.5.5), but the sparsity of the data network is

1562 preserved in the null network. This is achieved by sampling from a probability distribution
 1563 for the creation or non-creation of each possible connection, then distributing the weight of
 1564 the data network randomly in this sparse network (Humphries et al., 2019). This is the null
 1565 network that we used when searching for additional structure in our data networks.

1566 **Spectral rejection**

1567 We made use of the spectral rejection algorithm as outlined in (Humphries et al., 2019). The
 1568 spectral rejection algorithm is a method for finding structure in a network not captured by a
 1569 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

$$\mathbf{B} = \mathbf{W} - \langle \mathbf{P} \rangle \quad (2.25)$$

1570 a common choice for $\langle \mathbf{P} \rangle$ in community detection is the ‘configuration model’ (Fosdick et
 1571 al., 2016; Humphries, 2011). The matrix \mathbf{B} is often called the configuration matrix, in this
 1572 context we will use the term ‘deviation matrix’ as it captures the deviation of \mathbf{W} from the
 1573 null model.

1574 To test for structure in the network represented by \mathbf{W} , we examine the eigenspectrum of \mathbf{B}
 1575 and compare it to the eigenspectrum of our null model. Firstly, note that since our data model
 1576 doesn’t allow self loops, and is not directed, the matrix representing the network will be
 1577 symmetric and positive semi-definite, and will therefore be invertible with real eigenvalues.
 1578 We selected a null model with the same characteristics.

1579 To find the eigenspectrum of the null model, we generated N samples from our null
 1580 model P_1, \dots, P_N , and we measured their deviation matrices B_1, \dots, B_N . We then calculated
 1581 the eigenspectrum of each of those samples. We calculated the upper bound of the null model
 1582 eigenspectrum by taking the mean of the largest eigenvalues of B_1, \dots, B_N . We calculated a
 1583 lower bound on the null model eigenspectrum by taking the mean of the smallest eigenvalues
 1584 of B_1, \dots, B_N .

1585 We then calculated the eigenspectrum of \mathbf{B} , our data network deviation matrix. If any of
 1586 those eigenvalues lay outside of the upper or lower bounds of the null model eigenspectrum,
 1587 this is evidence of additional structure not captured by the null model. If we chose the sparse
 1588 weighted configuration model (see section 2.5.5) as our null network model, then eigenvalues

1589 lying below the lower bound indicate k -partite structure in the network. For example, if one
1590 eigenvalue lay below the lower bound, this would indicate some bipartite structure in the data
1591 network. If any eigenvalues lay above the upper bound of the null model eigenspectrum, this
1592 is evidence of community structure in the data network. For example, one eigenvalue of \mathbf{B}
1593 lying above the upper bound of the null model eigenspectrum indicates the presence of two
1594 communities in the network (Humphries, 2011).

1595 **Node rejection**

1596 If there are d data eigenvalues lying outside of the null network eigenspectrum, the d eigen-
1597 vectors corresponding to these eigenvalues will form a vector space. If we project the nodes
1598 of our network into this vector space, by projecting either rows or columns of the data ma-
1599 trix, we can see how strongly each node contributes to the vector space. Nodes that contribute
1600 strongly to the additional structure will project far away from the origin, nodes that do not
1601 contribute to the additional structure will project close to the origin. We want to use this
1602 information to discard those nodes that do not contribute.

1603 We can test whether a node projects *far* away from the origin or *close* to the origin
1604 using the eigenvalues and eigenvectors of B_1, \dots, B_N . The j th eigenvector and eigenvalue
1605 of B_i gives a value for a null network's projection into the j th dimension of the additional
1606 structure vector space. The matrices B_1, \dots, B_N give N projections into that dimension.
1607 These projections are a distribution of the null networks' projections. If the data node's
1608 projection exceeds that of the null network projections this node is judged to project *far* from
1609 the origin, and therefore contribute to the additional structure. Otherwise, the node is judged
1610 to project *close* to the origin, and is therefore rejected (Humphries et al., 2019).

1611 **Community detection**

1612 Another application for this d dimensional space is community detection. We first project
1613 all of the nodes into this d -dimensional space, then perform the clustering in this space. The
1614 clustering and community detection procedure is described in (Humphries, 2011).

1615 In practice, the procedure is carried out n times (we chose $n = 100$ times), this returns n
1616 clusterings. We resolve these n clusterings to one final clustering using *consensus clustering*.
1617 We used the consensus clustering method that uses an explicit null model for the consensus
1618 matrix, as outlined in (Humphries et al., 2019).

2.5.6 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 $k, l \in \{1, \dots, K\}$:

$$C_k \cap C_l = \emptyset \quad (2.26)$$

$$\bigcup_{k=1}^K C_k = D \quad (2.27)$$

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

Adjusted Rand Index

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

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

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

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

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

- 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

$$RI = \frac{N_{11} + N_{00}}{N_{11} + N_{00} + N_{10} + N_{01}} = \frac{N_{11} + N_{00}}{\binom{n}{2}} \quad (2.28)$$

The Rand Index is 1 when the clusterings are identical, and 0 when the clusterings are completely different.

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

This is defined as

$$ARI = \frac{2(N_{00}N_{11} - N_{01}N_{10})}{(N_{00} + N_{01})(N_{01} + N_{11}) + (N_{00} + N_{10})(N_{10} + N_{11})} \quad (2.29)$$

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

1636 **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

$$P(K = k) = \frac{n_k}{n} \quad (2.30)$$

1637 this defines a probability distribution, which makes the clustering a random variable. Any 1638 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 2.5.4 with respect to clusterings. For example, the entropy of a clustering is

$$H(\mathcal{C}) = - \sum_{k=1}^K \frac{n_k}{n} \log \frac{n_k}{n} \quad (2.31)$$

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

$$P(K = k, K' = k') = \frac{|C_k \cap C'_{k'}|}{n} \quad (2.32)$$

1639 The joint distribution allows us to define the mutual information between two clusterings, 1640 $I(\mathcal{C}; \mathcal{C}')$ (Meilă, 2007).

1641 **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)

$$NMI_{joint} = \frac{I(\mathcal{C}; \mathcal{C}')}{H(\mathcal{C}, \mathcal{C}')} \quad (2.33)$$

$$NMI_{max} = \frac{I(\mathcal{C}; \mathcal{C}')}{\max\{H(\mathcal{C}), H(\mathcal{C}')\}} \quad (2.34)$$

$$NMI_{sum} = \frac{2I(\mathcal{C}; \mathcal{C}')}{H(\mathcal{C}) + H(\mathcal{C}')} \quad (2.35)$$

$$NMI_{sqrt} = \frac{I(\mathcal{C}; \mathcal{C}')}{\sqrt{H(\mathcal{C})H(\mathcal{C}')}} \quad (2.36)$$

$$NMI_{min} = \frac{I(\mathcal{C}; \mathcal{C}')}{\min\{H(\mathcal{C}), H(\mathcal{C}')\}} \quad (2.37)$$

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

$$AMI_{sum} = \frac{I(\mathcal{C}; \mathcal{C}') - E\{I(\mathcal{C}; \mathcal{C}')\}}{\frac{1}{2}[H(\mathcal{C}) + H(\mathcal{C}')] - E\{I(\mathcal{C}; \mathcal{C}')\}} \quad (2.38)$$

1642 The first term in the denominator, taking the average of the marginal entropies, can be re-
1643 placed by taking the maximum, minimum, or the geometric mean (Vinh, Epps, and Bailey,
1644 2010).

1645 Information based metrics

The variation of information between two clusterings $VI(\mathcal{C}; \mathcal{C}')$ (see section 2.5.4) is a metric on the space of clusterings (Meilă, 2007). That is,

$$VI(\mathcal{C}; \mathcal{C}') \geq 0 \quad (2.39)$$

$$VI(\mathcal{C}; \mathcal{C}') = 0 \iff \mathcal{C} = \mathcal{C}' \quad (2.40)$$

$$VI(\mathcal{C}; \mathcal{C}') = VI(\mathcal{C}'; \mathcal{C}) \quad (2.41)$$

$$VI(\mathcal{C}; \mathcal{C}'') \leq VI(\mathcal{C}; \mathcal{C}') + VI(\mathcal{C}'; \mathcal{C}'') \quad (2.42)$$

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

$$D_{max} = \max\{H(\mathcal{C}), H(\mathcal{C}')\} - I(\mathcal{C}; \mathcal{C}') \quad (2.43)$$

Both of these can be normalised

$$NVI(\mathcal{C}; \mathcal{C}') = 1 - \frac{I(\mathcal{C}; \mathcal{C}')}{H(\mathcal{C}, \mathcal{C}')} \quad (2.44)$$

$$d_{max} = 1 - \frac{I(\mathcal{C}; \mathcal{C}')}{\max\{H(\mathcal{C}), H(\mathcal{C}')\}} \quad (2.45)$$

1646 Comparing detected communities and anatomical divisions

1647 In order to quantify the difference or similarity between the communities detected in our cor-
1648 relation network and the anatomical classification of the cells in that network, we considered
1649 the communities and the anatomical regions as clusters in two different clusterings, \mathcal{C}_{comm}
1650 and \mathcal{C}_{anat} , respectively. We then measured the similarity between the clusterings using the
1651 mutual information, the adjusted mutual information, and the normalised mutual informa-
1652 tion. We measured the difference between, or the distance between, the clusterings using the

1653 variation of information, the normalised variation of information, and the normalised infor-
1654 mation distance. We also measured the difference between the clusterings using the adjusted
1655 Rand Index, just to use a non-information based measure.

1656 We took all of these measures for communities detected using different time bin widths.
1657 This gave us an idea of the effect of time bin width on correlation networks in neural ensem-
1658 bles relative to anatomical regions within those ensembles.

¹⁶⁵⁹ **Chapter 3**

¹⁶⁶⁰ **A simple two parameter distribution
for modelling neuronal activity and
capturing neuronal association**

1663 *Abstract*

1664 Recent developments in electrophysiological technology have lead to an increase in the size
1665 of electrophysiology datasets. Consequently, there is a requirement for new analysis tech-
1666 niques that can make use of these new datasets, while remaining easy to use in practice. In
1667 this work, we fit some one or two parameter probability distributions to spiking data collected
1668 from a mouse exposed to visual stimuli. We show that the Conway-Maxwell-binomial dis-
1669 tribution is a suitable model for the number of active neurons in a neuronal ensemble at any
1670 given moment. This distribution fits these data better than binomial or beta-binomial distribu-
1671 tions. It also captures the correlated activity in the primary visual cortex induced by stimulus
1672 onset more effectively than simply measuring the correlations, at short timescales (< 10ms).
1673 We also replicate the finding of Churchland et al (2010) relating to stimulus onset quenching
1674 neural variability in cortical areas, and we show a correspondence between this quenching
1675 and changes in one of the parameters of the fitted Conway-Maxwell-binomial distributions.

1676 **3.1 Introduction**

1677 Recent advances in electrophysiological technology, such as ‘Neuropixels’ probes (Jun et al.,
1678 2017) have allowed extracellular voltage measurements to be collected from larger numbers
1679 of cells than traditional methods, in multiple brain regions simultaneously, and routinely.
1680 These larger datasets require innovative methods to extract information from the data in a
1681 reasonable amount of time, ‘reasonable’ being subjective in this case.

1682 Theoretically, all the information at any given moment in an electrophysiological dataset
1683 with n neurons could be captured by calculating the probability distribution for every possi-
1684 ble spiking pattern. This would require defining a random variable with 2^n possible values, a
1685 task that quickly becomes impossible as n increases. Attempts at approximating this random
1686 variable often involve measuring pairwise or higher order correlations (Schneidman et al.,
1687 2006; Flach, 2013; Ganmor, Segev, and Schneidman, 2011). But pairwise correlations may
1688 not be enough to characterise instantaneous neural activity (Tkačik et al., 2014). Further-
1689 more, these kinds of models tend to ignore the temporal structure of neuronal data, in favour
1690 of smaller model size, and scalability.

1691 Higher order correlations would be helpful here, but defining these correlations can be
1692 tricky, never-mind quantifying them. If we use the interaction parameters arising from the
1693 exponential family model as measures of higher order correlations, measuring these correla-
1694 tions becomes computationally impractical quite quickly also (the number of ‘three neuron
1695 correlations’ to measure scales with $(^n_3)$). In this paper, we dispense with measuring correla-
1696 tions directly, and attempt to characterise correlated behaviour by measuring ‘association’; a
1697 more general concept that includes correlation.

1698 In this work, we examined the ability of simple distributions to model the number of
1699 active (spiking) neurons in a neuronal ensemble at any given timepoint. We compared a
1700 little-known distribution named the Conway-Maxwell-binomial distribution to the binomial
1701 distribution and the beta-binomial distribution. The binomial distribution is a probability dis-
1702 tribution over the number of successes in a sequence of independent and identical Bernoulli
1703 trials. The beta-binomial distribution is similar, but allows for a bit more flexibility while still
1704 being a model for heterogeneity. Similar to the binomial and beta-binomial, the Conway-
1705 Maxwell-binomial distribution is a probability distribution over the number of successes in a
1706 series of Bernoulli trials, but allows over- and under-dispersion relative to the binomial dis-
1707 tribution. This distribution should therefore be a good candidate for our purposes. We found
1708 that Conway-Maxwell-binomial distribution was usually the best candidate of the three that

1709 we examined.

1710 We also observed some interesting changes in the number of active neurons in the primary
1711 visual cortex and hippocampus at stimulus onset and some changes in this activity in the
1712 thalamus which were sustained for the full duration of the stimulus presentation. This let us
1713 know that there were some responses to model.

1714 We found that fitting a Conway-Maxwell-binomial distribution was a better method of
1715 capturing association between neurons than measuring the spike count correlation for the
1716 short time bins that we used (< 10ms).

1717 Finally, we also wanted to investigate parallels between the parameters of the Conway-
1718 Maxwell-binomial distribution and quantities that have been established as relevant to sen-
1719 sory processing. So, we replicated the findings made by Churchland et al. (2010) relating
1720 to a reduction in neural variability at stimulus onset in the macaque cortical regions, but for
1721 data taken from the mouse primary visual cortex. We compared these findings to the values
1722 of the fitted Conway-Maxwell-binomial distribution parameters.

1723 3.2 Data

1724 We used data collected by Nick Steinmetz and his lab ‘CortexLab at UCL’ (Steinmetz, Caran-
1725 dini, and Harris, 2019). The data can be found online ¹ and are free to use for research
1726 purposes.

1727 Two ‘Phase3’ Neuropixels (Jun et al., 2017) electrode arrays were inserted into the brain
1728 of an awake, head-fixed mouse for about an hour and a half. These electrode arrays recorded
1729 384 channels of neural data each at 30kHz and less than $7\mu\text{V}$ RMS noise levels. The sites
1730 are densely spaced in a ‘continuous tetrode’-like arrangement, and a whole array records
1731 from a 3.8mm span of the brain. One array recorded from visual cortex, hippocampus, and
1732 thalamus, the other array recorded from motor cortex and striatum. The data were spike-
1733 sorted automatically by Kilosort and manually by Nick Steinmetz using Phy. In total 831
1734 well-isolated individual neurons were identified.

1735 3.2.1 Experimental protocol

1736 The mouse was shown a visual stimulus on three monitors placed around the mouse at right
1737 angles to each other, covering about ± 135 degrees azimuth and ± 35 degrees elevation.

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

1738 The stimulus consisted of sine-wave modulated full-field drifting gratings of 16 drift di-
1739 rections ($0^\circ, 22.5^\circ, \dots, 337.5^\circ$) with 2Hz temporal frequency and 0.08 cycles/degree spatial
1740 frequency displayed for 2 seconds plus a blank condition. Each of these 17 conditions were
1741 presented 10 times in a random order across 170 different trials. There were therefore 160
1742 trials with a drifting-grating visual stimulus present, and 10 trials with a blank stimulus.

1743 **3.3 Methods**

1744 **3.3.1 Binning data**

1745 We converted the spike times for each cell into spike counts by putting the spike times into
1746 time bins of a given ‘width’ (in milliseconds). We used time bins of 1ms, 5ms, and 10ms.
1747 We used different time bin widths to assess the impact of choosing a bin width.

1748 **3.3.2 Number of *active* neurons**

1749 To count the number of active neurons in each neuronal ensemble, we split the time interval
1750 for each trial into bins of a given width. We counted the number of spikes fired by each cell
1751 in each bin. If a cell fired *at least* one spike in a given bin, we regarded that cell as active in
1752 that bin. We recorded the number of active cells in every bin, and for the purposes of further
1753 analysis, we recorded each cell’s individual spike counts.

1754 It should be noted that when we used a bin width of 1ms, the maximum number of
1755 spikes in any bin was 1. For the wider time bins, some bins had spike counts greater than
1756 1. Consequently when using a bin width of 1ms, the number of active neurons and the total
1757 spike count of a given bin were identical. But for wider bin widths, the total spike count was
1758 greater than the number of active neurons.

1759 So for the 1ms bin width, the activity of a neuron and the number of spikes fired by that
1760 neuron in any bin can be modelled as a Bernoulli variable. But for wider time bins, only the
1761 activity can be modelled in this way.

1762 **3.3.3 Moving windows for measurements**

1763 When taking measurements (e.g. moving average over the number of active neurons) or
1764 fitting distributions (eg. the beta binomial distribution) we slid a window containing a certain
1765 number of bins across the data, and made our measurements at each window position. For
1766 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 3.1: Details of the different bin width and analysis window sizes used when binning spike times, and analysing those data.

1767 the window across the time interval for each trial moving 10 bins at a time. So that for
 1768 3060ms of data, we made 296 measurements.

1769 For the 5ms bin width data, we used windows containing 40 bins, and slid the window 2
 1770 bins at a time when taking measurements.

1771 For the 10ms bin width data, we used windows containing 40 bins, and slid the window
 1772 1 bin at a time when taking measurements (see table 3.1 for concise details).

1773 By continuing to use windows containing 40 bins, we retained statistical power but sac-
 1774 rificed the number of measurements taken.

1775 There was an interval between each trial with a grey image in place of the moving of
 1776 the moving bar stimulus. This interval varied in time. But we included some of this interval
 1777 when recording the data for each trial. We started recording the number of active neurons,
 1778 and the number of spikes from each neuron from 530ms before each trial until 1030ms after
 1779 each trial. This way, we could see the change in our measurements at the onset of a stimulus
 1780 and the end of stimulus presentation.

1781 As mentioned in section 3.3.2, we recorded the number of active neurons in each bin, and
 1782 the spike count for each neuron in each bin. The actual measurements we took using these
 1783 data in each window were as follows:

1784 **Moving average** The average number of active cells in each window.

1785 **Moving variance** The variance of the number of active cells in each window.

1786 **Average correlation** We measured the correlation between the spike counts of each pair of
 1787 cells in the ensemble, and took the average of these measurements.

1788 **Binomial p** We fitted a binomial distribution to the data in each window and recorded the
 1789 fitted probability of success, p in each case.

1790 **Beta-binomial α, β** We fitted a beta-binomial distribution to the data in each window, and
 1791 recorded the values of the fitted shape parameters, α and β , of each distribution.

1792 **Conway-Maxwell-binomial distribution p, ν** We fitted a Conway-Maxwell-binomial dis-
 1793 tribution to the data in each window, and recorded the fitted values of p and ν for each
 1794 distribution.

1795 **Log-likelihoods** We also recorded the log-likelihood of each of the fitted distributions for
 1796 each window.

1797 **3.3.4 Fano factor**

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

$$F = \frac{\sigma^2}{\mu} \quad (3.1)$$

1798 We measured the Fano factor of the spike count of a given cell by measuring the mean and
 1799 variance of the spike count across trials, and taking the ratio of those two quantities. When
 1800 calculated in this way the Fano factor can be used as a measure of neural variability that
 1801 controls for changes in the firing rate. This is similar to the calculation used in (Churchland
 1802 et al., 2010).

1803 **3.3.5 Probability Distributions suitable for modelling ensemble activity**

1804 We present here three different probability distributions that could be suitable to model the
 1805 number of active neurons in an ensemble. Each distribution has the set $\{0, \dots, n\}$ as its sup-
 1806 port, where n is the number of neurons in the ensemble. These are simple distributions with
 1807 either two or three parameters each. However, we regard n as known when using these dis-
 1808 tributions for modelling, so in effect each distribution has either one or two free parameters.

1809 **Association**

1810 *Association* between random variables is similar to the correlation between random variables
 1811 but is more general in concept. The correlation is a measure of association; and association
 1812 doesn't have a mathematical definition like correlation does. Essentially, the association
 1813 between two random variables is their tendency to take the same or similar values. Positively
 1814 associated variables tend to take the same value, and negatively associated variables tend to
 1815 take different values. In this research, we work with probability distributions of the number of
 1816 successes in a set of Bernoulli trials. These Bernoulli variables may or may not be associated.

1817 A probability distribution over the number of successes in n Bernoulli trials, where the
 1818 Bernoulli variables may be associated, could constitute a good model for the number of active
 1819 neurons in an ensemble of n neurons.

1820 **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

$$P(k; n, p) = \binom{n}{k} p^k (1 - p)^{n-k} \quad (3.2)$$

1821 As a model for the activity of a neuronal ensemble, the main problem with the binomial
 1822 distribution is that it treats each neuron, represented as a Bernoulli trial, as independent. It is
 1823 well known that neurons are not independent, and that correlated behaviour between neurons
 1824 is vital for representing sensory information (Cohen and Maunsell, 2009). The binomial dis-
 1825 tribution falls short in this regard, but it is useful as performance benchmark when assessing
 1826 the performance of other models.

1827 **Beta-binomial distribution**

1828 The beta distribution is the conjugate distribution of the binomial distribution. The beta-
 1829 binomial distribution is the combination of the beta distribution and the binomial distribution,
 1830 in that the probability of success for the binomial distribution is sampled from the beta dis-
 1831 tribution. This allows the beta-binomial distribution to capture some over dispersion relative
 1832 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

$$P(k; n, \alpha, \beta) = \binom{n}{k} \frac{B(k + \alpha, n - k + \beta)}{B(\alpha, \beta)} \quad (3.3)$$

1833 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

$$\pi = \frac{\alpha}{\alpha + \beta} \quad (3.4)$$

$$\rho = \frac{1}{\alpha + \beta + 1} \quad (3.5)$$

1834 This reparametrisation is useful because π acts as a location parameter analogous to the p
 1835 parameter of a binomial distribution. A value of $\rho > 0$ indicates over-dispersion relative to a
 1836 binomial distribution.

1837 As a model for the activity of a neuronal ensemble, the beta-binomial distribution is
 1838 more suitable than a binomial distribution because the over-dispersion of the beta-binomial
 1839 distribution can be used to model positive association between the neurons. An extreme
 1840 example of this over-dispersion/positive association can be seen in figure 3.1b. In this figure,
 1841 the neurons are positively associated and so tend to take the same value, consequently the
 1842 probability mass of the beta-binomial distribution builds up close to $k = 0$ and $k = n$. It is
 1843 worth noting that the location parameter for each distribution has the same value, $p = \pi =$
 1844 0.5.

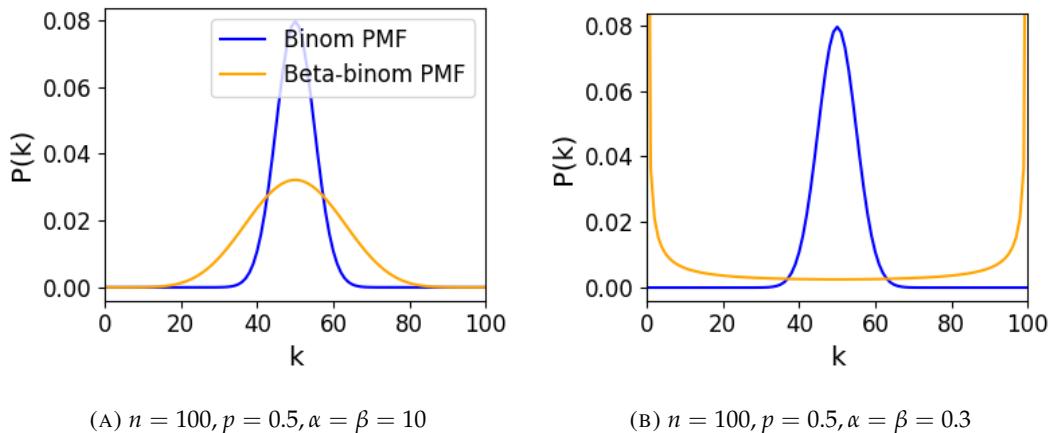


FIGURE 3.1: Figures showing the over-dispersion possible for a beta-binomial distribution relative to a binomial distribution. Parameters are shown in the captions.

1845 Conway-Maxwell-binomial distribution

1846 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

$$P(k; n, p, \nu) = \frac{1}{S(n, p, \nu)} \binom{n}{k}^{\nu} p^k (1-p)^{n-k} \quad (3.6)$$

where

$$S(n, p, \nu) = \sum_{j=0}^n \binom{n}{j}^{\nu} p^j (1-p)^{n-j} \quad (3.7)$$

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 3.2d 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

$$D_{KL}(P_{COMb}(k; n, p, \nu) || P_{Bin}(k; n, p)) = (\nu - 1) E_{P_{COMb}(k; n, p, \nu)} \left[\log \binom{n}{k} \right] \quad (3.8)$$

$$- \log S(n, p, \nu) \quad (3.9)$$

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 3.2b.

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 3.2a.

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 3.2c. 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 3.2: Relative dispersion of the COMb distribution, and association between Bernoulli variables as represented by the value of the ν parameter.

1868 Since the COMb distribution has the potential to capture positive and negative associa-
 1869 tions between the neurons/Bernoulli variables, it should be an excellent candidate for mod-
 1870 elling the number of active neurons in a neuronal ensemble.

1871 We wrote a dedicated Python package to enable easy creation and fitting of COMb dis-
 1872 tribution objects. The format of the package imitates the format of other distribution objects
 1873 from the `scipy.stats` Python package. The COMb package can be found here:

1874 https://github.com/thomasjdelaney/Conway_Maxwell_Binomial_Distribution

1875 3.3.6 Fitting

1876 We fitted binomial, beta-binomial, and Conway-Maxwell-binomial (COMb) distributions to
 1877 the neural activity in each of the overlapping windows covering each trial. To fit the distribu-
 1878 tions we minimised the appropriate negative log likelihood function using the data from the
 1879 window.

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

$$\hat{p} = \frac{1}{n} \sum_{i=1}^N k_i \quad (3.10)$$

1880 We minimised the negative log likelihood function of the beta-binomial distribution nu-
 1881 merically. We calculated the negative log likelihood for a sample directly, by taking the sum
 1882 of the log of the probability mass function for each value in the sample. We minimised the
 1883 negation of that function using the `minimise` function of the `scipy.optimize` Python
 1884 package.

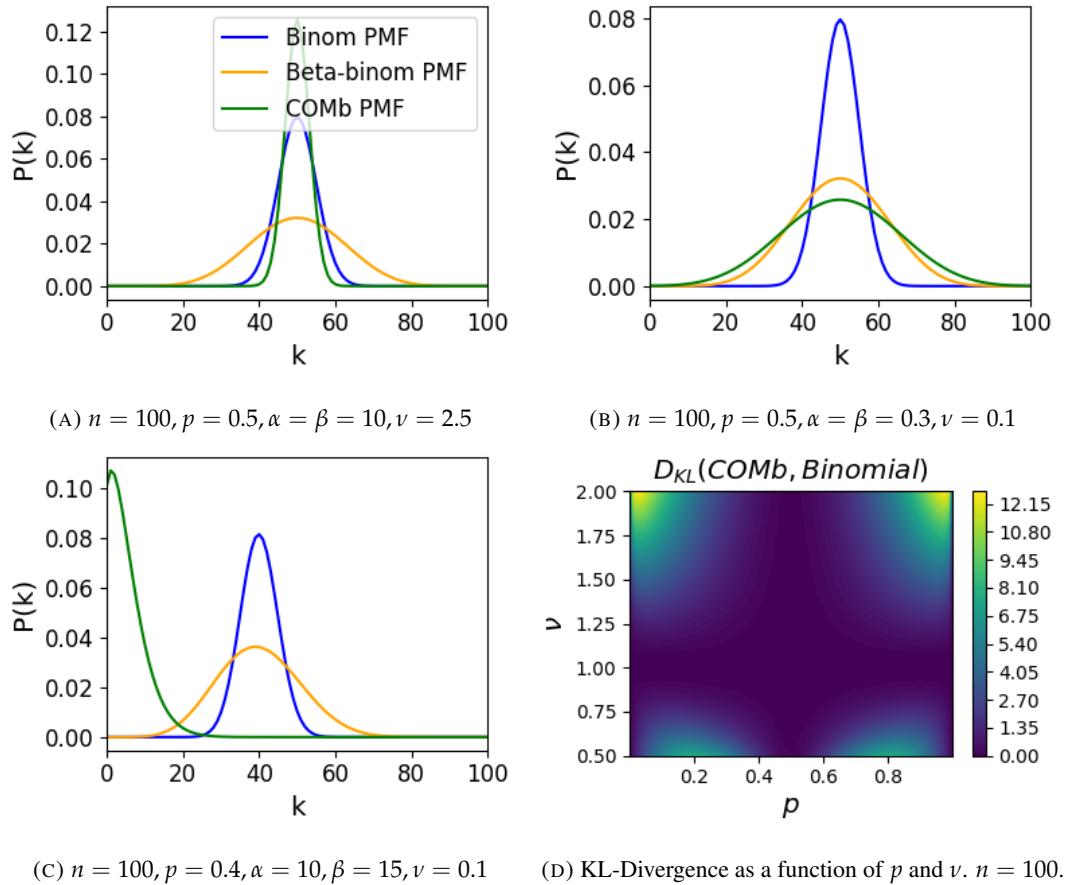


FIGURE 3.2: Figures showing (A) the under-dispersion and (B) over-dispersion permitted by the COMb distribution relative to a binomial distribution. (C) illustrates that the p parameter of the COMb distribution does not correspond to the mean of the distribution, as it does for the binomial and beta-binomial 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

$$\ell(p, \nu | k_1, \dots, k_N) = N [n \log(1 - p) - \log S(n, p, \nu)] \quad (3.11)$$

$$+ \log \frac{p}{1 - p} \sum_{i=1}^N k_i \quad (3.12)$$

$$+ \nu \sum_{i=1}^N \log \binom{n}{k_i} \quad (3.13)$$

1885 We minimised the negation of this function using numerical methods. More specifically, we
 1886 used the `minimise` function of the `scipy.optimize` Python package.

1887 **3.3.7 Goodness-of-fit**

1888 After fitting, we measured the goodness-of-fit of each model/distribution with their log like-
 1889 lihood. We calculated this directly using the `logpmf` functions of the distribution objects in
 1890 Python.

1891 **3.4 Results**

1892 We defined a neuron as *active* in a time bin if it fires at least one spike during the time interval
 1893 covered by that bin. We measured the number of active neurons in the primary visual cortex
 1894 of a mouse in 1ms bins across 160 trials of a moving bar visual stimulus. We then slid a
 1895 100ms window across these 1ms bins taking measurements, and fitting distributions along
 1896 the way. We did the same for neurons in the thalamus, hippocampus, striatum, and motor
 1897 cortex. We repeated the analysis for 5ms time bins with 40 bin windows, and 10ms time bins
 1898 with 40 bin windows.

1899 **3.4.1 Increases in mean number of active neurons and variance in number of
 1900 active neurons at stimulus onset in some regions**

1901 We measured the average number of active neurons, and the variance of the number of active
 1902 neurons in a 100ms sliding window starting 500ms before stimulus onset until 1000ms after
 1903 stimulus onset. We found differences in the response across regions. There were no observed
 1904 changes in response to the stimulus in the motor cortex or the striatum. The changes in the
 1905 other regions are detailed below.

1906 Primary visual cortex

1907 We found a transient increase in both the average and variance of the number of active neu-
1908 rons at stimulus onset, followed by a fall to pre-stimulus levels, followed by another transient
1909 increase (see figure 3.3). The oscillation in both of these measurements appear to reflect the
1910 frequency of the stimulus (see Data section 3.2.1), and it is known that stimulus structure can
1911 influence response structure(Litwin-Kumar, Chacron, and Doiron, 2012). We see a similar
1912 but lower amplitude oscillation at the end of the stimulus presentation.

1913 Hippocampus

1914 In the hippocampus we observed a transient increase in the average number of active neurons
1915 and in the variance of the number of active neurons at stimulus onset (see figure 3.4). The
1916 increase lasted about 125ms, and the subsequent fall to baseline took the a similar amount of
1917 time.

1918 Thalamus

1919 In the thalamus we observed a transient increase in the both the average and variance of
1920 the number of active neurons on stimulus onset, followed by a fall to pre-stimulus levels,
1921 followed by a sustained increase until the stimulus presentation ends.

1922 As one you might expect for a visual stimulus, the change in the average number of active
1923 neurons was greatest in the primary visual cortex. In this region, this quantity doubled on
1924 stimulus onset. In contrast, in the hippocampus and the thalamus, the average number of
1925 active neurons only increased by a fraction of the unstimulated baseline value. The duration
1926 of the response in V1 and the hippocampus at stimulus onset was 300 – 400ms, but the
1927 response in the thalamus appeared to last for the duration of stimulus presentation. The V1
1928 also showed a change in the average number of active neurons at stimulus end. The change
1929 was similar to that observed at stimulus onset, but smaller in magnitude (see figures 3.3, 3.4,
1930 and 3.5)

**1931 3.4.2 Conway-Maxwell-binomial distribution is usually a better fit than bino-
1932 mial or beta-binomial**

1933 Since the Conway-Maxwell-binomial distribution has not been fitted to neuronal data before,
1934 it is not clear that it would be a better fit than the binomial or beta-binomial distributions.
1935 In order to find out which parametric distribution was the best fit for the largest proportion

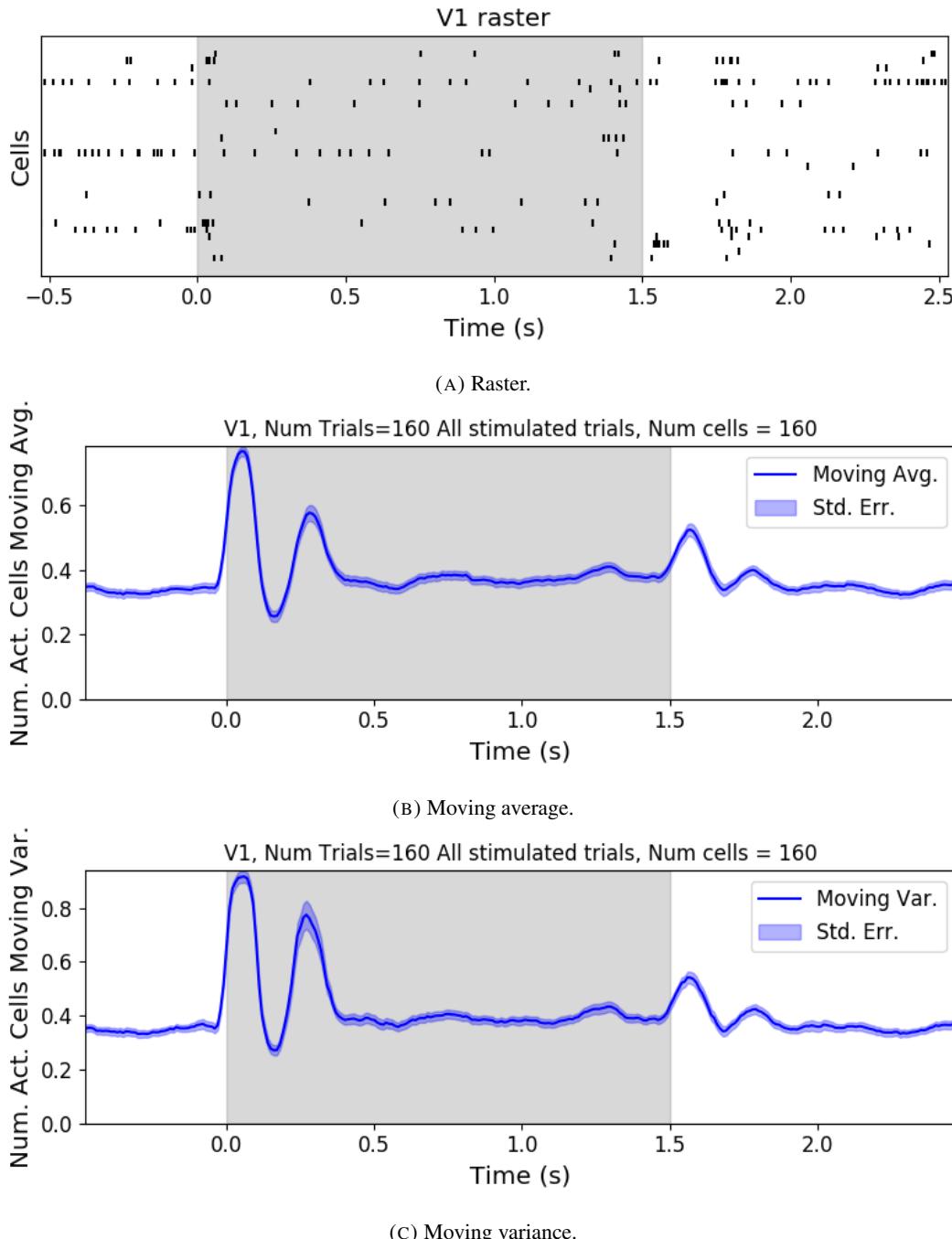


FIGURE 3.3: (A) Raster plot showing the spikes fired by 33 randomly chosen neurons in the primary visual cortex. (B-C) (B) average and (C) variance of the number of active neurons, measured using a sliding window 100ms wide, split into 100 bins. The midpoint of the time interval for each window is used 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.

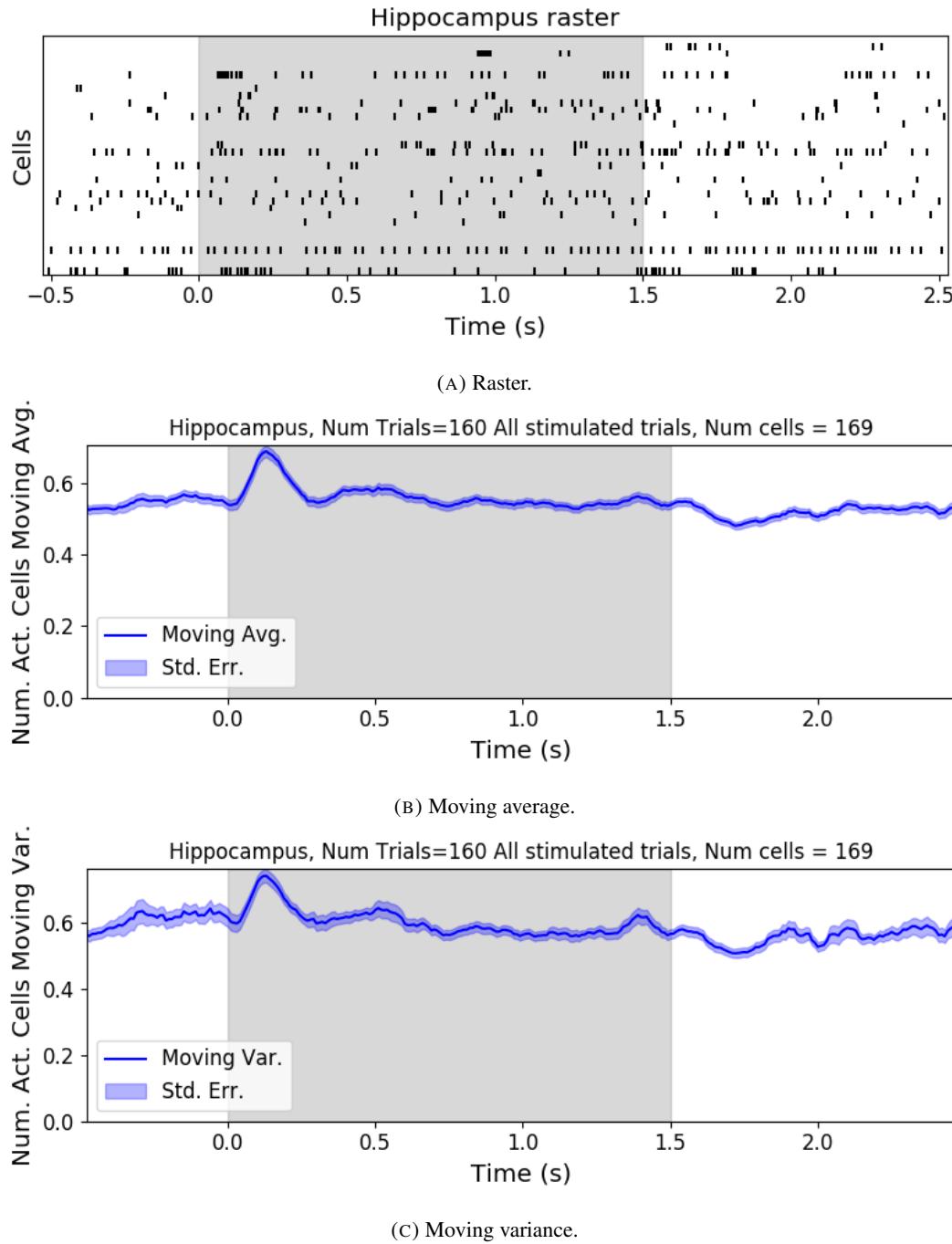


FIGURE 3.4: (A) Raster plot showing the spikes fired by 33 randomly chosen neurons in the hippocampus. (B-C) (B) average and (C) variance of the number of active neurons, measured using a sliding window 100ms wide, split into 100 bins. The midpoint of the time interval for each window is used as the 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.

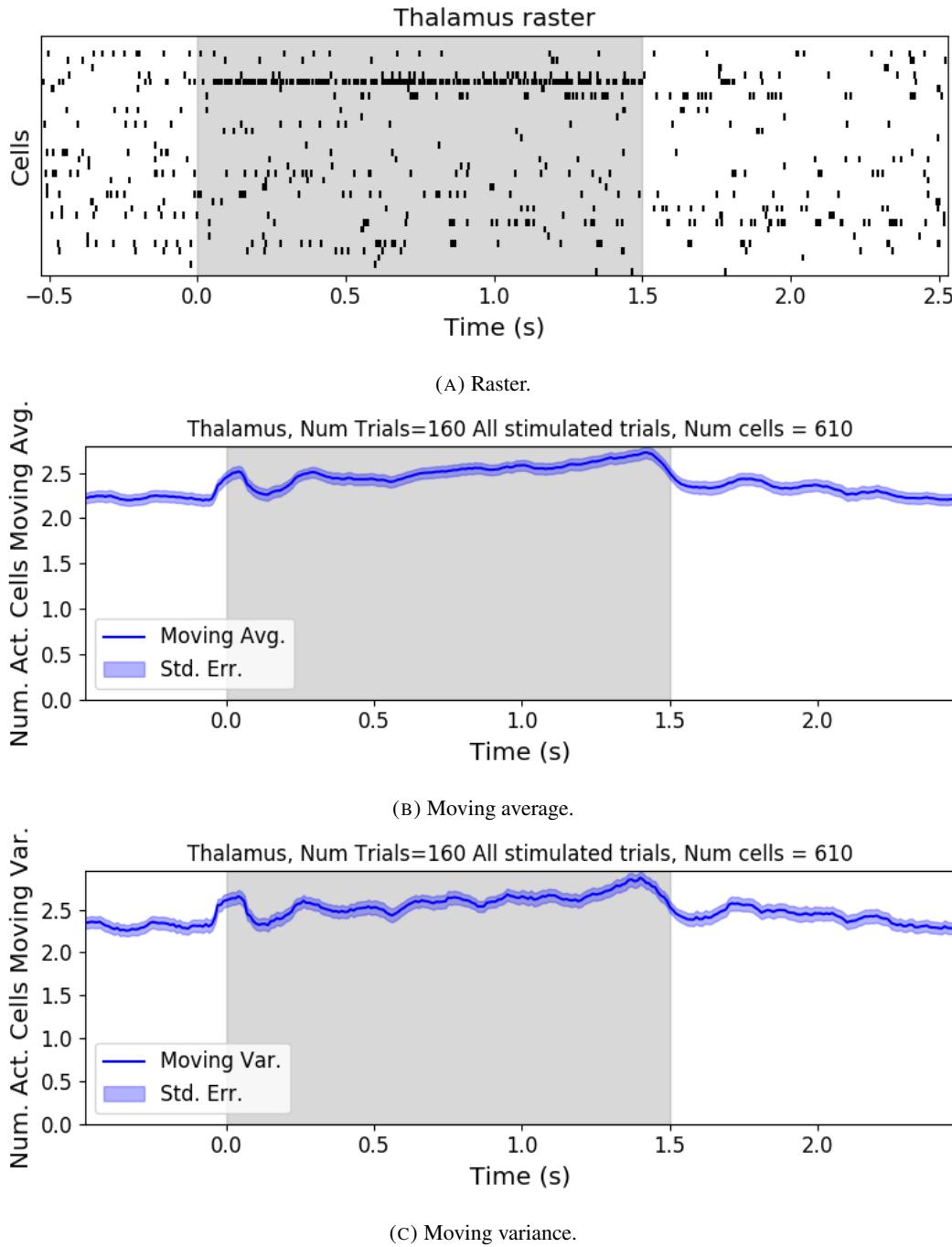
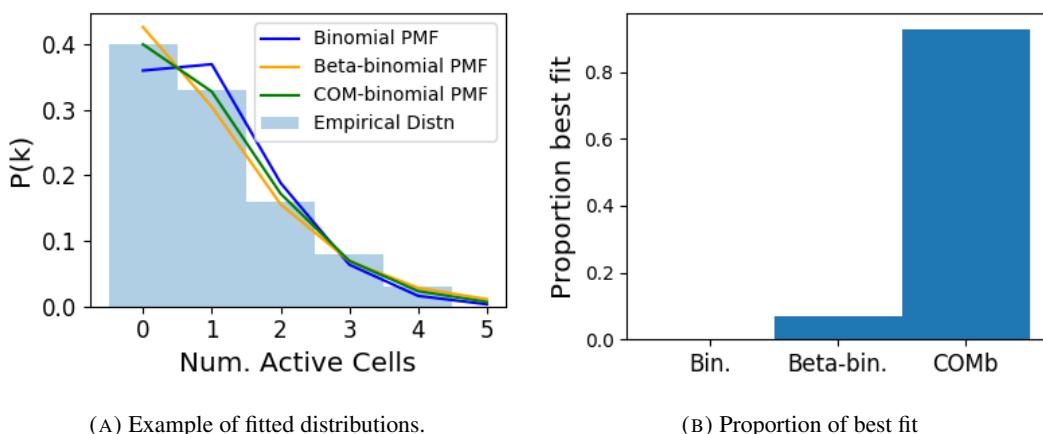


FIGURE 3.5: (A) Raster plot showing the spikes fired by 33 randomly chosen neurons in the thalamus. (B-C) (B) average and (C) variance of the number of active neurons, measured using a sliding window 100ms wide, split into 100 bins. The midpoint of the time interval for each window is used as the timepoint (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 3.3. 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 3.6: (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 3.3: 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.

1953 3.4.3 Conway-Maxwell-binomial distribution captures changes in association 1954 at stimulus onset

1955 We fit a Conway-Maxwell-binomial (COMb) distribution to the number of active neurons in
1956 the 1ms time bins in a 100ms sliding window. We also measured the correlation coefficient
1957 between the spike counts of all possible pairs of neurons, and took the average of these
1958 coefficients. We did this for all the trials with a visual stimulus. We observed a reduction in
1959 the COMb distribution's ν parameter at stimulus onset from around 1 to between 0 and 1 (see
1960 figure 3.7a). A value of ν less than 1 indicates positive association between the neurons (see
1961 section 3.3.5). We might expect to see this positive association reflected in the correlation
1962 coefficients, but this is not the case. We see no change in the time series of average correlation
1963 measures at stimulus onset.

1964 This may be due to the very short time bin we used in this case. We know that using small
1965 time bins can artificially reduce correlation measurements (Cohen and Kohn, 2011). In this
1966 case, fitting the COMb distribution may be a useful way to measure association in a neuronal
1967 ensemble over very short timescales (< 10ms).

1968 3.4.4 Replicating stimulus related quenching of neural variability

1969 Churchland et al. (2010) inspected the effect of a stimulus on neural variability. One of the
1970 measures of neural variability that they employed was the Fano factor of the spike counts of
1971 individual cells (see section 3.3.4). They found a reduction in neural variability as measured
1972 by the Fano factor in various cortical areas in a macaque at the onset of various visual stimuli,
1973 or a juice reward (Churchland et al., 2010).

1974 We measured the Fano factor of the spike count of each cell in each brain region, during
1975 each trial. We measured the mean and standard error of these Fano factors from 500ms
1976 before stimulus onset until 1000ms after stimulus end. For the primary visual cortex, we
1977 found a transient reduction in the Fano factor immediately after stimulus onset. We used
1978 a Mann-Whitney U test to check that the Fano factors measured in a window starting at

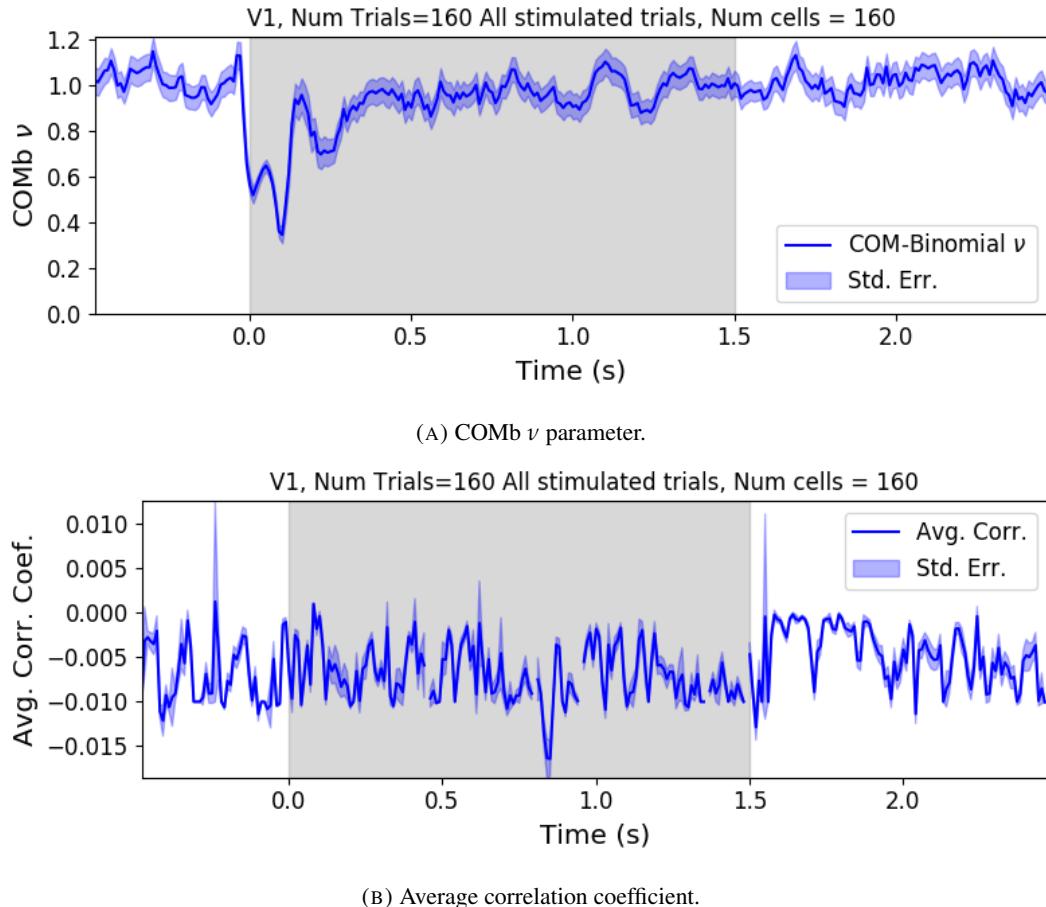


FIGURE 3.7: (A) We fit a Conway-Maxwell-binomial distribution to the number of active neurons in 1ms time bins of a 100ms sliding window. We did this for all trials with a visual stimulus and took the average across those trials. We see a transient drop in value for the distribution’s ν parameter at 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.

1979 stimulus onset and ending 100ms later were significantly lower than the factors measured
 1980 in a window ending at stimulus onset ($p < 0.001$, see figure 3.8a). We did not get this
 1981 statistically significant result in any other region.

1982 Our findings agree with those of Churchland et al. for the primary visual cortex. However
 1983 Churchland also found a reduction in the Fano factor in the dorsal premotor cortex (PMd) at
 1984 stimulus onset. Our measurements from the mouse motor cortex show no change at stimulus
 1985 onset (see figure 3.8b). This could indicate some difference in the functionality of the motor
 1986 cortex in a macaque and the motor cortex of a mouse.

1987 Similar to these findings in the Fano factor, we found a reduction in the ν parameter of
 1988 the COMB distribution on stimulus onset in V1 (figure 3.7a) and in no other region from

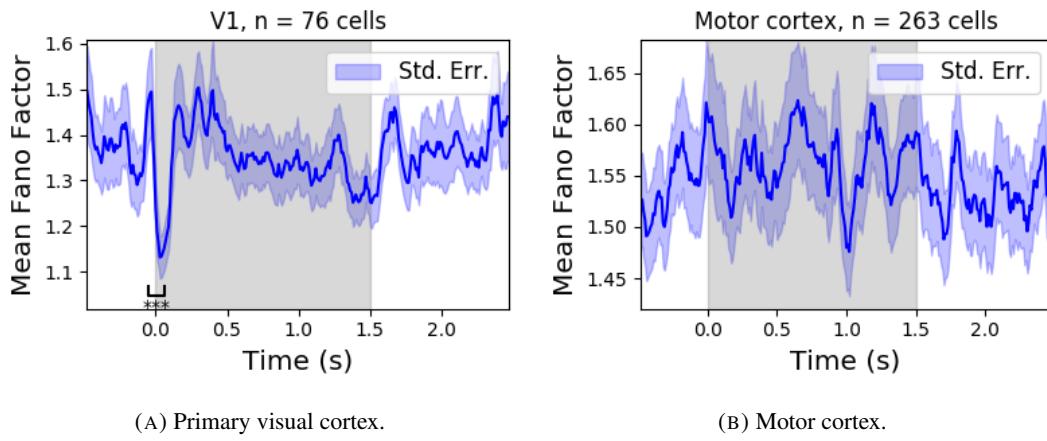


FIGURE 3.8: (A) The mean Fano factor of the spike counts of the cells in the primary visual cortex. Means were taken across cells first, then across trials. There was a significant decrease in the Fano factors immediately after stimulus onset. (B) The mean Fano factor of the spike counts of the cells in the motor cortex. No significant change in measurements at any point.

1989 which we had data. Specifically, the ν parameter reduced from around 1, to between 1 and 0.
 1990 This represents a change from no association between the neurons, to a positive association.
 1991 It is possible that this positive association may be responsible for the reduction in the Fano
 1992 factor.

1993 3.5 Discussion

1994 Our aim in this research was to develop a new statistical method for analysing the activity
 1995 of a neuronal ensemble at very short timescales. We wanted our method to use information
 1996 taken from the whole ensemble, but we also wanted the method to be quick and easy to
 1997 implement. It is likely that analysis methods with these characteristics will become valuable
 1998 as electrophysiological datasets include readings from more cells over longer time periods. In
 1999 this case, we used the number of active, or spiking, neurons in a very short time bin ($< 10\text{ms}$)
 2000 as a measure of ensemble activity.

2001 First of all, we showed that there were changes in response that we could model at these
 2002 very short time scales in some of the brain regions from which we had recordings. We ob-
 2003 served changes in the average number of active neurons, and the variance of the number of
 2004 active neurons in three different brain regions in response to visual stimuli. Since we know
 2005 that correlated behaviour is associated with sensory perception (deCharms and Merzenich,
 2006 1996), we might hope to measure the pairwise correlations within the neuronal population
 2007 in order to further investigate these responses. But, using such short time bins can produce

2008 artificially small spike count correlation measurements (Cohen and Maunsell, 2009). Over-
2009 coming this limitation was one of our objectives for our new method. In order to do this, we
2010 abandoned the idea of measuring the correlations directly and embraced the concept of *asso-*
2011 *ciation*. In order to quantify the association between neurons, we used the Conway-Maxwell-
2012 binomial distribution to model the number of active (spiking) neurons in an ensemble as a
2013 sum of possibly associated Bernoulli random variables.

2014 We showed that the Conway-Maxwell-binomial distribution performed better than the
2015 more common options of the binomial and beta-binomial distributions. Furthermore, we
2016 showed that the positively associated behaviour between neurons in the primary visual cortex
2017 could be captured by fitting a Conway-Maxwell-binomial distribution, but was not captured
2018 by the more standard approach of measuring the spike count correlation. The associated
2019 behaviour could not be measured using spike count correlations, because of the very short
2020 bins required to capture short timescale behaviour.

2021 We replicated a famous result from Churchland et al (2010) relating to the quenching of
2022 neural variability in cortical areas at stimulus onset, and in doing so, we established a corre-
2023 spondence between the association quantifying parameter of the Conway-Maxwell-binomial
2024 (COMB) distribution and the neural variability as measured by the Fano factor. We found a
2025 reduction in the ν parameter of the COMB distribution at stimulus onset, indicating a change
2026 from no association to positive association between neurons in V1. We found a corresponding
2027 reduction in the Fano factor of the individual cells in V1. The positive association between
2028 neurons induced by the stimulus would constrain the neurons to fire at the same time. The
2029 stimulus also induced a larger number of neurons to spike. These two actions combined could
2030 cause an increase in the firing rate of individual cells that is greater in magnitude than the in-
2031 crease in firing rate variability. If this is indeed the case, then the association as captured by
2032 the COMB distribution could be regarded as one of the ‘natural parameters’ of the ensemble
2033 response for short timescales. That is, a quantity that directly measures some aspect of the
2034 behaviour of the ensemble. In this case, it the correlated behaviour of the individual neurons
2035 is captured.

2036 This work could be just a first step in creating analysis methods based on the Conway-
2037 Maxwell-binomial distribution, or similar statistical models. One way to extend the method
2038 would be to pair it up with the ‘Population Tracking model’ (O’Donnell et al., 2017). This
2039 model attempts to characterise the interaction between an ensemble and each member of the
2040 ensemble by quantifying the probability of spiking for a given a cell, given the number of
2041 active cells in the whole population. Combining this model with the COMB distribution

2042 would give us a model that could accurately fit the number of active neurons at any moment,
2043 and that gives a probability of firing for each cell, and therefore probabilities for full spiking
2044 patterns, without adding a huge number of parameters to fit.

2045 A more complex way to extend the model would be to fit a Conway-Maxwell-binomial
2046 distribution to data recorded from multiple brain regions simultaneously, with a different fit
2047 for each region, then to analyse the temporal relationship between the fitted parameters of
2048 each region. If we analysed the time series of the COMB distribution parameters from the
2049 different regions, looking at cross-correlations between regions, this may give some results
2050 relating to the timescales in which information is processed in different brain regions.

2051 **Chapter 4**

2052 **Studies with practical limitations &**
2053 **negative results**

2054

Abstract

2055 Here I will present some details on research topics that I started, but that unfortunately did not
2056 lead anywhere useful. There are two pieces of research, based on two papers. Each paper is
2057 related to the overall theme of my PhD of analysing and modelling behaviours of populations
2058 of neurons. The first part is based on a model of parallel spike trains including higher order
2059 interactions by Shimazaki et al (2012). The second part is based on a multiscale model for
2060 making inferences on hierarchical data.

2061 **4.1 Dynamic state space model of pairwise and higher order neu-
2062 ronal correlations**

2063 In their paper Shimazaki et al (2012) aimed to model spike trains from populations of neurons
2064 in parallel, with pairwise and higher order dynamic interactions between the trains. They
2065 modelled the spike trains as multi-variate binary processes using a log-linear model, and they
2066 extracted spike interaction parameters using a Bayesian filter/EM-algorithm. They developed
2067 a goodness-of-fit measure for the model to test if including these higher order correlations
2068 is necessary for an accurate model. Their measure was based on the Bayes factor but they
2069 also assessed the suitability of higher order models using the AIC and BIC. So the increase
2070 in the number of parameters associated with fitting higher order interactions was taken into
2071 account. They tested the performance of the model on synthetic data with known higher
2072 order correlations. They used the model to look for higher order correlations in data from
2073 awake behaving animals. They use the model to demonstrate dynamic appearance of higher
2074 order correlations in the monkey motor cortex (Shimazaki et al., 2012).

2075 We used the available Python repository to implement the model, and we successfully
2076 worked through the tutorial provided. But we found that the model did not scale well to
2077 larger populations. We attempted to fit the model to a population of 10 neurons and found we
2078 didn't manage to finish the process. Since, our goal was to find a model to scale to hundreds
2079 or thousands of neurons, we decided that this model was no longer worth pursuing.

2080 **4.2 A multiscale model for hierarchical data applied to
2081 neuronal data**

2082 In their paper Kolaczyk et al (2001) developed a framework for a modelling hierarchically
2083 aggregated data, and making inferences based on a model arising from this framework. They
2084 assumed that a hierarchical aggregation existed on the data in question, where each element at
2085 each level of the hierarchy had some associated measurements, an associated mean process,
2086 which was the expected value of these measurements. They also assumed that the measure-
2087 ments of each parent were equal to the sum of the measurements from all of its children.
2088 They showed that these assumptions gave rise to a relationship between parent and child
2089 measurements across all levels of the hierarchy, where the product of the likelihood of the
2090 parameters of the lowest level of the hierarchy can be expressed as products of conditional
2091 likelihoods of the elements of higher levels of the hierarchy (Kolaczyk and Huang, 2010).

2092 They gave examples of these expressions for measurements sampled from Gaussian dis-
2093 tributions, and Poisson distributions, and showed the definitions of the hierarchical param-
2094 eters which reparametrise the distribution of these data taking the hierarchy into account.
2095 They go on to suggest prior distributions for this multiscale model, and integrate these priors
2096 to give posterior distributions for the measurements from each element at each level in the hi-
2097 erarchy, and expressions for the MAP estimated parameters of each the associated processes
2098 (Kolaczyk and Huang, 2010).

2099 We implemented their model in Python by creating some synthetic data from Poisson
2100 distributions, and defining a hierarchy by agglomerating these data. We calculated the MAP
2101 estimates using our knowledge of the hierarchy, and using the expressions given in the paper.
2102 We found that the MAP estimates were far less accurate than would be achieved by simply
2103 ignoring the hierarchy during estimation, and using a maximum likelihood approach. After
2104 that, we decided to move on.

2105 4.3 Discussion

2106 Ideas (not in order):

2107 • Calcium imaging limitations (spike inference not always possible)

2108 • Judging by Greenberg biophysical modelling is the way to go.

2109 • our fluorescence model could be useful in a number of situations.

2110 • Applied new network science to new electrophysiological dataset.

2111 • Abandon calcium

2112 • Multi region calcium imaging dataset would be useful?

2113 • Results might be intuitive, but are new nonetheless (as far as I know)

2114 • Potential for more network science applications?

2115 • Applied the COMB distribution to neuronal data for the first time.

2116 • captures correlated behaviour by quantifying *association*.

2117 • Abandon correlations.

2118 • coupling with existing models could yield some nice models.

2119 • More statistical invention could be useful.

2120

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