

1

UNIVERSITY OF BRISTOL

2

DOCTORAL THESIS

3

4

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

5

6

Author:

Thomas J. DELANEY

Supervisors:

Dr. Cian O'DONNELL

Dr. Michael C. ASHBY

7

9 *A thesis submitted in fulfillment of the requirements*

10 *for the degree of Doctor of Philosophy*

11 *in the*

12 Biological Intelligence & Machine Learning Unit

13 Department of Computer Science

14 August 19, 2020

15 Word count: 39000

¹⁶ **Declaration of Authorship**

¹⁷ I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes
¹⁸ and that it has not been submitted for any other academic award. Except where indicated by
¹⁹ specific reference in the text, the work is the candidate's own work. Work done in collabora-
²⁰ tion with, or with the assistance of, others, is indicated as such. Any views expressed in the
²¹ dissertation are those of the author.

²² Signed:

²³ _____

²⁴ Date:

²⁵ _____

Acknowledgements

28 I would like to thank my supervisors, Cian O'Donnell and Mike Ashby, for their help, en-
29 couragement, advice, and patience over the last four years. This includes not only helping
30 with research, but also enabling and encouraging me to make the most of my opportunities
31 during that time. Without their help, I would not have grown as much as I have done in those
32 years. I very grateful for their time and effort.

33 I would also like to thank the members of the Bristol Computational Neuroscience Unit
34 for introducing me to all the various aspects of computer science, neuroscience, and machine
35 learning, of which I otherwise would not have heard. As the first person to introduce me to
36 the concept of mathematical neuroscience during my undergraduate days, and a great source
37 of advice and guidance during my PhD, I would also like to thank Conor Houghton.

38 Personally, I would like to thank my girlfriend Ashley, who has been nothing but helpful since
39 I met her.

40 Finally, I would like to thank my father, mother, brother and sister. I am truly fortunate to
41 have such a good family. I thank them for their love, encouragement, and excellent example.

42 *Abstract*

43 Since the use of multi-electrode recording in neuroscience began, the number neurons
44 being recorded in parallel has been increasing. Recently developed methods using calcium
45 or voltage imaging have also contributed to the growth in neuronal datasets. As datasets grow,
46 the need for new analysis methods also grows. In this research we attempted to address some
47 of the problems associated with reading from large neuronal ensembles using fluorescent
48 calcium indicators, and some of the problems with analysing data read from large neuronal
49 ensembles.

50 We created a biophysical model for the fluorescence trace produced by a calcium indicator
51 responding to a given spike train. Our model reproduced the characteristics of a real
52 fluorescence trace recognised by spike inference algorithms. This model will be useful for
53 anyone using or considering calcium imaging.

54 To find order in the correlated behaviour of a large multi-region neuronal ensemble, we
55 applied a novel method from network science to detect structure and communities in corre-
56 lated behaviour. We investigated the similarities between these communities and their brain
57 anatomy. Our results indicate local correlated networks function at shorter timescales (<
58 50ms), while multi-region correlated networks function over longer timescales (> 100ms).
59 This result agrees with previous findings from EEG data, but has not been shown before using
60 spiking data.

61 We developed a statistical model for the number of neurons spiking in a neuronal ensem-
62 ble based on the Conway-Maxwell-binomial distribution. Our aim was to capture correlated
63 activity in a neuronal population without measuring correlation coefficients directly. The
64 model captured correlated activity at very short timescales better than measuring correlation
65 coefficients. We also replicated one of the findings of Churchland et al. (2010) relating to
66 the quenching of neural variability at stimulus onset. We propose a connection between this
67 result and the changes in association captured by our model.

68 **Contents**

| | | |
|----|--|-----|
| 69 | Declaration of Authorship | iii |
| 70 | Acknowledgements | v |
| 71 | Abstract | vii |
| 72 | 1 Introduction | 1 |
| 73 | 1.1 Overview | 1 |
| 74 | 1.2 Modelling the fluorescence of calcium indicators | 2 |
| 75 | 1.3 Functional networks | 4 |
| 76 | 1.4 A new statistical model for capturing correlated behaviour | 6 |
| 77 | 2 Sensitivity of the spikes-to-fluorescence transform to calcium indicator and neu- | |
| 78 | ron properties | 9 |
| 79 | 2.1 Introduction | 10 |
| 80 | 2.2 Methods | 12 |
| 81 | 2.2.1 Calcium dynamics model | 12 |
| 82 | Photon release & capture | 14 |
| 83 | 2.2.2 Parameter optimisation | 15 |
| 84 | Fixed parameters | 17 |
| 85 | 2.2.3 Julia | 17 |
| 86 | 2.2.4 Spike inference | 18 |
| 87 | Comparing spike inference quality | 21 |
| 88 | 2.2.5 Perturbation analysis | 22 |
| 89 | 2.2.6 Signal-to-noise ratio | 22 |
| 90 | 2.2.7 Data sources | 22 |
| 91 | 2.3 Results | 23 |
| 92 | 2.3.1 A biophysical computational model can generate accurate fluores- | |
| 93 | cence traces from spike trains | 23 |

| | | | |
|-----|----------|--|-----------|
| 94 | 2.3.2 | Spike inference algorithms perform similarly on real data compared with time series simulated from the model | 24 |
| 95 | 2.3.3 | Relative effects of various buffers to the fluorescence signal | 25 |
| 96 | 2.3.4 | Spike inference accuracy is sensitive to indicator properties, and likely varies within and between cells | 27 |
| 97 | 2.3.5 | Single spike inference accuracy drops for high firing rates, but firing rate itself can be estimated from mean fluorescence amplitude | 31 |
| 98 | 2.4 | Discussion | 34 |
| 102 | 3 | Functional networks expand across anatomical boundaries as correlation time- scale increases | 37 |
| 103 | 3.1 | Introduction | 38 |
| 104 | 3.2 | Data | 39 |
| 105 | 3.2.1 | Brain regions | 39 |
| 106 | 3.2.2 | Video recordings | 40 |
| 107 | 3.3 | Methods | 40 |
| 108 | 3.3.1 | Binning data | 40 |
| 109 | 3.3.2 | Correlation coefficients | 41 |
| 110 | | Total correlations, r_{SC} | 41 |
| 111 | | Shuffled total correlations | 42 |
| 112 | | Separating Correlations & Anti-correlations | 42 |
| 113 | 3.3.3 | Conditioning on behavioural data | 42 |
| 114 | | Linear regression | 42 |
| 115 | | Elastic net regularisation | 43 |
| 116 | | Conditional covariance | 44 |
| 117 | | Measures of conditional correlation | 44 |
| 118 | 3.3.4 | Information Theory | 45 |
| 119 | | Entropy $H(X)$ | 45 |
| 120 | | Maximum entropy limit | 46 |
| 121 | | Mutual Information $I(X; Y)$ | 47 |
| 122 | | Variation of Information $VI(X, Y)$ | 49 |
| 123 | | Measuring entropies & mutual information | 49 |
| 124 | 3.3.5 | Network analysis | 49 |
| 125 | | Correlation networks | 49 |

| | | |
|-----|--|-----------|
| 127 | Rectified correlations | 50 |
| 128 | Sparsifying data networks | 50 |
| 129 | Communities | 50 |
| 130 | Weighted configuration model | 51 |
| 131 | Sparse weighted configuration model | 51 |
| 132 | Spectral rejection | 51 |
| 133 | Node rejection | 52 |
| 134 | Community detection | 53 |
| 135 | 3.3.6 Clustering Comparison | 53 |
| 136 | Adjusted Rand Index | 53 |
| 137 | Clusterings as random variables | 54 |
| 138 | Information based similarity measures | 55 |
| 139 | Information based metrics | 56 |
| 140 | Comparing detected communities and anatomical divisions | 56 |
| 141 | 3.4 Results | 57 |
| 142 | 3.4.1 Average correlation size increases with increasing time bin width | 57 |
| 143 | 3.4.2 Goodness-of-fit for Poisson and Gaussian distributions across increasing time bin widths | 58 |
| 144 | 3.4.3 Differences between and inter- and intra- regional correlations decrease with increasing bin width | 60 |
| 145 | 3.4.4 Connected and divided structure in correlation based networks reduces in dimension with increasing bin width | 61 |
| 146 | 3.4.5 Detecting communities in correlation based networks | 65 |
| 147 | 3.4.6 Functional communities resemble anatomical division at short timescales | 65 |
| 148 | 3.4.7 Conditional correlations & signal correlations | 67 |
| 149 | 3.4.8 Absolute correlations and negative rectified correlations | 72 |
| 150 | 3.5 Discussion | 75 |
| 151 | 4 A simple two parameter distribution for modelling neuronal activity and capturing neuronal association | 79 |
| 152 | 4.1 Introduction | 80 |
| 153 | 4.2 Data | 81 |
| 154 | 4.2.1 Experimental protocol | 81 |
| 155 | 4.3 Methods | 82 |

| | | | |
|-----|----------|---|------------|
| 160 | 4.3.1 | Binning data | 82 |
| 161 | 4.3.2 | Number of <i>active</i> neurons | 82 |
| 162 | 4.3.3 | Moving windows for measurements | 82 |
| 163 | 4.3.4 | Fano factor | 84 |
| 164 | 4.3.5 | Probability Distributions suitable for modelling ensemble activity | 84 |
| 165 | | Association | 84 |
| 166 | | Binomial distribution | 85 |
| 167 | | Beta-binomial distribution | 85 |
| 168 | | Conway-Maxwell-binomial distribution | 86 |
| 169 | 4.3.6 | Fitting | 88 |
| 170 | 4.3.7 | Goodness-of-fit | 90 |
| 171 | 4.4 | Results | 90 |
| 172 | 4.4.1 | Increases in mean number of active neurons and variance in number of active neurons at stimulus onset in some regions | 90 |
| 173 | | Primary visual cortex | 91 |
| 174 | | Hippocampus | 91 |
| 175 | | Thalamus | 91 |
| 176 | 4.4.2 | Conway-Maxwell-binomial distribution is usually a better fit than binomial or beta-binomial | 91 |
| 177 | 4.4.3 | Relative goodness-of-fit for binomial, beta-binomial, and COMb distributions | 96 |
| 178 | | Log likelihoods of distributions fitted to stimulated and unstimulated responses | 96 |
| 179 | | Distribution of fitted parameters for stimulated and unstimulated responses | 98 |
| 180 | | Examples of empirical distributions and fitted distributions | 100 |
| 181 | 4.4.4 | Conway-Maxwell-binomial distribution captures changes in association at stimulus onset | 100 |
| 182 | 4.4.5 | Replicating stimulus related quenching of neural variability | 100 |
| 183 | 4.4.6 | Effects of greater bin widths | 103 |
| 184 | 4.5 | Discussion | 104 |
| 191 | 5 | Studies with practical limitations & negative results | 107 |
| 192 | 5.1 | Dynamic state space model of pairwise and higher order neuronal correlations | 108 |

| | | |
|-----|--|------------|
| 193 | 5.2 A multiscale model for hierarchical data applied to neuronal data | 108 |
| 195 | 6 Discussion | 111 |
| 196 | Bibliography | 119 |

197 **List of Figures**

| | | |
|-----|--|----|
| 198 | 2.1 A: Example spike train (blue) and the corresponding GCaMP6s fluorescence | |
| 199 | trace (green), data replotted from (Berens et al., 2018). Inset shows zoomed | |
| 200 | section of traces to highlight slow decay of GCaMP6s fluorescence relative | |
| 201 | to spike time intervals. B: Schematic diagram of the neuron calcium and | |
| 202 | GCaMP computational model. C: Good visual match of data fluorescence | |
| 203 | trace (green) and model simulated fluorescence (orange) in response to an | |
| 204 | identical spike train (blue). | 11 |
| 205 | 2.2 A: Workflow to compare spike inference for real versus simulated fluores- | |
| 206 | cence data. B: True positive rates achieved by three different spike inference | |
| 207 | algorithms when applied to observed spike trains, and simulated spike trains. | |
| 208 | Data points overlaid as blue circles. The performance is similar from real | |
| 209 | and simulated data for each of the algorithms. | 25 |
| 210 | 2.3 Calcium Buffering Dynamics (A) The proportions of bound and free cal- | |
| 211 | cium concentrations within a cell, with the associated spike train. (B)-(F) | |
| 212 | The dynamics of the concentration of (B) excited indicator bound calcium, | |
| 213 | (C) indicator bound calcium, (D) immobile endogenous buffer bound cal- | |
| 214 | cium, (E) mobile endogenous buffer bound calcium, and (F) free calcium in | |
| 215 | response to an action potential at $\sim 23.2\text{s}$ | 26 |

| | | |
|-----|--|----|
| 216 | 2.4 (A) An example trace for each of the five pairs of values used for the binding | |
| 217 | and unbinding rates of the fluorescent calcium indicator. (B) The signal-to- | |
| 218 | noise ratio of the modelled fluorescence traces using each of the four per- | |
| 219 | turbed value pairs, and the experimental value. The SNRs for the value pairs | |
| 220 | perturbed downward are lower than that for the unperturbed value pair or | |
| 221 | the higher value pairs. (C) The true-positive rates of the deconvolution al- | |
| 222 | gorithm's predictions when inferring from the observed data, and inferring | |
| 223 | from modelled traces using the perturbed and experimental values. We used | |
| 224 | the OASIS algorithm for spike inference here. The results from the other | |
| 225 | spike inference methods were similar, with their true positive rates scales | |
| 226 | similarly to figure 2.2 B. | 29 |
| 227 | 2.5 (A) An example trace for each of the five perturbed values for the concen- | |
| 228 | tration of fluorescent calcium indicator. The top two traces are produced | |
| 229 | by the lower perturbed values, the middle trace is produced by the experi- | |
| 230 | mental value, and the lowest two traces are produced when using the higher | |
| 231 | perturbed values. (B) The signal-to-noise ratio of the modelled fluorescence | |
| 232 | traces using each of the four perturbed values, and the experimental value. | |
| 233 | Extreme perturbations of the concentration either above or below the experi- | |
| 234 | mental level lowered the SNR. (C) The true-positive rates of the deconvolu- | |
| 235 | tion algorithm's predictions when inferring from the observed data, and | |
| 236 | inferring from modelled traces using the perturbed and experimental values. | |
| 237 | We found that the algorithms performs equally badly on the two most ex- | |
| 238 | treme values, and performs equally well on the experimental value, and the | |
| 239 | next higher perturbed value. We used the OASIS algorithm for spike infer- | |
| 240 | ence here. The results from the other spike inference methods were similar, | |
| 241 | with their true positive rates scales similarly to figure 2.2 B. | 30 |

| | | |
|-----|---|----|
| 242 | 2.6 (A) An example trace for each of the five perturbed values for the concentration of immobile endogenous buffer. (B) The signal-to-noise ratio of the modelled fluorescence traces using each of the four perturbed values, and the experimental value. The lower values for the immobile buffer produce the same SNR as the experimental value. But the higher perturbed values produce fluorescence traces with a lower SNR. (C) The true-positive rates of the deconvolution algorithm's predictions when inferring from the observed data, and inferring from modelled traces using the perturbed and experimental values. We used the OASIS algorithm for spike inference here. The results from the other spike inference methods were similar, with their true positive rates scales similarly to figure 2.2 B. | 32 |
| 253 | 2.7 Simulating fluorescence traces at different firing rates Example modelled traces created using simulated spike trains with a mean firing rate of 1Hz (left column), 5Hz (middle column), and 10Hz (right column). Note the difference in amplitude with different mean firing rates. | 33 |
| 254 | | |
| 255 | | |
| 256 | | |
| 257 | 2.8 Inference quality and $\Delta F / F_0$ vs Firing rate (left) The spike inference accuracy when applied to 30 traces created using simulated spike trains with mean firing rates of 1, 5, and 10 Hz. (right) The mean $\Delta F / F_0$ across those 30 traces for each frequency. | 33 |
| 258 | | |
| 259 | | |
| 260 | | |
| 261 | 3.1 Probe Locations: The locations of the probes in each of the three mouse brains (Stringer et al., 2019). | 40 |
| 262 | | |
| 263 | 3.2 Entropy Limit: The upper limit on entropy of binned spike count data as a function of the maximum observed spike count. The orange line is the analytical maximum. The blue line is the entropy of samples with $N = 1000$ data points taken from the discrete uniform distribution. | 47 |
| 264 | | |
| 265 | | |
| 266 | | |
| 267 | 3.3 (A) An example of the correlation coefficients between two different pairs of cells, one where both cells are in the same brain region (intra-regional pair), and one where both cells are in different brain regions (inter-regional pair). The correlation coefficients have been measured using different time bin widths, ranging from 5ms to 3s. Note the increasing amplitude of the correlations with increasing bin width. (B) A raster plot showing the spike times of each pair of cells. | 57 |
| 268 | | |
| 269 | | |
| 270 | | |
| 271 | | |
| 272 | | |
| 273 | | |

| | | | |
|-----|-----|--|----|
| 274 | 3.4 | Mean correlation coefficients measured from pairs of 50 randomly chosen neurons. (A) All possible pairs, (B) positively correlated pairs, and (C) neg- atively correlated pairs. (D) Mean and standard error of χ^2 test statistics for Poisson and Gaussian distributions fitted to neuron spike counts. | 59 |
| 275 | | | |
| 276 | | | |
| 277 | | | |
| 278 | 3.5 | (Left)The mean intra-region and inter-region correlations using all possible pairs of ~ 500 neurons, spread across 9 different brain regions. (Right) Courtesy of Stringer et al. (2019), mean inter-regional (out-of-area) correla- tion 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. | 61 |
| 279 | | | |
| 280 | | | |
| 281 | | | |
| 282 | | | |
| 283 | | | |
| 284 | 3.6 | The mean intra-regional correlations (coloured dots) and mean inter-regional correlations (black dots) for a given region, indicated on the x-axis, for dif- ferent 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. | 62 |
| 285 | | | |
| 286 | | | |
| 287 | | | |
| 288 | | | |
| 289 | | | |
| 290 | | | |
| 291 | | | |
| 292 | 3.7 | Mean inter-regional (main diagonal) and intra-regional (off diagonal) corre- lation 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. | 63 |
| 293 | | | |
| 294 | | | |
| 295 | | | |
| 296 | | | |
| 297 | 3.8 | The number of dimensions in the k -partite and connected structure in the cor- relation based networks beyond the structure captured by a sparse weighted configuration null network model (see section 3.3.5), shown for different time bin widths. Note that the k -partite structure disappears for time bin width greater than 200ms for all three subjects. The dimension of the connected structure reduces with increasing bin width for 2 of the 3 subjects (top row). . | 64 |
| 298 | | | |
| 299 | | | |
| 300 | | | |
| 301 | | | |
| 302 | | | |

| | | |
|-----|--|----|
| 303 | 3.9 (A-B) Correlation matrices with detected communities indicated by white | |
| 304 | lines. Each off main diagonal entry in the matrix represents a pair of neu- | |
| 305 | rons. Those entries within a white square indicate that both of those neurons | |
| 306 | are in the same community as detected by our community detection proce- | |
| 307 | dure. Matrices shown are for 5ms and 1s time bin widths respectively. Main | |
| 308 | diagonal entries were set to 0. (C-D) Matrices showing the anatomical dis- | |
| 309 | tribution of pairs along with their community membership. Entries where | |
| 310 | both cells are in the same region are given a colour indicated by the colour | |
| 311 | bar. Entries where cells are in different regions are given the grey colour also | |
| 312 | indicated by the colour bar. | 66 |
| 313 | 3.10 (A) The variation of information is a measure of distance between cluster- | |
| 314 | ings. The distance between the anatomical ‘clustering’ and community de- | |
| 315 | tection ‘clustering’ increases with increasing time bin width. (B) The ad- | |
| 316 | justed Rand index is a normalised similarity measure between clusterings. | |
| 317 | The anatomical and community detection clusterings become less similar as | |
| 318 | the time bin width increases. | 67 |
| 319 | 3.11 Comparing the components of the total covariance across different values for | |
| 320 | the time bin width. We observed a consistent increase in $E[\text{cov}(X, Y Z)]$ as | |
| 321 | the time bin width increased. But we saw different trends for $\text{cov}(E[X Z], E[Y Z])$ | |
| 322 | for each mouse. | 68 |
| 323 | 3.12 Comparing the components of the total covariance across different values for | |
| 324 | the time bin width. We saw a consistent increase in $\rho_{X,Y Z}$ as the time bin | |
| 325 | width increased in all three subjects. But we saw different trends in ρ_{signal} for | |
| 326 | each of the subjects. | 69 |
| 327 | 3.13 Matrices showing the regional membership of pairs by colour, and the com- | |
| 328 | munities in which those pairs lie. (A-B) Detected communities and regional | |
| 329 | membership matrix for network based on rectified spike count correlation | |
| 330 | $\rho_{X,Y Z}$, using time bin widths of 0.005s and 1s respectively. (C-D) Detected | |
| 331 | communities and regional membership matrix for network based on rectified | |
| 332 | signal correlation ρ_{signal} , using time bin widths of 0.005s and 1s respectively. | 70 |

| | | |
|-----|---|----|
| 333 | 3.14 Distance and similarity measures between the anatomical division of the neu- | |
| 334 | rons, and the communities detected in the network based on the spike count | |
| 335 | correlations $\rho_{X,Y Z}$. (A) The variation of information is a ‘distance’ mea- | |
| 336 | sure between clusterings. The distance between the anatomical ‘clustering’ | |
| 337 | and the community clustering increases as the time bin width increases. (B) | |
| 338 | The adjusted Rand index is a similarity measure between clusterings. The | |
| 339 | detected communities become less similar to the anatomical division of the | |
| 340 | cells as the time bin width increases. | 71 |
| 341 | 3.15 Distance and similarity measures between the anatomical division of the neu- | |
| 342 | rons, and the communities detected in the network based on the signal cor- | |
| 343 | relations ρ_{signal} . (A) The variation of information is a ‘distance’ measure be- | |
| 344 | tween clusterings. The distance between the anatomical ‘clustering’ and the | |
| 345 | community clustering increases as the time bin width increases. (B) The ad- | |
| 346 | justed Rand index is a similarity measure between clusterings. The detected | |
| 347 | communities become less similar to the anatomical division of the cells as | |
| 348 | the time bin width increases. | 72 |
| 349 | 3.16 (A-B) Absolute correlation matrices with detected communities indicated by | |
| 350 | white lines. These communities are based on the absolute value of the total | |
| 351 | correlation between each pair of cells. Those entries within a white square in- | |
| 352 | dicate that both of those neurons are in the same community. Matrices shown | |
| 353 | are for 5ms and 1s time bin widths respectively. Main diagonal entries were | |
| 354 | set to 0. (C-D) Matrices showing the anatomical distribution of pairs along | |
| 355 | with their community membership. Regional membership is indicated by the | |
| 356 | colour bar. (E) Variation of information between the anatomical division of | |
| 357 | the cells, and the detected communities. (F) Adjusted Rand index between | |
| 358 | the anatomical division, and the detected communities. | 73 |

| | | |
|-----|--|----|
| 359 | 3.17 (A-B) Sign reversed rectified correlation matrices with detected communities indicated by white lines. Those entries within a white square indicate that both of those neurons are in the same community. Matrices shown are for 5ms and 1s time bin widths respectively. Main diagonal entries were set to 0. (C-D) Matrices showing the anatomical distribution of pairs along with their community membership. Regional membership is indicated by the colour bar. (E) Variation of information between the anatomical division of the cells, and the detected communities. (F) Adjusted Rand index between the anatomical division, and the detected communities. | 74 |
| 368 | 4.1 Figures showing the over-dispersion possible for a beta-binomial distribution relative to a binomial distribution. Parameters are shown in the captions. . . | 86 |
| 370 | 4.2 Figures showing (A) the under-dispersion and (B) over-dispersion permitted by the COMb distribution relative to a binomial distribution. (C) illustrates that the p parameter of the COMb distribution does not correspond to the mean of the distribution, as it does for the binomial and beta-binomial distributions. (D) shows a heatmap for the value of the Kullback-Liebler divergence between the COMb distribution and the standard binomial distribution with same value for n , as a function of p and ν . The point of this figure is to give the reader a sense of how the values of p and ν influence the difference between the COMb distribution and the binomial distribution. The divergence is smallest when $\nu \approx 1$, or when $p \approx 0.5$. When $\nu = 1$, the PMF for the COMb distribution is the same as the PMF for the binomial distribution. When $p = 0.5$ the mass of the distribution is centred around $n/2$ for both the COMb and the binomial distribution. The difference between the two distributions is controlled by the ν parameter. The further the p and ν parameters are from 0.5 and 1 respectively, the greater the difference between the COMb distribution and the binomial distribution. Parameters for all figures are shown in the captions. | 89 |

| | | |
|-----|---|----|
| 387 | 4.3 (A) Raster plot showing the spikes fired by 33 randomly chosen neurons in 388 the primary visual cortex. (B-C) (B) average and (C) variance of the number 389 of active neurons, measured using a sliding window 100ms wide, split into 390 100 bins. The midpoint of the time interval for each window is used as the 391 timepoint (x-axis point) for the measurements using that window. The grey 392 shaded area indicates the presence of a visual stimulus. The opaque line is 393 an average across the 160 trials that included a visual stimulus of any kind. 394 We can see a transient increase in the average number of active neurons and 395 the variance of this number, followed by a fluctuation and another increase. | 92 |
| 396 | 4.4 (A) Raster plot showing the spikes fired by 33 randomly chosen neurons in 397 the hippocampus. (B-C) (B) average and (C) variance of the number of active 398 neurons, measured using a sliding window 100ms wide, split into 100 bins. 399 The midpoint of the time interval for each window is used as the timepoint (x- 400 axis point) for the measurements using that window. The grey shaded area 401 indicates the presence of a visual stimulus. The opaque line is an average 402 across the 160 trials that included a visual stimulus of any kind. We can see 403 a transient increase in the average number of active neurons and the variance 404 of this number at stimulus onset. | 93 |
| 405 | 4.5 (A) Raster plot showing the spikes fired by 33 randomly chosen neurons in 406 the thalamus. (B-C) (B) average and (C) variance of the number of active 407 neurons, measured using a sliding window 100ms wide, split into 100 bins. 408 The midpoint of the time interval for each window is used as the timepoint (x- 409 axis point) for the measurements using that window. The grey shaded area 410 indicates the presence of a visual stimulus. The opaque line is an average 411 across the 160 trials that included a visual stimulus of any kind. We can 412 see in immediate increase at stimulus onset, a subsequent fall, and another 413 sustained increased until the stimulus presentation ends. | 94 |

| | | | |
|-----|---|-----|--|
| 414 | 4.6 (A) An example of the binomial, beta-binomial, and Conway-Maxwell-binomial | | |
| 415 | distributions fitted to a sample of neural activity. The Conway-Maxwell- | | |
| 416 | binomial distribution is the best fit in this case. The histogram shows the | | |
| 417 | empirical distribution of the sample. The probability mass function of each | | |
| 418 | distribution is indicated by a different coloured line. (B) Across all samples | | |
| 419 | in all trials, the proportion of samples for which each fitted distribution was | | |
| 420 | the the best fit. The Conway-Maxwell-binomial distribution was the best fit | | |
| 421 | for 93% of the samples taken from V1 using a bin width of 1ms. | 95 | |
| 422 | 4.7 Number of active neurons data from the primary visual cortex, 1ms bin | | |
| 423 | widths. (Left column) Histograms of log likelihoods for the binomial, beta- | | |
| 424 | binomial, and COMb distributions fitted to windows where no visual stimu- | | |
| 425 | lus was present. 160 trials. (Right column) Similar histograms for windows | | |
| 426 | where a visual stimulus was present. 160 trials. In both cases, there are | | |
| 427 | marginal increases in the log likelihoods from binomial to beta-binomial to | | |
| 428 | COMb. The log likelihoods are larger for the unstimulated windows. | 97 | |
| 429 | 4.8 Histograms of fitted parameters for binomial, beta-binomial, and COMb dis- | | |
| 430 | tributions. Distributions fitted to data from the primary visual cortex, using | | |
| 431 | 1ms bin widths. (Left column) The distributions were fitted a window before | | |
| 432 | the onset of the visual stimulus. (Right column) The distributions were fitted | | |
| 433 | to a window immediately after the onset of the visual stimulus. | 99 | |
| 434 | 4.9 Examples of empirical and fitted distributions. At least one example from | | |
| 435 | each of the 5 brain regions from which we had data. | 101 | |
| 436 | 4.10 (A) We fit a Conway-Maxwell-binomial distribution to the number of active | | |
| 437 | neurons in 1ms time bins of a 100ms sliding window. We did this for all | | |
| 438 | trials with a visual stimulus and took the average across those trials. We see | | |
| 439 | a transient drop in value for the distribution's ν parameter at stimulus onset. | | |
| 440 | This shows an increase in positive association between the neurons. (B) We | | |
| 441 | measured the correlation coefficient between the spike counts of all possible | | |
| 442 | pairs of neurons in the same sliding window. The took the average of those | | |
| 443 | coefficients. We also did this for every visually stimulated trial, and took the | | |
| 444 | average across trials. The increase in positive association is not reflected with | | |
| 445 | an increase in average correlation. | 102 | |

| | | |
|-----|---|-----|
| 446 | 4.11 (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. | 103 |
| 451 | 4.12 (A) The mean ν parameter of the COMb distribution fitted to activity in the primary visual cortex. Mean taken across all stimulated trials. A bin width of 10ms was used to classify cells as active or inactive. The change in association at stimulus onset is still captured. Some high frequency fluctuations are filtered out by using the wider bins (compare to figure 4.10a) (B) The mean Fano factor of the cells in the primary visual cortex. The change in the mean Fano factor at stimulus onset is not significant when using a bin width of 10ms. | 105 |
| 457 | | |

458 List of Tables

| | | | |
|-----|-----|---|----|
| 459 | 2.1 | Fixed parameters A table of the parameters fixed before optimising the | |
| 460 | | model. The values of these parameters could be changed to model differ- | |
| 461 | | ent fluorescent calcium indicators. | 17 |
| 462 | 4.1 | Details of the different bin width and analysis window sizes used when bin- | |
| 463 | | ning spike times, and analysing those data. | 83 |
| 464 | 4.2 | Relative dispersion of the COMb distribution, and association between Bernoulli | |
| 465 | | variables as represented by the value of the ν parameter. | 88 |
| 466 | 4.3 | Proportion of samples for which each distribution was the best fit, grouped | |
| 467 | | by bin width. The COMb distribution is the best fit most of the time. | 96 |

List of Abbreviations

| | |
|--------------|--|
| COMb | Conway-Maxwell-binomial (distribution) |
| OASIS | Online active set method to infer spikes |
| SNR | Signal to noise ratio |
| NMI | Normalised mutual information |
| AMI | Adjusted mutual information |
| VI | Variation of information |

⁴⁶⁹ **List of Symbols**

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

470 **Chapter 1**

471 **Introduction**

472 **1.1 Overview**

473 Since Hodgkin and Huxley's squid experiments featuring a single axon (Hodgkin and Hux-
474 ley, 1939), to more recent research with spike sorted data from ~ 24000 neurons from 34
475 brain regions from 21 mice (Allen et al., 2019), the number of neurons contributing to elec-
476 trophysiological datasets has been growing. The number of simultaneously recorded neurons
477 has doubled approximately every seven years since the use of multi-electrode recording in
478 neuroscience began (Stevenson and Kording, 2011). Recording methods using two-photon
479 calcium imaging have also been used to extract data from populations containing over 10000
480 neurons (Peron et al., 2015). This dramatic growth in the number of neurons available for
481 analysis requires a dramatic change in analysis methods.

482 There are multiple methods for reading activity from neuronal ensembles: electrophysiolog-
483 ogy, calcium imaging, and voltage imaging. Electrophysiology involves inserting electrodes
484 into the brain of an animal. The electrodes read extra-cellular membrane potential, and using
485 these readings we observe activity in the ensemble. Calcium imaging and voltage imaging
486 use indicator dyes or fluorescent proteins that emit fluorescence traces that indicate either
487 the concentration of calcium in a neuron's cytoplasm, or the neuron's membrane potential.
488 In this project, we have attempted to address some of the difficulties in collecting data from
489 these large ensembles using fluorescent calcium indicators, and some of the difficulties in
490 analysing the collected data.

491 The rest of this introductory chapter will give some background about methods of record-
492 ing from the brain, and some background for the rest of the document. Chapter two describes
493 a biophysical model for the fluorescence trace induced by a given spike train in a cell con-
494 taining a fluorescent calcium indicator. Our third chapter describes our investigations into

495 the correlated activity across different regions of a mouse behaving spontaneously. We ap-
496 plied a novel community detection method (Humphries et al., 2019) from network science
497 to correlation based networks of neurons, and observed differences in the structure of these
498 correlations at different timescales. In our fourth chapter, we detail a new statistical model
499 for the number of neurons spiking in a neuronal ensemble at any given moment. With this
500 model, we attempted to capture correlated activity in a new way. The fifth chapter is a brief
501 description of the work that yielded negative results or was abandoned. The final chapter is a
502 discussion of our work and results from the previous chapters and their implications.

503 1.2 Modelling the fluorescence of calcium indicators

504 To focus on calcium imaging for a start, a neuron that contains a fluorescent calcium indicator
505 in its cytoplasm will fluoresce when bombarded with photons. The amount that the cell
506 will fluoresce is dependent on the concentration of fluorescent indicator within the cell, and
507 the concentration of calcium within the cell. When a neuron fires an action potential, the
508 influx of free calcium ions causes an increase in fluorescence when those ions bond with the
509 fluorescent indicator and those bounded molecules are bombarded with photons. After the
510 action potential, as calcium is extruded from the cell the fluorescence returns to a baseline
511 level. This is the basic mechanism of fluorescent calcium indicator based imaging.

512 This method has some advantages over electrophysiology as measure of neuronal ensem-
513 ble activity. Many of the problems with electrophysiology are within the processes used to
514 isolate spikes in the extracellular voltage readings, and assign these spikes to individual cells.
515 These processes are collectively called ‘spike sorting’. A comparison of many different spike
516 sorting algorithms found that these algorithms only agreed on a fraction of cases (Buccino
517 et al., 2019). Furthermore, because electrodes measure extracellular voltage, neurons that do
518 not spike will not be detected. Isolating individual neurons is easier and more reliable when
519 using calcium imaging data, because cells will emit a baseline level of fluorescence when not
520 firing action potentials. Another advantage is that calcium imaging sites can be re-used for
521 weeks for longitudinal studies (Chen et al., 2013). One of the methods of delivering the flu-
522 orescent indicator is by adeno-associated viruses, consequently there can be problems with
523 indicator gradients around the infection site, and expression levels will change in individual
524 cells over weeks (Tian et al., 2009; Chen et al., 2013). This delivery method can also cause
525 cell pathology, and nuclear filling (Zariwala et al., 2012), but these problems can be solved by
526 using lines of transgenic mice (Dana et al., 2014). The fluorescence signal itself can serve a a

527 good indicator of cell activity, but similarly to electrophysiology, the aim of calcium imaging
528 is often spike detection.

529 If the imaging data is collected at a high enough frequency, and the signal-to-noise ratio
530 of the fluorescence trace is high enough, it should be possible to infer the spike times to some
531 level of accuracy. For example, the calmodulin based indicator GCaMP6s has a sufficiently
532 high signal-to-noise ratio that isolated action potentials can be detected and inferred (Chen
533 et al., 2013). Many spike inference algorithms exist (Vogelstein et al., 2010; Pnevmatikakis
534 et al., 2016; Friedrich and Paninski, 2016; Pnevmatikakis et al., 2013; Pnevmatikakis et al.,
535 2014; Deneux et al., 2016; Greenberg et al., 2018), and some of these can perform both cell
536 isolation and spike detection simultaneously (Vogelstein et al., 2010; Pnevmatikakis et al.,
537 2016; Pnevmatikakis et al., 2014; Deneux et al., 2016). But the relationship between spik-
538 ing and fluorescence change is not fully understood. For example, the fluorescent indicator
539 will act like an additional calcium buffer within the cell cytoplasm and will compete with
540 the other endogenous buffers to bind with free calcium ions. Therefore, the concentration
541 of those endogenous buffers, and the binding dynamics of those buffers will have an effect
542 on the change in fluorescence in response to an action potential. Furthermore, the binding
543 dynamics of the fluorescent indicator itself will have an effect on the change in fluorescence.
544 For example, the GCaMP series of fluorescence indicators are based on the calcium buffer
545 protein calmodulin. This protein has four binding sites, whose affinities interact non-linearly.
546 But most of the spike inference algorithms model the fluorescence as a linear function of
547 a calcium trace, and they model this calcium trace as a first or second order autoregression
548 with a pulse input to represent action potentials. Deneux et al. (2016) developed two dif-
549 ferent calcium fluorescence models behind their spike inference algorithm (MLspike) with a
550 more biological inspiration. For their simpler model, they take a physiological approach and
551 account for baseline calcium indicator dynamics. They end up with a system of first order
552 differential equations defining the dynamics of calcium concentration, baseline fluorescence,
553 and fluorescence. For their more complicated model specifically for genetically encoded cal-
554 cium indicators, they also took into account indicator binding and unbinding rates, which
555 added another equation to their system of equations. The algorithms that use the autore-
556 gression model and the MLspike algorithm are outperformed by the most recently published
557 spike inference algorithm (Greenberg et al., 2018). This algorithm takes into account the
558 binding dynamics of both the endogenous buffers and fluorescent calcium indicator, and the
559 concentrations of free calcium, indicator, and endogenous buffer within the cell cytoplasm.
560 The performance of this algorithm shows that there is value in more biologically inspired

561 models of fluorescent calcium indicators.

562 In light of the growing popularity of two-photon calcium imaging, and the lack of bio-
563 logically inspired spike inference algorithms ((Greenberg et al., 2018) developed their spike
564 inference algorithm in parallel to our work), we decided to develop a biologically inspired
565 model for fluorescent calcium indicator fluorescence. The idea being that our model would
566 take a spike train, or simply spike times, provided by the user, and return the fluorescence
567 trace that would be induced by this spike train or spike times. The model contains parameters
568 for concentrations of indicator and endogenous buffers, as well as affinity and unbinding rates
569 for these buffers. There are also parameters for the baseline concentration of free calcium in
570 the cell cytoplasm, and the cell radius (as a means for calculating the cell volume). With this
571 model, we hoped that experimentalists would be able to test out different calcium indicators
572 on the types of spike trains that they expect to encounter. This way they could decide ahead
573 of time which indicator suited their situation best. Since the output of our model is a fluo-
574 rescence trace, the spike inference models mentioned above can be applied to the modelled
575 fluorescence. This means that the model could also be used to benchmark the performance
576 of these spike inference algorithms, and to investigate the impact of variations in the model
577 on spike inference accuracy.

578 1.3 Functional networks

579 We have outlined some of the advantages that calcium imaging has over electrophysiology.
580 But electrophysiology is more useful in some situations. One particular drawback for two-
581 photon calcium imaging is that usually it can only be used for imaging near to the surface
582 of the brain. This problem can be solved by removing the tissue around the area to be im-
583 aged, and custom building a two-photon microscope Dombeck et al., 2010. Imaging with
584 three (or presumably more) photons may solve this problem in the future (Ouzounov et al.,
585 2017). A better option for reading activity from neurons beyond the surface of the brain is to
586 use Neuropixels probes (Jun et al., 2017). These probes can be used to read from thousands
587 of neurons simultaneously in many different areas of the brain (Allen et al., 2019; Stringer
588 et al., 2019; Steinmetz, Carandini, and Harris, 2019; Steinmetz et al., 2019). This brings us
589 to another problem for which we require new innovations in our analysis methods. Specif-
590 ically, analysing correlated behaviour in neural ensembles consisting of neurons from many
591 different brain regions.

592 Until the invention of new technologies such as the Neuropixels probes, most elec-
593 trophysiology datasets read from neurons in only one or two regions. Therefore most of
594 the research on interactions between neurons in different regions is limited to two regions
595 (Wierzynski et al., 2009; Patterson et al., 2014; Girard, Hupé, and Bullier, 2001). In chapters
596 3 and 4 we used datasets with neurons from 9 and 5 different brain regions respectively. In
597 their review of the interaction between growing the number of neurons in datasets and the
598 analysis methods applied to those dataset, Stevenson and Kording (2011) assert that an im-
599 portant objective of computational neuroscience is to find order in these kinds multi-neuron
600 of datasets. This was our main aim for the research described in chapter 3.

601 In light of recent findings based on correlated behaviour showing that spontaneous be-
602 haviours explain activity in many different parts of the brain that would otherwise be regarded
603 as noise (Stringer et al., 2019), that satiety is represented brain wide (Allen et al., 2019), and
604 that exploratory and non-exploratory states are represented in the amygdala (Gründemann
605 et al., 2019), it was clear that state representation or motor control had an influence on cor-
606 related behaviour in areas of the brain not usually associated with these tasks. Also, given
607 differences in timescales of fluctuations in different brain regions (Murray et al., 2014), and
608 different timescales for event representation in different brain regions (Baldassano et al.,
609 2017), we decided to investigate brain wide correlated behaviour at timescales ranging from
610 5ms up to 3s.

611 We started off measuring the correlations in spike counts between individual neurons in
612 our ensemble. These measurements induced a weighted undirected graph where each node
613 represented a neuron, and the weight of each edge was the strength of the correlation be-
614 tween the neurons represented by the nodes at either end of that edge. In order to put the
615 neurons into groups with correlated behaviour, we applied a novel community detection al-
616 gorithm (Humphries et al., 2019) to this graph. We repeated this analysis for timescales
617 from milliseconds to seconds. Bear in mind that our correlation based graph was completely
618 agnostic of the anatomical regions in which our cells resided. We then compared our corre-
619 lated communities to their anatomy at each timescale. In this way, we used a novel method,
620 never applied neuronal data before, to analyse the makeup of correlated communities across
621 different regions at different timescales.

622 1.4 A new statistical model for capturing correlated behaviour

623 Many important findings have been made by measuring the correlations between binned
624 spike counts, but there are some problems with this method of analysis. Firstly, the width
625 of the bins used to bin spike times into spike counts has an effect on the magnitude of the
626 correlations measured. Using a short bin width can cause your measurements to be artificially
627 small (Cohen and Kohn, 2011). This may not be an issue if one is considering relative size of
628 correlations when using the same bin width, but it is still not ideal. Secondly, while pairwise
629 correlations can capture most of the information in a small network (up to 40 cells) of highly
630 correlated cells (Schneidman et al., 2006), a model based on pairwise correlations alone will
631 fail to capture the activity of larger (~ 100 cells) networks, higher order correlated activity
632 is required (Ganmor, Segev, and Schneidman, 2011). One problem with these higher order
633 correlations is that they may be defined in different ways (Staude, Grün, and Rotter, 2010).
634 Furthermore if we want to include them in a model this usually involves greatly increasing the
635 number of parameters to fit, which increases the dimension of the parameter space leading
636 to the ‘curse of dimensionality’. Some models attempt to sidestep these problems while
637 still capturing higher-order correlations. These models attempt to capture the relationship
638 between each individual neuron in the ensemble, and the ensemble as a whole. Okun et al
639 (2015) called the strength of this relationship the ‘population coupling’, and demonstrated
640 that this quantity can predict an individual neuron’s response to optogenetic stimulation of
641 the whole ensemble. They also showed that this quantity was an indicator of the neuron’s
642 synaptic connectivity (Okun et al., 2015). With the ‘population tracking model’, O’Donnell
643 et al. (2016) linked the probability of firing an action potential for each individual neuron
644 with the distribution of the number of active neurons. This allowed model fitting for a large
645 number of neurons, as well as calculation of full pattern probabilities, and population entropy
646 (O’Donnell et al., 2017).

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

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

662 We fit a COMb distribution to spike sorted electrophysiological data taken from five
663 different regions in the brain of an awake mouse exposed to visual stimuli (Steinmetz et al.,
664 2019). We examined whether or not a model based on the COMb distribution was able to
665 capture changes in the number of active neurons in these neuronal ensembles in response to
666 the stimuli. We also investigated the relationship between the changes as captured by the
667 COMb model and the change in neural variability as measured by Churchland et al. in their
668 famous paper (Churchland et al., 2010).

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

675 **Chapter 2**

676 **Sensitivity of the**
677 **spikes-to-fluorescence transform to**
678 **calcium indicator and neuron**
679 **properties**

680 *Abstract*

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

694 **2.1 Introduction**

695 Although fluorescent calcium indicators such as GCaMP are widely used to monitor neuronal
696 activity, the relationship between the fluorescence signal and the underlying action potential
697 firing is imperfect. The fluorescence signal has a low signal-to-noise ratio, and most indica-
698 tors' kinetics are slow relative to the millisecond-timescale dynamics of the membrane volt-
699 age (example in figure 2.1A). This makes spike inference difficult. Furthermore, the effects
700 of the indicator and cell properties on the fluorescence signal are unknown. For example,
701 genetically encoded indicators can accumulate within neurons over weeks and months (Chen
702 et al., 2013). Studies using calcium-sensitive fluorescent dyes have shown that indicator con-
703 centration has substantial effects on the spike-to-fluorescence relationship (Maravall et al.,
704 2000). Therefore spike rates inferred from GCaMP fluorescence signals may give mislead-
705 ing results if comparing across imaging sessions. More generally, the poor understanding of
706 the spike-to-fluorescence transform means experimenters may not know whether to trust the
707 outputs of spike train inference methods in any given application.

708 Spike trains are usually inferred from the time series of intensity values of one pixel of the
709 fluorescence image, where the pixel is located at the cell's soma. The problems of identifying
710 these pixels, and inferring spikes from their time series can solved separately or together.
711 When attempting to infer spikes, the fluorescence trace is modelled as a linear combination of
712 calcium concentration dynamics, a baseline calcium concentration, and some Gaussian noise.
713 The calcium concentration dynamics are modelled as an autoregressive process of degree 1
714 or 2 with a pulse input corresponding to the spike train, or the number of spikes fired in a
715 time step. The model includes no dynamics for the fluorescent indicator itself. Furthermore,
716 in order to make this model into an easily solvable linear programming problem the number
717 of spikes fired in a timestep is not restricted to non-negative integers but to arbitrary non-
718 negative values (Vogelstein et al., 2010; Pnevmatikakis et al., 2016; Friedrich and Paninski,
719 2016; Pnevmatikakis et al., 2013; Pnevmatikakis et al., 2014). More biologically inspired
720 spike inference models do exist (Deneux et al., 2016), but their fundamentals are very similar.
721 In this work, we investigated the effect of changing dynamics and buffer concentrations on
722 the accuracy of the inference algorithms based on these models.

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

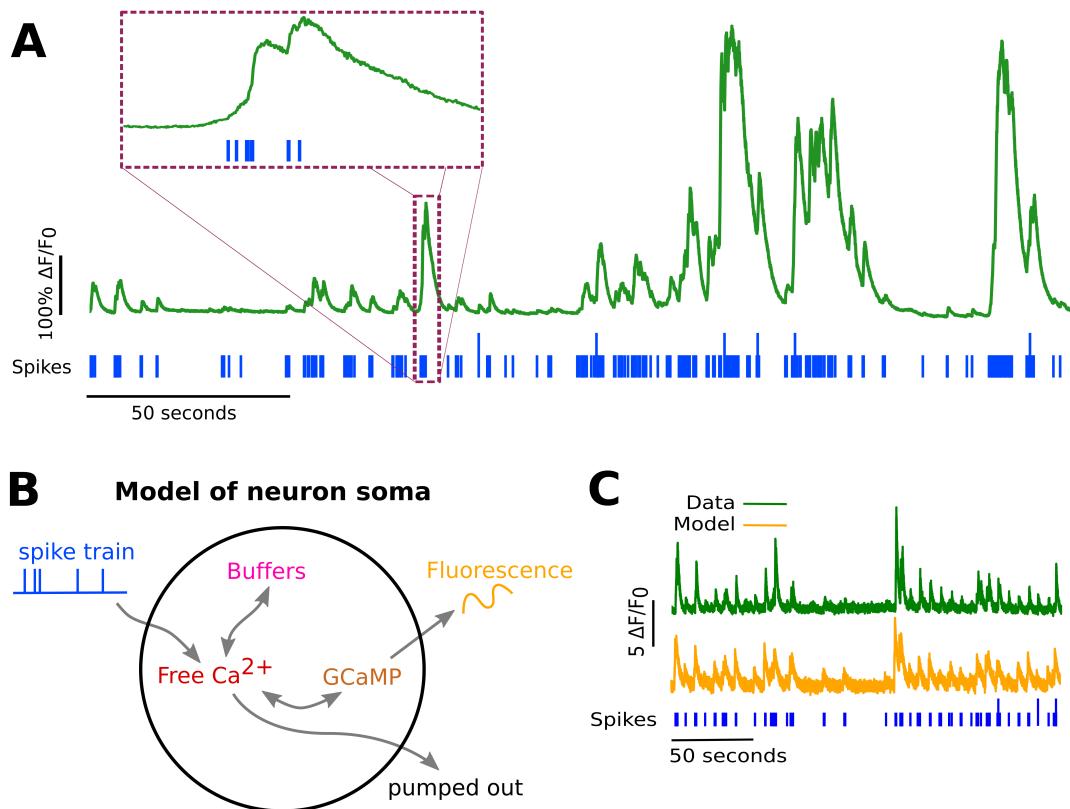


FIGURE 2.1:

- A: Example spike train (blue) and the corresponding GCaMP6s fluorescence trace (green), data replotted from (Berens et al., 2018). Inset shows zoomed section of traces to highlight slow decay of GCaMP6s fluorescence relative to spike time intervals.
- B: Schematic diagram of the neuron calcium and GCaMP computational model.
- C: Good visual match of data fluorescence trace (green) and model simulated fluorescence (orange) in response to an identical spike train (blue).

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

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

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

738 2.2 Methods

739 2.2.1 Calcium dynamics model

740 We wrote a biophysical model of the calcium dynamics within a cell soma. When a neuron
741 fires an action potential, voltage-dependent calcium ion-channels open up that allow a current
742 of calcium ions (Ca^{2+}) to flow into the neuron (Koch, 1999). The increase in the free calcium
743 ion concentration inside of the cell, along with changes in the concentration of potassium
744 and sodium, causes the change in cell membrane potential, which must be repolarised. The
745 repolarising process consists of free calcium ions leaving the cell through open ion channels,
746 or binding to molecules within the cell called buffers, or calcium storage by organelles such
747 as the endoplasmic reticulum. A diagram illustrating the cell, its channels, and its buffers
748 can be seen in figure 2.1A. There are several different types of calcium buffer, each with
749 different dynamics and different concentrations within different types of excitable cell. The
750 fluorescent calcium indicator is another calcium buffer, with the useful property that when it
751 is bound to a calcium ion, the bound molecule may become excited by a photon and release
752 a photon in return. This is what creates the fluorescence. After the action potential has taken
753 place, the free calcium concentration within the cell will return to a baseline level (Maravall
754 et al., 2000).

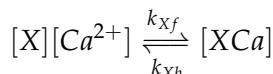
755 We modelled the the dynamics of five molecular concentrations,

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

2.2. Methods

- 757 • Fluorescent indicator bound calcium, $[BCa]$
- 758 • Endogenous mobile buffer bound calcium, $[ECa]$
- 759 • Endogenous immobile buffer bound calcium, $[ImCa]$
- 760 • Excited buffered calcium, $[BCa^*]$

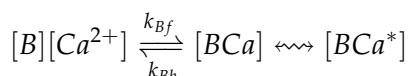
The term ‘buffering’ refers to free calcium ions coming into contact with buffer molecules followed by the binding of those molecules. Diagrammatically:



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

765 There are a number different endogenous buffers in any neuron. Which buffers are
766 present, and the buffers’ concentrations vary from cell to cell. In order to capture the ef-
767 fects of different kinds of buffers without modelling dozens of different individual buffers,
768 we modelled two different kinds of buffer only. These ‘mobile’ and ‘immobile’ buffers were
769 designed to be aggregations of the effects of multiple different buffers into two effective
770 buffers with different concentrations and binding rates. (Bartol et al., 2015) also divide cal-
771 cium buffers into mobile and immobile varieties. Note that since the model has no spatial
772 component, the mobile and immobile buffers only differ in their binding and unbinding rates.

The fluorescent calcium indicator behaves similarly to the other calcium buffers. The cal-
cium 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.



773 The released photons are captured by a photon collector. This gives us the fluorescence trace.

774 The system of equations we used to model all of these interactions is as follows:

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

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

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

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

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

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

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

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

791 Photon release & capture

792 We used a simple model for the photon release. The number of photons released at each time
793 step was controlled by the number of excited indicator bound calcium molecules in the cell

2.2. Methods

794 and a parameter called the ‘release rate’. The release rate is an optimised free parameter of
795 the model.

796 As for the photon capture, in two-photon excitation microscopy the photons scattered
797 by the fluorescent indicator get scattered in all directions. Therefore the number of photons
798 detected is stochastic. This made the process for capturing photons the natural source of
799 noise in the system. The number of photons captured, and therefore the intensity of the
800 fluorescence, is modelled using a binomial distribution. The number of photons released was
801 used as the number of trials. The probability of success, or ‘capture rate’ was a free parameter
802 of the model that we optimised.

803 2.2.2 Parameter optimisation

804 The free parameters of the model are as follows:

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

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

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

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

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

814 1. The frequency power spectrum of the trace was measured.

815 2. The power spectrum was smoothed using a boxcar smoother (aka. sliding average, box
816 smoother).

817 3. The log of the smoothed power spectrum was measured.

818 4. Use the model to create a modelled fluorescence trace.

819 5. Apply steps 1, 2, and 3 to the modelled fluorescence trace.

820 6. Calculate the root mean squared difference between the log power of the actual fluo-
821 rescence trace, and the log power of the modelled fluorescence trace.

822 7. Calculate the root mean squared difference between the actual fluorescence trace and
823 the modelled fluorescence trace.

824 8. Use an optimisation algorithm to reapply this process, attempting to minimize the sum
825 of the two root mean squared differences at each iteration.

826 Using the root mean squared difference of the log power spectra as part of the objective
827 function forces the model to match the noise frequency of the actual fluorescence. Using
828 the root mean squared difference of the traces themselves forces the model to match the
829 amplitude of the fluorescence trace more accurately. Using both of these terms as part of our
830 objective function was designed to make our model match the change in $\Delta F / F_0$ in response
831 to an action potential as well as the signal-to-noise ratio of the the actual fluorescence trace.
832 We weighted both of these terms equally.

833 In order to minimise the objective function, a suite of meta-heuristic optimisation (aka.
834 black-box optimisation) algorithms were implemented on each of the traces in the dataset.
835 These methods were chosen because they don't require a gradient for the objective function
836 (gradient-free) and they are particularly useful for minimising stochastic objective functions
837 like the one we used here. The free parameters were optimised for each individual fluores-
838 cence trace. The most successful method for each trace was recorded. The method that was
839 most often successful was probabilistic descent, and the second most successful method was
840 generating set search. Both of these methods are examples of pattern search. These two
841 methods were the best optimisers on about 75% of the traces in the dataset. The other meth-
842 ods were differential evolution (with and without radius limited sampling, adaptive and not
843 adaptive), natural evolution strategy, and random search for comparison.

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

2.2. Methods

| Parameter | Description | Value | Source |
|-------------|---|--|--------------------------|
| baseline | The baseline concentration of free calcium within the cell soma | $4.5 \times 10^{-8} \text{M}$ | (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 | $7.87 \times 10^{-5} \text{M}$ | (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 | 160s^{-1} | (Bartol et al., 2015) |
| k_{Bf} | The binding rate of the fluorescent calcium indicator | $7.77 \times 10^8 \text{s}^{-1} \text{M}^{-1}$ | (Bartol et al., 2015) |
| k_{Eb} | The unbinding rate of the endogenous mobile buffer | 10^4s^{-1} | (Bartol et al., 2015) |
| k_{ef} | The binding rate of the endogenous mobile buffer | $10^8 \text{s}^{-1} \text{M}^{-1}$ | (Bartol et al., 2015) |
| k_{Imb} | The unbinding rate of the endogenous immobile buffer | 524s^{-1} | (Bartol et al., 2015) |
| k_{Imf} | The binding rate of the endogenous immobile buffer | $2.47 \times 10^8 \text{s}^{-1} \text{M}^{-1}$ | (Bartol et al., 2015) |
| peak | The increase in free calcium concentration within the cell induced by an action potential | $2.9 \times 10^{-7} \text{M}$ | (Maravall et al., 2000) |

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

855 **Fixed parameters**

856 As well as the optimised parameters mentioned in section 2.2.2, the model also has thirteen
 857 fixed parameters. Please see table 2.1 for details of these parameters and their values. In
 858 an application of the model, these parameters can be changed in order to model any given
 859 fluorescent calcium indicator, or even prospective indicators that only exist in theory.

860 **2.2.3 Julia**

861 The programming language used to write and execute the model was ‘Julia’. Julia is a dy-
 862 namic programming language designed for technical computing. Julia was designed specif-
 863 ically to provide a convenient high-level dynamic language similar to MATLAB, or Python,
 864 with improved performance. Julia’s type system and Julia’s direct interfaces with C and

865 Fortran allow this aim to be achieved (Bezanson et al., 2012). The Julia version of the
866 ‘Sundials’ package for ODE solving was used to solve the system of equations above. The
867 BlackBoxOptim.jl package for Julia was used to perform the optimisation.

868 **2.2.4 Spike inference**

869 We used spike inference algorithms to compare the quality of spike inference using the mod-
870 elled traces to the quality of spike inference using the observed traces. We also used the
871 spike inference algorithms to assess the effect of parameter perturbation on the spike infer-
872 ence. Three algorithms were used:

873 **Constrained non-negative matrix deconvolution algorithm (aka CNMD algorithm)** The
874 underlying model models the fluorescence as a linear function of a calcium trace with
875 additional noise. This calcium trace is a first order autoregression with a pulse input to
876 represent action potentials. This algorithm uses a constrained version of non-negative
877 Weiner deconvolution to infer a calcium signal and a ‘spiking activity signal’ from the
878 fluorescence trace (Vogelstein et al., 2010; Pnevmatikakis et al., 2016). The spiking ac-
879 tivity signal is a non-negative vector of real numbers reflecting the cell’s activity rather
880 than an actual spike train. We inferred a spike train by choosing an optimised thresh-
881 old for the spiking activity signal. Whenever the spiking activity signal exceeded that
882 threshold, an action potential was inferred. The threshold was optimised by minimis-
883 ing the difference between the number of spikes observed and the number of spikes
884 predicted.

885 **MLSpike algorithm** Deneux et al. (2016) developed two different calcium fluorescence
886 models behind their spike inference algorithm (MLspike) with a more biological in-
887 spiration. For their simpler model, they take a physiological approach and account for
888 baseline calcium indicator dynamics. They end up with a system of first order differen-
889 tial equations defining the dynamics of calcium concentration, baseline fluorescence,
890 and fluorescence. For their more complicated model specifically for genetically en-
891 coded calcium indicators, they also took into account indicator binding and unbinding
892 rates, which added another equation to their system of equations. This algorithm uses a
893 generalised version of the Viterbi algorithm to return the spike train that maximises the
894 likelihood of producing the given fluorescence trace. The Viterbi algorithm is an algo-
895 rithm for estimating the most likely sequence of hidden states resulting in a sequence
896 of observed states in a discrete-time finite-state Markov process (Forney, 1973). In this

2.2. Methods

897 case, each hidden state is defined by the presence or absence of an action potential, and
898 each observed state is the value of the fluorescence trace at each time step. (Deneux
899 et al., 2016).

900 We used the autocalibration algorithm provided with this spike inference algorithm to
901 estimate values for the standard deviation of the white noise in the fluorescence trace, the
902 increase in fluorescence in response to an action potential, and the decay time constant
903 of the fluorescence trace. The standard deviation of the noise in the trace was estimated
904 as the mean of power spectral density of the trace in the frequency range between 3 and
905 20Hz. The authors of this algorithm believed that the frequencies lower than this range
906 will contain information about the calcium dynamics, while the frequencies above this
907 range contain correlated noise and are therefore unsuitable for estimating white noise.

908 The increase in fluorescence in response to an action potential and the decay time
909 constant were estimated in parallel. Firstly, a ‘calcium events’ trace was extracted
910 from the fluorescence trace using a modified version of the spike inference algorithm
911 with fixed values for the increase in calcium in response to a calcium event and de-
912 cay time constant. The temporally isolated calcium events with moderate amplitude
913 ($\Delta F/F \leq .25$) are extracted, and the other events are removed. The idea here is to
914 extract a trace of the calcium concentration produced by isolated groups of action po-
915 tentials. The event amplitude and the time decay constant are then estimated from
916 this trace of ‘good’ events. Next, a histogram of the event amplitudes is constructed.
917 An estimate of the number of spikes in each event is estimated from the peaks of this
918 histogram. A number of spikes is then assigned to each calcium event based on this
919 estimate. Finally, the autocalibrated values for the change in fluorescence and the decay
920 time constant are calculated by fitting to the modified calcium event trace.

921 Many values that may be influential in the autocalibration process were hard-coded by
922 the authors of the algorithm. Particularly, defining ‘moderate’ calcium events as those
923 that produce $\Delta F/F \leq .25$ may be suitable for indicators with lower signal-to-noise
924 ratios than GCaMP6s. This could be reason for this algorithms poor performance in
925 our application (see Results section 2.3.2).

926 **Online Active Set method to Infer Spikes (OASIS)** This algorithm is once again based on
927 an auto-regressive model of the fluorescence trace, but can be generalised to any or-
928 der. Both the first and second order versions can be fit to a spike train in a reasonable
929 time. The algorithm itself is a generalisation of the pool adjacent violators algorithm

930 (PAVA) that is used in isotonic regression. The OASIS algorithm works through the
931 fluorescence trace from beginning to end, this combined with the speed of the algo-
932 rithm means that it could be used for real-time online spike inference (Friedrich and
933 Paninski, 2016).

934 This algorithm required the standard deviation of the noise component of the fluo-
935 rescence trace and the fluorescence trace decay time constant to be estimated. The
936 standard deviation of the noise was estimated by calculating the power spectral den-
937 sity of the fluorescence trace, then taking the mean of the density across the frequency
938 range between 0.25 and 0.5 times the Nyquist rate for the fluorescence trace. These
939 frequencies were judged to be most likely to contain uncorrelated noise, rather than
940 signal or correlated noise, by the developers of the algorithm. The time constant of the
941 fluorescence trace was estimated from the autocovariance of the denoised fluorescence
942 trace. In practice, we used the `estimate_parameters` function provided by the
943 authors of this algorithm to estimate these parameters.

944 This algorithm also required one more parameter, a constant that indicated the minimal
945 non-zero activity within a bin in order for a spike to be assigned to that bin. When
946 applying the algorithm, we used 10 different values for this parameter, and counted
947 the total number of spikes inferred by the algorithm each time. We then chose the
948 value that gave the smallest difference between the total number of inferred spikes
949 and the total number of actual spikes. Calibrating the algorithm in this way gives the
950 algorithm an advantage for inferring the number spikes, but not for inferring the timing
951 of the spikes. This kind of calibration makes the results of the different algorithms
952 incomparable, but the results from the same algorithm applied to different fluorescence
953 traces are still comparable.

954 In order to quantify the quality of spike inference for a given algorithm, we ran that algorithm
955 on all of the fluorescence traces in dataset number eight of the spike finder datasets. These
956 datasets contained fluorescence traces from neurons containing a fluorescent calcium indica-
957 tor (either OGB-1 or GCaMP6s) and spike trains from those cells simultaneously recorded
958 using loose-patch electrophysiological recordings. This provided us with a *ground-truth* for
959 spike inference from the fluorescence traces. Then we measured some binary classification
960 measures on the results. These measures included

- 961 ● Accuracy
962 ● True positive rate (aka recall, sensitivity, hit rate)

2.2. Methods

963 ● True negative rate (aka specificity)

964 ● Precision

965 ● Negative predicted value

966 ● False negative rate (aka miss rate)

967 ● False positive rate (aka fall-out)

968 ● False discovery rate

969 ● False omission rate

970 In making these measurements, we allowed a tolerance of two subsequent time bins for spike
971 prediction. For example, the spike train data is a vector of 0s and 1s, with one element
972 for each time bin, and time bin being 10ms. A ‘0’ denotes inactivity, a ‘1’ denotes the
973 presence of at least one action potential. The inferred spike trains produced by the spike
974 inference algorithms take the same form. In our analysis, if a spike appeared in the inferred
975 spike train up to two time frames after a spike in the observed spike train, that spike was
976 considered correctly inferred i.e. a true positive. However, once a spike in the inferred
977 spike train was matched to a spike from the observed spike train, the inferred spike could
978 not be matched to another observed spike. To illustrate, if two spikes were inferred in the
979 two time bins following an isolated observed spike, the first inferred spike was considered
980 correctly inferred, but the second inferred spike was considered incorrectly inferred, i.e. a
981 false positive.

982 The most useful measure was the true positive rate. This is because the spiking is sparse
983 and this measurement is sensitive to the number of spikes observed and inferred, but is not
984 affected by the true negative or false negative rates. After optimising the parameters for each
985 fluorescence trace we measured the spike inference quality for the observed fluorescence
986 traces, and compared this to the spike inference quality for the modelled traces.

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

991 **Comparing spike inference quality**

992 In order to compare spike inference quality we had to use methods for comparing samples.

993 When comparing the true positive rate distributions arising from two different datasets, or

994 two different algorithms on the same dataset, we compared the distributions using a paired
995 t-test.

996 **2.2.5 Perturbation analysis**

997 In order to measure the sensitivity of spike inference to changes in a given model parameter,
998 we perturbed the parameter and compared the quality of spike inference with the perturbed
999 parameters to the quality of spike inference with the experimental or optimised parameters.
1000 In order to maximise the possibility of observing a difference due to the perturbation, we
1001 perturbed the chosen parameter by a relatively large amount. For example, the experimen-
1002 tal value for the molar concentration of the fluorescent indicator within the cell was 10^{-4}M
1003 (Maravall et al., 2000). The perturbed values used for this parameter were 10^{-2}M , 10^{-3}M ,
1004 10^{-5}M , and 10^{-6}M . The quality of the inference was compared by measuring the true posi-
1005 tive rate for each perturbed value and using a t-test to compare the distributions of the results.

1006 **2.2.6 Signal-to-noise ratio**

1007 To assess the effect of perturbation on the modelled traces, we measured and compared the
1008 signal to noise ratio (SNR) on each of the modelled traces. We calculated the SNR as the
1009 peak change in fluorescence divided by the standard deviation of the baseline fluctuation of
1010 the fluorescence trace (Tada et al., 2014). We measured these values by running the model
1011 on a spike train consisting a long period of inactivity followed by one action potential. We
1012 ran the model on this spike train one hundred times. We then measured the mean change
1013 in fluorescence and standard deviation of baseline activity across the one hundred modelled
1014 fluorescence traces, and calculated the SNR.

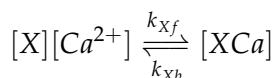
1015 **2.2.7 Data sources**

1016 All of the data used in this project was sourced from the ‘Spike Finder’ project
1017 (spikefinder.codeneuro.org). The data consisted of a collection of datasets with simultane-
1018 ously measured fluorescence traces and action potentials (Berens et al., 2018).

2.3 Results

1019 **2.3.1 A biophysical computational model can generate accurate fluorescence**
 1020 **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



1022 where X is the buffer concentration and Ca^{2+} is the calcium concentration. Each species
 1023 could therefore exist in two states: either bound with calcium or unbound. To model the
 1024 imaging process, we also added a third, excited state to the indicator. When in the calcium-
 1025 bound state, the indicator could be converted to an excited state, corresponding to the absorp-
 1026 tion of a photon. The rate of this excitation process could be interpreted as the intensity of
 1027 the light illuminating the sample. Once excited, the species decayed back to the unexcited
 1028 state at a fixed rate, corresponding to the spontaneous emission of photons. The total emitted
 1029 fluorescence signal was interpreted as proportional to this de-excitation flux. To represent
 1030 experimental noise in the photon capture process, we drew a random number of captured
 1031 photons at each time step from a binomial distribution, parameterised by a number p that
 1032 corresponds to the mean fraction of released photons that are captured.

1033 The model had 17 parameters in total describing the molecules' concentrations and re-
 1034 action rates (Methods). We set 13 of these parameters to values from the literature. The
 1035 remaining 4 parameter values we fit to publicly-available data (Berens et al., 2018), briefly
 1036 explained as follows (see Methods for full details). Single neurons from acute rat cortical
 1037 slices expressing GCaMP6s were imaged with two-photon microscopy while the membrane
 1038 potentials of the somata of the same neurons were simultaneously recorded via whole-cell
 1039 patch clamp electrophysiology. In this dataset, the electrical recordings give unambiguous
 1040 information about neurons' spike times. To do the parameter fitting, we feed these spike
 1041 trains as inputs to the computational model. After running, the model returns a simulated
 1042 fluorescence trace. We aimed to find the model parameter values that give the best match
 1043 between this simulated fluorescence trace and the real fluorescence time series recorded in

1044 the corresponding neuron. To do this we used a suite of optimisation procedures to jointly
1045 fit both the real neuron's fluorescence time series and power spectrum, which capture com-
1046 plementary information about the spikes-to-fluorescence mapping (Methods). We performed
1047 the fitting procedure independently for each of the 20 neurons in the spikefinder dataset
1048 (<http://spikefinder.org>). After fitting, the model produced realistic-looking fluorescence time
1049 series (Figure 2.1).

1050 Given that fluorescence traces are often modelling using a linear combination of a first
1051 degree autoregressive process and white noise, it could be argued that a four parameter model
1052 contains some redundancy in the parameter space. But, our aim was to create a biophysical
1053 model that could be useful for interpreting the role of cell dynamics behind the production of
1054 the fluorescence trace. Because of that we felt that our redundancy was justified.

1055 **2.3.2 Spike inference algorithms perform similarly on real data compared with
1056 time series simulated from the model**

1057 Researchers often pass the fluorescence time series through a spike inference tool before per-
1058 forming further statistical analyses. These spike inference algorithms take the fluorescence
1059 trace as input and attempt to estimate the neuronal spike train that triggered them (Vogelstein
1060 et al., 2010; Pnevmatikakis et al., 2016; Friedrich and Paninski, 2016; Pnevmatikakis et al.,
1061 2013; Pnevmatikakis et al., 2014; Deneux et al., 2016). Part of our motivation for building
1062 this model was to allow us to investigate which properties of the cell and the calcium indi-
1063 cator affect the quality of spike inference? In order to trust the conclusions from our model,
1064 we should first be confident that spike inference from our simulated fluorescence traces is
1065 similar to that from the real data. To test this we passed each of the simulated fluorescence
1066 traces through three previously published spike inference algorithms, quantified their perfor-
1067 mance against the ground-truth electrophysiology data, repeated the procedure for the real
1068 calcium fluorescence time series, and compared the accuracy of the inference processes in
1069 all cases. The *true positive rate*, also known as the *recall*, the *sensitivity*, or the *probabil-
1070 ity of detection* of spike inference varied across the three inference algorithms we tried (p
1071 value and statistical test here). The constrained non-negative matrix deconvolution algorithm
1072 (Pnevmatikakis et al., 2016) (CNMD algorithm) correctly detected approximately 45% of the
1073 true spikes, the OASIS algorithm (Friedrich and Paninski, 2016) correctly detected approx-
1074 imately 35% of the true spikes, and the ML spike algorithm (Deneux et al., 2016) correctly
1075 detected approximately 15% of the true spikes (see figure 2.2). Notably, for two of the three
1076 inference algorithms, the quality of inference was also fairly consistent for individual spike

2.3. Results

1077 trains, not just the group means ($p > 0.05$, paired t-test). This demonstrates that the models
 1078 were generating fluorescence time series that were similarly difficult to decode as the real
 1079 data, in ways that were not specific to any one inference algorithm. This is evidence that the
 models captured real aspects of the spikes-to-fluorescence transform.

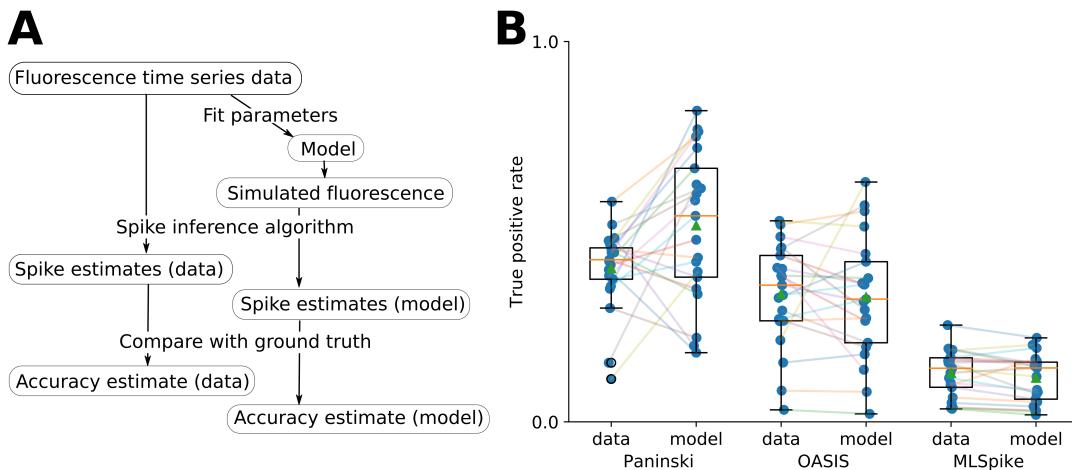


FIGURE 2.2:
 A: Workflow to compare spike inference for real versus simulated fluorescence data.
 B: True positive rates achieved by three different spike inference algorithms when applied to observed spike trains, and simulated spike trains. Data points overlaid as blue circles. The performance is similar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

1080

2.3.3 Relative effects of various buffers to the fluorescence signal

1082 One of the benefits of computational models over laboratory experiments is that we can
 1083 observe all the variables in the simulation to gain insight into the system's dynamics, which
 1084 can be difficult to do in the lab. We plotted the concentrations of the various species over
 1085 time for a version of the model fit to one data set, in response to the same train of spikes used
 1086 for fitting (figure 2.3). Figure 2.3a shows the absolute values of the species concentrations,
 1087 summed. Consistent with experimental estimates (Maravall et al., 2000), only a small fraction
 1088 ($\sim 0.1\%$) of calcium is free and unbound to any buffer. Of the bound calcium, the vast
 1089 majority, ($\sim 96\%$) is bound to the GCaMP indicator. The two types of endogenous buffer
 1090 are bound to the remaining calcium ($\sim 4\%$). An influx of calcium from a single spike adds
 1091 very little to the total calcium, in relative terms (red line in Figure 3a).

1092 When calcium entered the model neuron it was rapidly buffered (Bartol et al., 2015).
 1093 However the relative fractions of which buffer molecules bound to the influxed calcium was

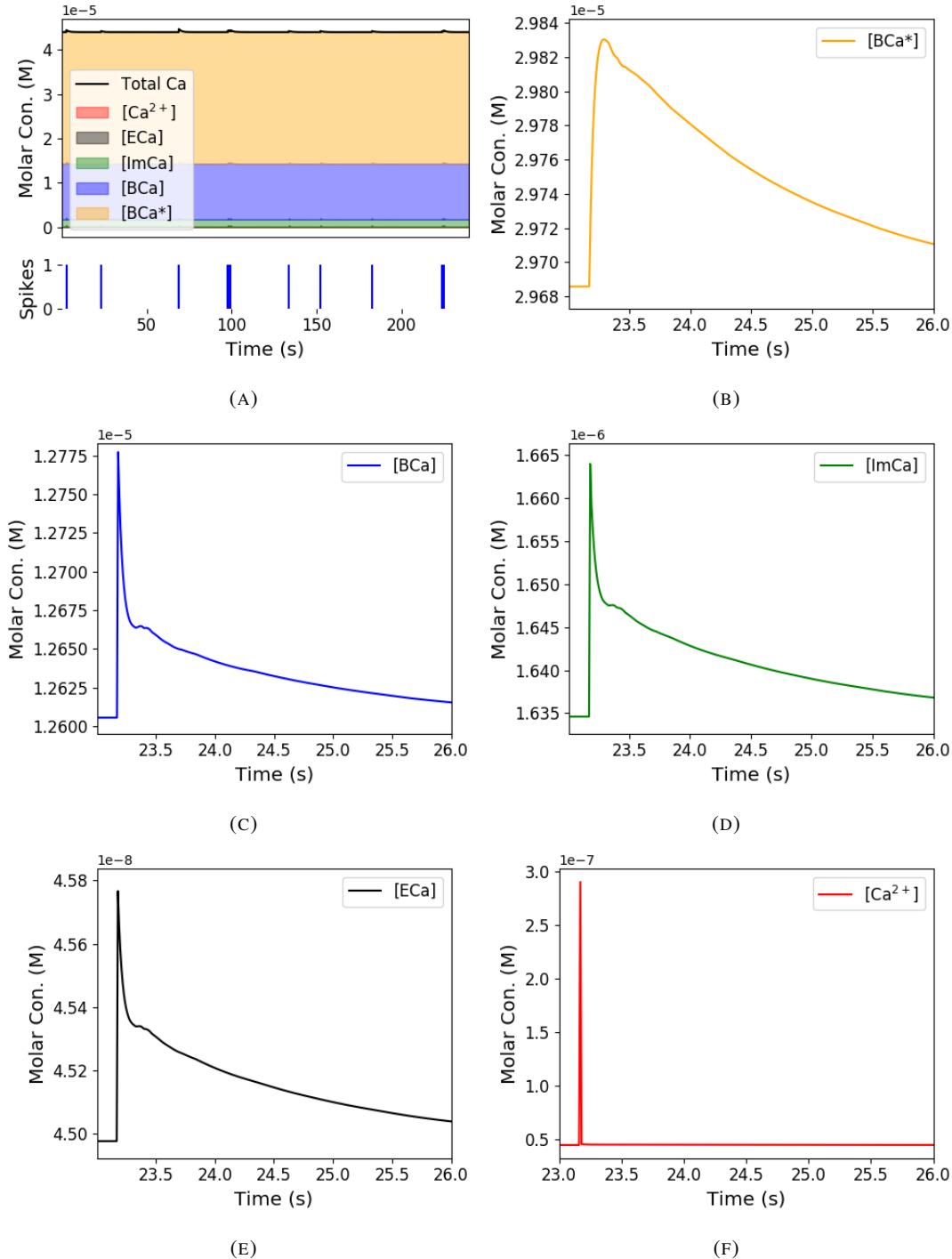


FIGURE 2.3: **Calcium Buffering Dynamics** (A) The proportions of bound and free calcium concentrations within a cell, with the associated spike train. (B)-(F) The dynamics of the concentration of (B) excited indicator bound calcium, (C) indicator bound calcium, (D) immobile endogenous buffer bound calcium, (E) mobile endogenous buffer bound calcium, and (F) free calcium in response to an action potential at $\sim 23.2s$.

2.3. Results

dynamic, and changed over time. Figure 2.3 (b-f) shows the time course of the various species over time in response to a calcium influx event from a single action potential. Crucially, the indicator $[BCa]$ competed with the endogenous buffers $[ImCa]$ and $[ECa]$ – all three bind calcium on similar timescales. This implies that the timecourse and amplitude of the $[BCa]$ variable will also depend on the binding rates and availabilities of the endogenous buffers. For example if we decreased the concentration of an endogenous buffer, we might expect both a faster rise time and greater peak amplitude of the $[BCa]$ signal in response to a calcium influx event. The slowest component of the decay had a similar time constant for $[BCa]$, $[ImCa]$ and $[ECa]$, which in turn matched the $[Ca]$ extrusion time constant in our model ($\sim 6.29 \times 10^{-22} \text{Ms}^{-1}$). This implies that the buffers and the indicator had reached a dynamic equilibrium and were jointly tracking the free calcium concentration as calcium was slowly extruded from the cell.

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

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

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

Several variants of GCaMP itself have been made that differ in calcium binding kinetics, baseline fluorescence, fluorescence efficiency, and other factors. For example, GCaMP6f has a decay time constant of $\sim 1\text{s}$, while GCaMP6s has a decay time constant of $\sim 2\text{s}$ (Chen et al., 2013). Here we asked how these differences in binding kinetics affect spike inference. We jointly varied the calcium binding and unbinding rates of the indicator by the same factor over a range from 100-fold slower to 100-fold faster from the fitted values, and simulated the

1127 fluorescence response for each of the parameter settings in response to the same spike trains
1128 as before (figure 2.4). Notably this manipulation does not affect the indicators affinity, and
1129 therefore would not affect steady-state responses to prolonged changes in calcium. Instead
1130 it is likely to affect its sensitivity to the spike train dynamics. We computed two summary
1131 measures from the simulated fluorescence traces: the signal-to-noise ratio for a single spike
1132 (Methods, section 2.2.6), and the accuracy of spike inference for each of the spike trains. We
1133 observed a reduction in the signal-to-noise ratio and the spike inference quality when we set
1134 the binding and unbinding rates were set to one hundredth of their fitted values, and to one
1135 tenth of their fitted values. When we increased the value of both binding rates, we observed
1136 no change in these measurements. The reduction in both rates lead to smaller increases in
1137 fluorescence in response to an action potential and a longer decay time (figure 2.4a), this
1138 caused the reduction in signal-to-noise ratio. As both rates were increased, the change in
1139 $\Delta F/F_0$ in response to an action potential increased and the decay time decreased slightly,
1140 but the fluorescence trace created by these values was very similar to the trace created by the
1141 fitted values.

1142 Second, the overall concentrations of GCaMP often varies from cell to cell. For exam-
1143 ple different cells, even of the same type in the same tissue, can express different levels of
1144 GCaMP, due to proximity to the infection site, or the cell becoming ‘nuclear-filled’ (Tian et
1145 al., 2009; Chen et al., 2013). Also, GCaMP is often used for longitudinal experiments where
1146 the same cells are re-imaged across multiple days or weeks. However since GCaMP expres-
1147 sion typically ramps up over time (Chen et al., 2013), the accuracy of spike inference may
1148 differ across multiple longitudinal recordings in the same cell. We addressed this by varying
1149 the concentration of calcium indicator in the model, simulating spike trains and measuring
1150 signal-to-noise ratio and spike inference accuracy on the resulting fluorescence traces. Both
1151 increasing and decreasing the concentration of the indicator had effects on the fluorescence
1152 trace, signal-to-noise ratio, and spike inference. The signal-to-noise ratio and spike inference
1153 quality decreased with decreased indicator concentration, and both showed a decrease when
1154 the indicator concentration was increased to 100 times its fitted value (figure 2.5). The signal-
1155 to-noise ratio showed an increase when the indicator concentration was increased to 10 times
1156 its fitted value, but there was no corresponding change in the spike inference quality. The
1157 decrease in indicator concentration caused a reduction in the increase in $\Delta F/F_0$ in response
1158 to an action potential, and an increase in the decay time of this increase (figure 2.5a). The
1159 increase in indicator concentration had the opposite effect, it caused an increase in the change
1160 in $\Delta F/F_0$ in response to an action potential, and a decrease in the decay time.

2.3. Results

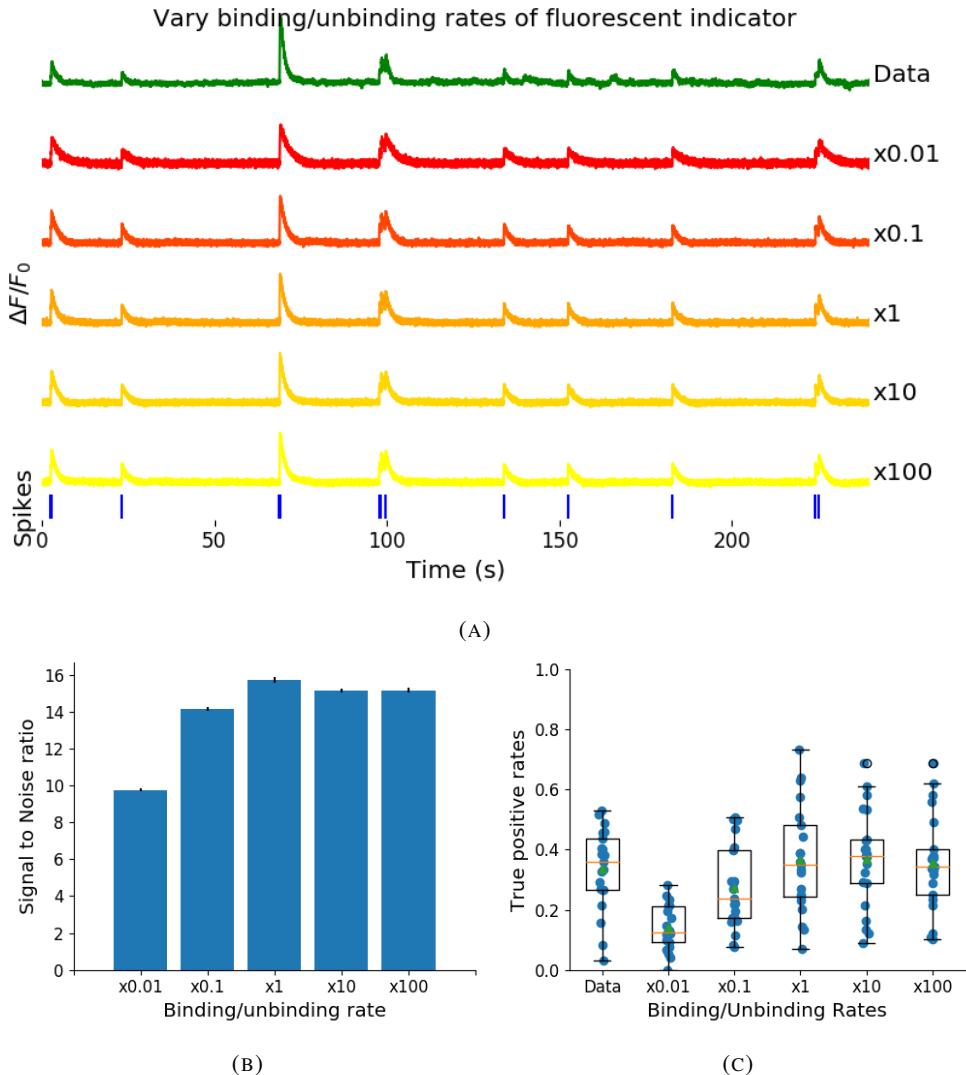


FIGURE 2.4: (A) An example trace for each of the five pairs of values used for the binding and unbinding rates of the fluorescent calcium indicator. (B) The signal-to-noise ratio of the modelled fluorescence traces using each of the four perturbed value pairs, and the experimental value. The SNRs for the value pairs perturbed downward are lower than that for the unperturbed value pair or the higher value pairs. (C) The true-positive rates of the deconvolution algorithm's predictions when inferring from the observed data, and inferring from modelled traces using the perturbed and experimental values. We used the OASIS algorithm for spike inference here. The results from the other spike inference methods were similar, with their true positive rates scales similarly to figure 2.2 B.

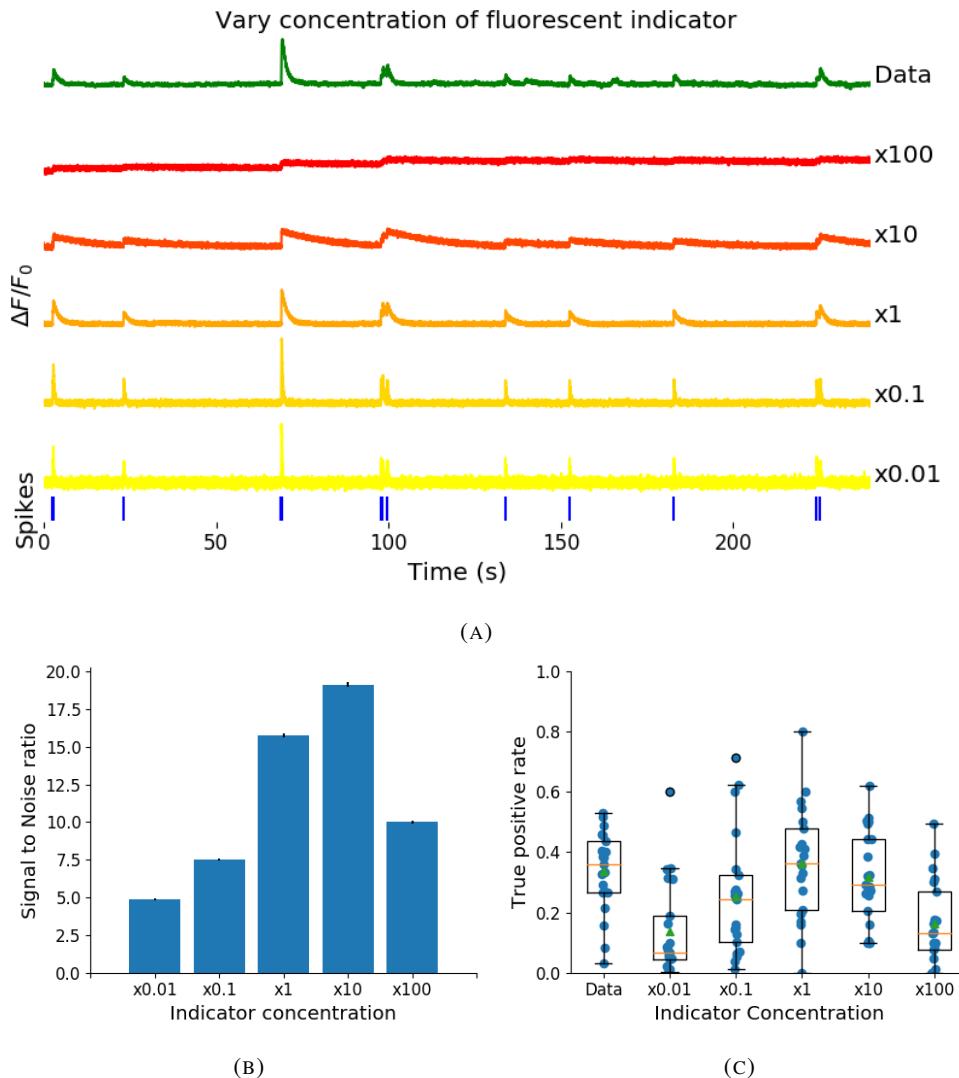


FIGURE 2.5: (A) An example trace for each of the five perturbed values for the concentration of fluorescent calcium indicator. The top two traces are produced by the lower perturbed values, the middle trace is produced by the experimental value, and the lowest two traces are produced when using the higher perturbed values. (B) The signal-to-noise ratio of the modelled fluorescence traces using each of the four perturbed values, and the experimental value. Extreme perturbations of the concentration either above or below the experimental level lowered the SNR. (C) The true-positive rates of the deconvolution algorithm's predictions when inferring from the observed data, and inferring from modelled traces using the perturbed and experimental values. We found that the algorithms performs equally badly on the two most extreme values, and performs equally well on the experimental value, and the next higher perturbed value. We used the OASIS algorithm for spike inference here. The results from the other spike inference methods were similar, with their true positive rates scales similarly to figure 2.2 B.

2.3. Results

1161 Third, the concentration and types of endogenous calcium buffers also vary from neuron
1162 to neuron, both within and between cell types (Bartol et al., 2015; Maravall et al., 2000;
1163 Neher and Augustine, 1992). Since the calcium buffer capacity of neurons is high, around
1164 50-70 (Lee et al., 2000) in excitatory hippocampal pyramidal cells, around 100-250 (Lee et
1165 al., 2000) in inhibitory hippocampal pyramidal cells, and 900-200 in Purkinje cells (depend-
1166 ing on the age of the subject), these endogenous buffers compete with GCaMP for binding
1167 to calcium, and variations in endogenous buffer concentration may affect GCaMP signal and
1168 therefore spike inference. To address this we varied the concentration of the endogenous
1169 buffer in the model neuron over five orders of magnitude from 0.8 to 8000 μM , simulated
1170 calcium fluorescence traces in response to the same set of spike trains, and performed spike
1171 inference on the resulting fluorescence time series. Increasing the endogenous buffer con-
1172 centration had a substantial effect on the GCaMP fluorescence signal, both decreasing its
1173 amplitude and slowing its kinetics (figure 2.6(a)). This corresponded with a decrease in both
1174 single-spike signal-to-noise ratio (figure 2.6(b)) and spike inference accuracy (figure 2.6(c)).
1175 In contrast, decreasing endogenous buffer capacity from the fitted value had little effect on
1176 either the GCaMP signal or spike inference (figure 2.6).

1177 2.3.5 Single spike inference accuracy drops for high firing rates, but firing rate 1178 itself can be estimated from mean fluorescence amplitude

1179 The fluorescence signal recorded from neurons using calcium indicators is typically much
1180 slower than changes in membrane potential for two reasons: first, because the calcium and
1181 the indicator have slow binding and unbinding kinetics, the signal is a low-pass filtered ver-
1182 sion of the membrane potential. Second, neuronal two-photon imaging experiments are often
1183 performed in scanning mode, which limits their frame rate to $\sim 10\text{Hz}$ or slower. This im-
1184 plies that multiple spike events that occur close in time might be difficult to resolve from a
1185 calcium indicator time series. Many cells, especially several types of inhibitory interneurons,
1186 fire tonically at rates higher than 10Hz. We used the model to test whether spike inference
1187 accuracy depended on the neuron's firing frequency by driving the cell with spike trains sam-
1188 pled from a Poisson processes of varying frequency. We simulated a variable firing rate using
1189 an Ornstein-Uhlenbeck process, and simulated the spike trains using a Poisson distribution
1190 with its rate taken from this process. Because of the high frequency firing rate of these spike
1191 trains, we used the accuracy as the measure of spike inference quality. We simulated 30
1192 spike trains at average firing rate of 1, 5, and 10Hz, and measured the spike inference quality
1193 of all these traces. Spike inference accuracy decreased with increasing firing rate, for up to

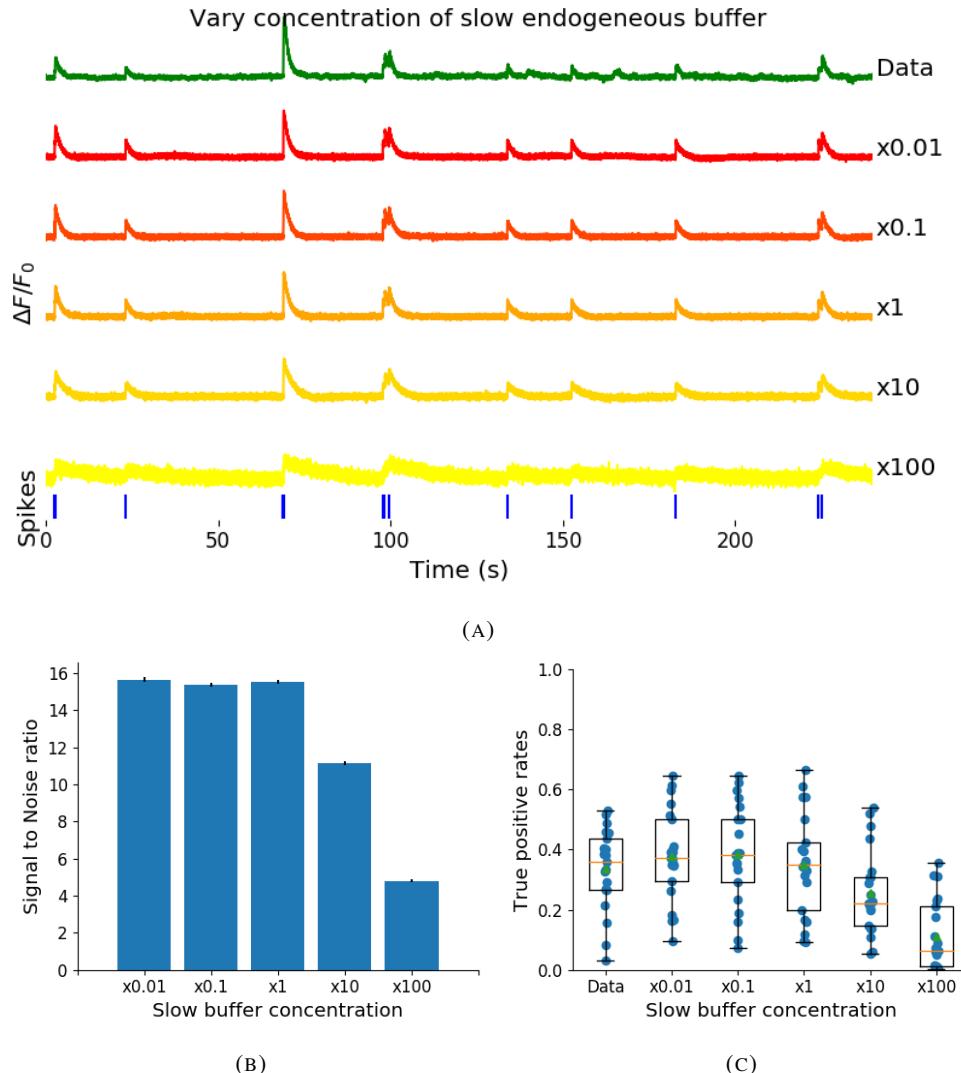


FIGURE 2.6: (A) An example trace for each of the five perturbed values for the concentration of immobile endogenous buffer. (B) The signal-to-noise ratio of the modelled fluorescence traces using each of the four perturbed values, and the experimental value. The lower values for the immobile buffer produce the same SNR as the experimental value. But the higher perturbed values produce fluorescence traces with a lower SNR. (C) The true-positive rates of the deconvolution algorithm’s predictions when inferring from the observed data, and inferring from modelled traces using the perturbed and experimental values. We used the OASIS algorithm for spike inference here. The results from the other spike inference methods were similar, with their true positive rates scales similarly to figure 2.2 B.

2.3. Results

10Hz Poisson spike trains (figure 2.8(left)). Although the accuracy remained above 90% for each of the three frequencies. We also plotted the average $\Delta F/F_0$ as a function of stimulation firing rate. We found that it increased monotonically as a function of firing rate (figure 2.8(right)).

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

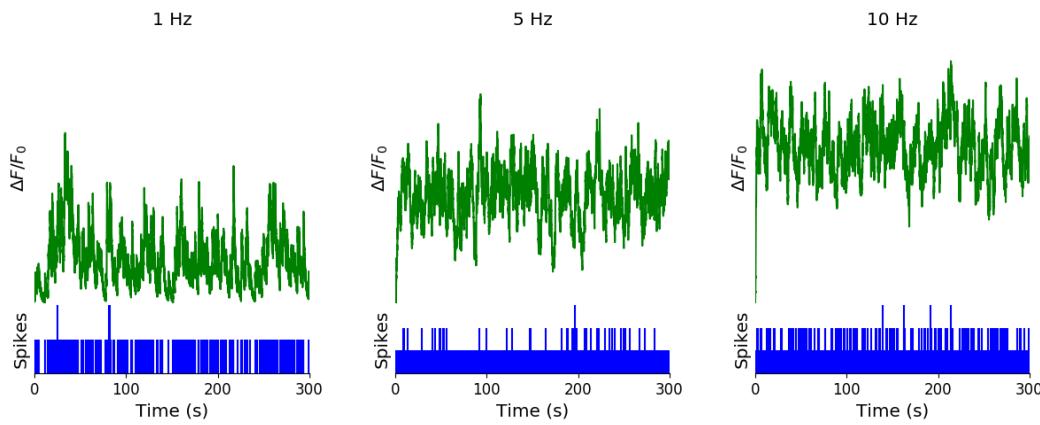


FIGURE 2.7: Simulating fluorescence traces at different firing rates Example modelled traces created using simulated spike trains with a mean firing rate of 1Hz (left column), 5Hz (middle column), and 10Hz (right column). Note the difference in amplitude with different mean firing rates.

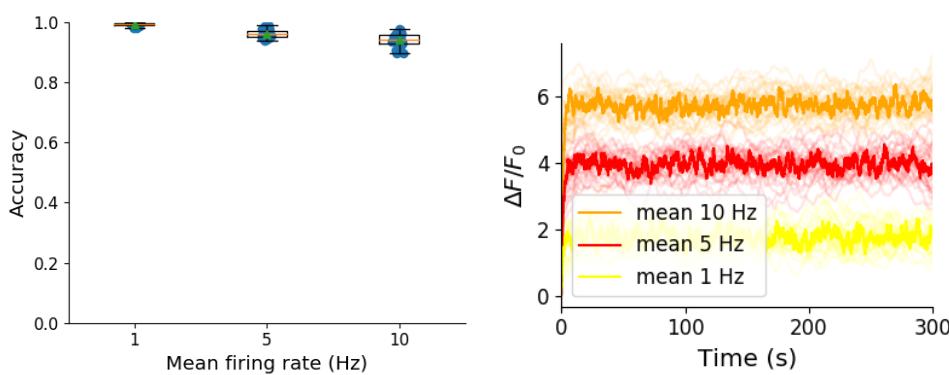


FIGURE 2.8: Inference quality and $\Delta F/F_0$ vs Firing rate (left) The spike inference accuracy when applied to 30 traces created using simulated spike trains with mean firing rates of 1, 5, and 10 Hz. (right) The mean $\Delta F/F_0$ across those 30 traces for each frequency.

1203 **2.4 Discussion**

1204 We designed a biophysical model for the changes in free calcium and bound calcium con-
1205 centrations within a cell soma with a fluorescent calcium indicator. We used this model to
1206 model the fluorescence trace resulting from a spike train in this cell. We fit the free parame-
1207 ters of the model by matching the power spectrum and amplitude of fluorescence traces with
1208 simultaneously measured spike trains. We inferred spikes from real fluorescence traces and
1209 modelled fluorescence traces, and measured the quality of the spike inference in both cases.
1210 We found that the spike inference quality was similar in both cases. We perturbed the concen-
1211 tration of the calcium buffers in the model, and the binding/unbinding rates of those buffers
1212 in the model, and measured the effect on the signal-to-noise ratio (SNR) of the modelled
1213 fluorescence traces and the spike inference quality.

1214 For the fluorescent calcium indicator, we found that any large perturbation away from the
1215 value taken from the literature led to a reduction in SNR, and spike inference quality. For
1216 the binding/unbinding rates, we kept the ratio of these rates constant, but altered their values
1217 in parallel. The lower values caused a reduction in SNR, and a reduction in spike inference
1218 quality. For the endogenous buffer concentration, an increase above the experimental value
1219 caused a reduction in SNR and spike inference quality.

1220 We perturbed the concentration of the indicator, the binding/unbinding rates, and the en-
1221 dogenous buffer concentration to values 100 times smaller and 100 times larger than than
1222 the value taken from the literature. Given that the indicator concentration can be controlled,
1223 at least to some extent, we thought it was worthwhile to simulate these extreme perturba-
1224 tions. For the binding/unbinding rates, such extreme values in these rates are unlikely for
1225 fluorescent calcium indicators or endogenous buffers. But these extreme perturbations are
1226 still useful for studying what kind of fluorescence trace an indicator with such extreme bind-
1227 ing/unbinding rates produce. Similarly, these extreme values in endogenous buffer are also
1228 unlikely to occur in neurons. But, using these values in our model allows us to analyse the
1229 interplay between the endogeneous buffer concentration and the fluorescence trace produced.

1230 Although the model produced visually similar time series to the real data, there were a
1231 few aspects it did not capture. First, the real data featured some low-frequency components
1232 that did not appear related to the spike events. These were not captured by the models we
1233 used in this study, but could be added in future by adding a suitable low-frequency term to
1234 the resulting time series. Second, the real data seemed to have some non-linearities not cap-
1235 tured in the model, for example the response to two nearby spikes was greater than expected

2.4. Discussion

from the linear sum of two single spikes. This may be due to the co-operative binding of calmodulin to calcium, which gives calmodulin a supra-linear sensitivity to calcium concentration (Faas et al., 2011). The non-linear dynamics of this binding have been included in a recently developed spike inference model (Greenberg et al., 2018). Our model, in contrast, behaved much more linearly but could be extended in future to include such non-linearities.

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

The concentration of free calcium ions in the neuron cytoplasm enables calcium signalling, which has a vital role in neuronal energy metabolism, and neurotransmission in neurons (Brini et al., 2014). Our model allowed us to examine to which calcium buffer the incoming free calcium ions, due to an action potential, bound. We found that around 95% of the calcium ions bound to the fluorescent calcium indicator. This suggests that the introduction of the fluorescent calcium indicator has a dramatic effect on the free calcium concentration within the cell cytoplasm. This could have a downstream effect on the functionality of the cell.

As well as the optimised parameters, the model has 13 fixed parameters than can be changed to simulate different types of calcium indicators. This model could be used to test the theoretical performance of proposed new types of calcium indicator. The model could also be used by developers of spike inference algorithms to test the effects of changing calcium indicator parameters on spike inference, or to test the affects of changing spiking characteristics on spike inference. For example, high firing rate vs low firing rate, or bursting vs no bursting. Given the increasing amplitude of the fluorescence trace with increasing mean firing rate, it would be possible to build a spike inference algorithm on this principle at least

1270 in part.

1271 Our model has already been used as a tool by our colleagues, for simulating fluorescence
1272 traces in response to cells that can fire with a continuous rate between 10 and 20Hz, but do
1273 not always do so. Our colleagues found that a combination of the amplitude and the variance
1274 of the simulated fluorescence trace was the best indicator of firing rate. For example, when
1275 a cell was not firing, the amplitude and variance of the fluorescence trace was relatively
1276 low. When the cell fired with a low firing rate $\sim 1\text{Hz}$, the mean amplitude was still low
1277 but the variance of the fluorescence trace was high, and for high firing rate 10 – 20Hz, the
1278 fluorescence amplitude was high, and the variance was low. In this way, our model may be
1279 useful for investigating firing rates underlying real fluorescence traces in response to cells
1280 which can fire in these rage ranges.

1281 A recent paper by Greenberg et al (2018) described a biophysical model for spike train
1282 inference called the ‘Sequential binding model’. Their model for spike inference was sim-
1283 ilar to our model for fluorescence traces in that their model included parameters for two
1284 types of endogenous buffer. But this model also included dynamics for calcium binding to
1285 and unbinding from these endogenous buffers. Furthermore, this model included dynamics
1286 for calcium binding to and unbinding from the four binding sites present on a GCaMP6s
1287 molecule. In the accuracy measurements specified in that paper, this model performed better
1288 than the MLspike algorithm, which is also partially a biophysically model, and it performed
1289 better than the constrained non-negative deconvolution algorithm. The sequential binding
1290 model also has biophysically interpretable parameters, and its fitted parameters for quantities
1291 such as buffering capacity and calcium influx upon action potential firing fall in line with
1292 experimental values (Greenberg et al., 2018). Biophysical models like this appear to be the
1293 way forward for spike inference algorithms, and would make a good complimentary tool to
1294 our fluorescence model.

1295 **Chapter 3**

1296 **Functional networks expand across**
1297 **anatomical boundaries as correlation**
1298 **time-scale increases**

1299 *Abstract*

1300 Decades of research has established that correlated spiking plays a crucial role in represent-
1301 ing sensory information. One drawback associated with the recent improvement in recording
1302 technology and consequent large datasets is the difficulty in analysing higher order correla-
1303 tions in large neuronal ensembles. One benefit of these datasets that has not yet been explored
1304 is the opportunity to compare correlations within anatomical regions to correlations across
1305 anatomical regions. In this work, we measured correlations between neurons residing in
1306 nine different brains regions in three awake and behaving mice. Using the these correlation
1307 measurements, we created weighted undirected graph networks and applied network science
1308 methods to detect functional communities in our neural ensembles. We compared these func-
1309 tional communities to their anatomical distribution. We repeated the analysis, using different
1310 timescales for our correlation measurements, and found that functional communities were
1311 more likely to be dominated by neurons from a single brain region at shorter timescales
1312 (< 100ms).

1313

3.1 Introduction

1314 Decades of research has established that correlations play a crucial role in representing sen-
1315 sory information. For example, the onset of visual attention has been shown to have a greater
1316 affect on the correlations in the macaque V4 region than on the firing rates in that region
1317 (Cohen and Maunsell, 2009). Recent findings show that spontaneous behaviours explain cor-
1318 relations in parts of the brain not associated with motor control (Stringer et al., 2019), that
1319 satiety state appears to have a brain wide representation (Allen et al., 2019), and that subject
1320 exploratory and non-exploratory states are represented in the amygdala (Gründemann et al.,
1321 2019). So, behavioural states are likely represented across many regions of the brain, not just
1322 motor related areas. In order to understand the brain, we must understand the interactions
1323 between neurons and regions.

1324 Because of limitations in recording technology almost all research has explored corre-
1325 lations between neurons within a given brain region, or within only two regions at most
1326 (Wierzynski et al., 2009; Patterson et al., 2014; Girard, Hupé, and Bullier, 2001). Rela-
1327 tively little is known about correlations between neurons in many different brain regions.
1328 However, the recent development of ‘Neuropixels’ probes (Jun et al., 2017) has allowed
1329 extracellular voltage measurements to be collected from multiple brain regions simultane-
1330 ously routinely, and in much larger numbers than traditional methods. In this project we
1331 used a publicly-available Neuropixels dataset to analyse correlations between different brain
1332 regions (Stringer et al., 2019).

1333 A drawback associated with the improvement in recording technology is an increase in
1334 the difficulty in analysing these data. For example, analysing the i th order interactions of
1335 N neurons generally requires estimation of N^i parameters. A number that becomes astro-
1336 nomical for large N . New methods are required for analysing these new large datasets. We
1337 attempted to address this requirement in this piece of research by applying a cutting-edge
1338 network science community detection method to neural data.

1339 Another unexplored area of research is the changes in cell interactions at different timescales.
1340 Studies have shown different timescales for fluctuations in spiking activity (Murray et al.,
1341 2014), and different time scales for event representation (Baldassano et al., 2017) across dif-
1342 ferent brain regions. Still most studies focus on quantifying interactions at a given timescale.
1343 But neurons may interact differently, or may interact with different neurons at different
1344 timescales. Here we explore correlated communities of neurons at different timescales.

1345 In this work, we measured correlations between binned spike counts from neurons from

3.2. Data

1346 nine different regions of the mouse brain. These measurements induced a weighted undi-
1347 rected graph or network where each neuron is represented by a node, and the strength of
1348 the connection between these nodes/neurons is the strength of the correlation between their
1349 spike counts. We then applied newly invented network methods (Humphries et al., 2019)
1350 to this network to find any community structure, and place the neurons in these correlation
1351 based communities. Finally, we compared these functional communities to the anatomical
1352 membership of the neurons.

1353 To investigate the functional communities and their relationship with anatomy at different
1354 time scales, we repeated these analyses using different length bin widths when binning spike
1355 times.

1356 To find and analyse functional networks while controlling for the subject’s behaviour, we
1357 conditioned the binned spike counts on data from a video of the subject’s face, and repeated
1358 our analysis for spike count correlations (or noise correlations) and signal correlations.

1359 3.2 Data

1360 The data that we used in this project were collected by Nick Steinmetz and his lab members
1361 (Stringer et al., 2019; Steinmetz et al., 2019).

1362 3.2.1 Brain regions

1363 Neuropixels probes were used to collect extracellular recordings (Jun et al., 2017) from three
1364 different mice. The mice were awake, headfixed, and engaging in spontaneous behaviour.
1365 The mice were of different sexes and different ages. One mouse was ‘wild-type’, the others
1366 were mutants. Details as follows:

- 1367 1. male, wild type, P73.
- 1368 2. female, TetO-GCaMP6s, Camk2a-tTa, P113
- 1369 3. male, Ai32, Pvalb-Cre, P99

1370 Eight probes were used to collect readings from 2296, 2668, and 1462 cells respectively.
1371 Data were collected from nine brain regions in each mouse:

- 1372 • Caudate Putamen (CP)
- 1373 • Frontal Motor Cortex (Frmocxt)
- 1374 • Hippocampal formation (Hpf)

- 1375 ● Lateral Septum (Ls)
- 1376 ● Midbrain (Mb)
- 1377 ● Superior Colliculus (Sc)
- 1378 ● Somatomotor cortex (Sommotcx)
- 1379 ● Thalamus (Th)
- 1380 ● Primary visual cortex (V1)

1381 Readings were continuous and lasted for about 1 hour (Stringer et al., 2019; Steinmetz et al.,
1382 2019). Locations of each of the probes can be seen in figure 3.1.

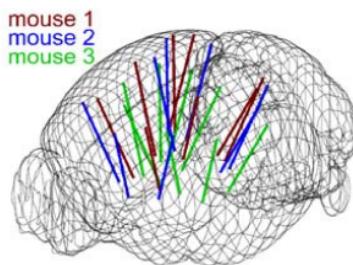


FIGURE 3.1: **Probe Locations:** The locations of the probes in each of the three mouse brains (Stringer et al., 2019).

1383 3.2.2 Video recordings

1384 Video recordings of the mouse's face were taken during the spontaneous behaviour. We
1385 had access to the top 500 principal components and top 500 eigenvectors of the processed
1386 videos. The frequency of recording was slightly less than 40Hz. Each frame contained
1387 327×561 pixels (Stringer et al., 2019; Steinmetz, Carandini, and Harris, 2019). These
1388 principal components were used as behavioural data. We controlled for these components
1389 when taking measurements conditioned on behaviour.

1390 3.3 Methods

1391 3.3.1 Binning data

1392 We transformed the spike timing data into binned spike count data by dividing the experi-
1393 mental period into time bins and counting the spikes fired by each cell within the time period
1394 covered by each of those bins. The data were divided into time bins of various widths ranging
1395 from 0.005s to 4s.

3.3. Methods

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

3.3.2 Correlation coefficients

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

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

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

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

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

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

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

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

1410 Total correlations, r_{SC}

In this context, we defined the total correlation (r_{SC}) of two cells to be the correlation between the spike counts of those cells across the entire period of spontaneous behaviour.

1413 **Shuffled total correlations**

1414 We measured the shuffled total correlations between two neurons by randomly permuting one
 1415 of the neuron's spike counts and measuring the total correlations. These shuffled correlations
 1416 were useful when measuring the effect of time bin width on correlations, and when decid-
 1417 ing which correlations should be preserved when creating correlation networks (see section
 1418 [3.3.5](#)).

1419 **Separating Correlations & Anti-correlations**

1420 In order to compare the effect of bin width on measures of negative r_{SC} (anti-correlation) and
 1421 positive r_{SC} separately, we had to separate correlated and anti-correlated pairs. To do this, we
 1422 simply measured the mean r_{SC} , taking the mean across all the bin widths. If this quantity was
 1423 positive or zero we regarded the pair as positively correlated. If this quantity was negative
 1424 we regarded the pair as anti-correlated.

1425 **3.3.3 Conditioning on behavioural data**

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

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

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

1426 Given the 500 components, a naïve estimation of $P(Z_1, \dots, Z_{500})$ or $P(X, Z_1, \dots, Z_{500})$ by
 1427 histogramming was impossible. Therefore we modelled X as a linear combination of the
 1428 PCs.

1429 **Linear regression**

1430 We modelled the spike count of a given cell, X , as a linear combination of the PCs of the
 1431 video of the mouse's face, $\mathbf{Z} = Z_1, \dots, Z_{500}$. We tried three different types of regularization

1432 • $L1$ or 'Lasso'

1433 • $L2$ or 'Ridge regression'

3.3. Methods

- 1434 • ‘Elastic net’ regularisation (a linear combination of both $L1$ and $L2$ regularisation
1435 penalties)

1436 The elastic net regularisation performed the best, so we stuck with that.

1437 **Elastic net regularisation**

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

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

where

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

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

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

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

1438 We implemented the model using the `ElasticNetCV` method of Python’s
1439 `sklearn.linear_models` package. We chose to put equal weighting on the $L1$ and $L2$
1440 regression parts of equation 3.6. We used 10-fold cross validation to set an optimised value
1441 for $\lambda_1 = \lambda_2$.

1442 As well as using the PCs, we also tried fitting the models using the raw video data recon-
1443 structed from the PCs and eigenvectors. These models performed worse than those using the
1444 PCs. We expected this because each representation contains the same amount of information,
1445 but the raw video representation spreads this information across many more components.
1446 This requires more parameter fitting, but given the same information.

1447 **Conditional covariance**

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

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

1448 where these expected values are calculated with respect to the distribution of Z as a random
1449 variable.

1450 The law of total covariance breaks the covariance into two components. The first com-
1451 ponent $E[\text{cov}(X, Y|Z)]$ is the expected value, under the distribution of Z , of the conditional
1452 covariance $\text{cov}(X, Y|Z)$. This covariance could be interpreted as the unnormalised version
1453 of what Cohen et al. (2011) call the spike count correlation (Cohen and Kohn, 2011), aka.
1454 the noise correlation. In particular, this is the covariance of the spike counts in response to
1455 repeated presentation of identical stimuli.

1456 The second component is analogous to what Cohn et al. (2011) call the *signal correlation*
1457 (Cohen and Kohn, 2011). In particular, $\text{cov}(E[X|Z], E[Y|Z])$ is the covariance between
1458 spike counts in response to different stimuli.

1459 Our linear model gave us 500 coefficients, one for each of Z_1, \dots, Z_{500} . By summing
1460 the linear combination of these coefficients and a set containing one value for each Z_i , we
1461 obtained our model's estimate for the spike count of the cell represented by X . We interpreted
1462 our model as a function that takes a set of values $\{Z_1 = z_1, \dots, Z_{500} = z_{500}\}$ as input and
1463 returns $E[X|Z_1 = z_1, \dots, Z_{500} = z_{500}]$.

1464 Using our linear model, we calculated $E[X|Z_1, \dots, Z_{500}]$ for each cell X and for all sets
1465 of values for $\{Z_1, \dots, Z_{500}\}$ available to us. We used those values to calculate $\text{cov}(E[X|Z_1, \dots, Z_{500}], E[Y|Z_1, \dots, Z_{500}])$
1466 for each pair of cells (X, Y) . Then we proceeded to calculate

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

1467 **Measures of conditional correlation**

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

$$\rho_{XY|Z} = \frac{E[\text{cov}(X, Y|Z)]}{\sqrt{E[\text{var}(X|Z)]E[\text{var}(Y|Z)]}} \quad (3.12)$$

3.3. Methods

1468 Although this is not an actual correlation, it is an intuitive analogue to the correlation as a
1469 normalised version of the covariance.

For comparison, we also measured the ‘signal correlation’

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

1470 this is an actual correlation.

3.3.4 Information Theory

1472 We used an information theory based measure to measure the difference between the com-
1473 munities that we detected in the correlation based functional networks that we constructed
1474 and the anatomical division of the cells in our data. We treated these as clusterings, and mea-
1475 sured the distance between them. We also were planning on using the mutual information
1476 between the spike counts of cells as measure upon which to build functional networks. But
1477 our measurements turned out to be heavily biased. So we abandoned that approach.

1478 As a result, we have here a lot of background on information theory. This information
1479 is still useful for understanding our measure of distance between between clusterings. So I
1480 think it is worth keeping.

1481 Entropy $H(X)$

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

$$H(X) = - \sum_{n=1}^N p_n \log_2 p_n \quad (3.14)$$

1482 This quantity is also known as the information entropy or the ‘surprise’. It measures the
1483 amount of uncertainty in a random variable. For example, a variable with a probability of 1
1484 for one outcome, and 0 for all other outcomes will have 0 bits entropy, because it contains no
1485 uncertainty. But a variable with a uniform distribution will have maximal entropy as it is the
1486 least predictable. This quantity is analogous to the entropy of a physical system (Shannon,
1487 1948). Note that any base may be used for the logarithm in equation 3.14, but using base 2
1488 means that the quantity will be measured in ‘bits’.

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

$$H(X, Y) = - \sum_{n=1}^N \sum_{m=1}^M P(X = x_n, Y = y_m) \log_2 P(X = x_n, Y = y_m) \quad (3.15)$$

- 1489 If X and Y are independent then $H(X, Y) = H(X) + H(Y)$. Otherwise $H(X, Y) <$
 1490 $H(X) + H(Y)$. When X and Y are completely dependent and the mapping from X to Y
 1491 is one-to-one, $H(X, Y) = H(X) = H(Y)$.

The conditional entropy of Y conditioned on X is defined as

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

- 1492 When X and Y are independent $H(Y|X) = H(Y)$. Intuitively, we learn nothing of Y by
 1493 knowing X , so Y is equally uncertain whether we know X or not. If Y is totally dependent
 1494 on X , then the fraction in the logarithm is 1, which gives $H(Y|X) = 0$.

1495 These entropy measures are the basis of the mutual information measure.

1496 Maximum entropy limit

- 1497 Originally, we intended to measure the mutual information between the spike counts of cells.
 1498 We included this section to explain why using larger bin widths resulted in potentially larger
 1499 spike counts, containing potentially more information, corresponding with higher mutual
 1500 information values. The idea of measuring the mutual information between spike counts was
 1501 abandoned. But this section was kept as an illustration of how the number of values a random
 1502 variable can take affects the information conveyed by that variable.

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

3.3. Methods

this neuron will be

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

1503 Therefore, the maximum entropy of the binned spike counts of a neuron is $\log_2 (n_{\max} + 1)$.
 1504 Of course, it would be very unusual for a neuron to fire in accordance with the discrete
 1505 uniform distribution. Most measurements of entropy taken on binned spiking data will be
 1506 much lower than the maximum. See figure 3.2 to see the maximum entropy as a function of
 1507 the maximum observed spike count.

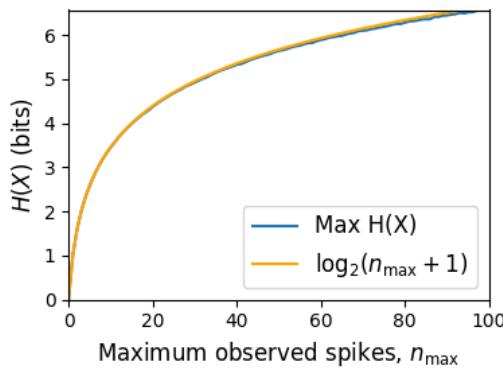


FIGURE 3.2: **Entropy Limit:** The upper limit on entropy of binned spike count data as a function of the maximum observed spike count. The orange line is the analytical maximum. The blue line is the entropy of samples with $N = 1000$ data points taken from the discrete uniform distribution.

1508 **Mutual Information** $I(X; Y)$

1509 The mutual information can be defined mathematically in a number of ways, all of which are
 1510 equivalent. These definitions illustrate the different ways of interpreting the mutual informa-
 1511 tion.

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

$$I(X; Y) = H(Y) - H(Y|X) \quad (3.17)$$

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

1512 Equation 3.17 fits with the following intuition: The mutual information between X and Y is
1513 the reduction in uncertainty about X gained by knowing Y , or vice versa. We could also say
1514 the mutual information is the amount of information gained about X by knowing Y , or vice
1515 versa.

Another useful entropy based definition for the mutual information is

$$I(X; Y) = H(X) + H(Y) - H(X, Y) \quad (3.19)$$

1516 This definition is useful because it does not require the calculation of conditional probabili-
1517 ties.

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

$$I(X; Y) = - \sum_{n=1}^N \sum_{m=1}^M P(X = x_n, Y = y_m) \log_2 \frac{P(X = x_n, Y = y_m)}{P(X = x_n)P(Y = y_m)} \quad (3.20)$$

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

$$I(X; Y) = D_{KL}(P(X, Y) || P(X)P(Y)) \quad (3.21)$$

1518 So, we can also think of the mutual information as a measure of the difference between
1519 the joint distribution of X and Y , and the product of their marginal distributions. Since the
1520 product of the marginal distributions is the joint distribution for independent variables, we
1521 can think of the mutual information as a measure of the variables' dependence on one another.

1522 The minimum value that $I(X; Y)$ can take is 0. This occurs when the random variables
1523 X and Y are independent. Then we have $H(X|Y) = H(X)$, and $H(Y|X) = H(Y)$, which
1524 according to equation 3.17, gives $I(X; Y) = 0$. We also have that $H(X, Y) = H(X) +$
1525 $H(Y)$ in this case, which according equation 3.19, gives $I(X; Y) = 0$. Finally, we also have
1526 $P(X, Y) = P(X)P(Y)$, which leaves us with 1 in the argument for the logarithm in equation
1527 3.20, which again gives $I(X; Y) = 0$.

3.3. Methods

1528 The mutual information reaches its maximum value when one of the variables X and
1529 Y is completely determined by knowing the value of the other. In that case $I(X;Y) =$
1530 $\min\{H(X), H(Y)\}$.

1531 **Variation of Information** $VI(X, Y)$

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

$$VI(X;Y) = H(X) + H(Y) - 2I(X;Y) \quad (3.22)$$

We can rewrite this as the summation of two positive quantities

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

1532 In English, the variation of information is the summation of the uncertainty in the random
1533 variables X and Y excluding the uncertainty shared by those variables.

1534 This measure will become more relevant when we go on to talk about clusterings because
1535 $VI(X;Y)$ forms a metric on the space of clusterings.

1536 **Measuring entropies & mutual information**

1537 In practice, we measured the mutual information between spike counts using Python and the
1538 python package `pyitlib`. We used the PT-bias correction technique to estimate the bias of
1539 our measurements when measuring the mutual information between the spike counts of two
1540 cells (Treves and Panzeri, 1995).

1541 When measuring the mutual information between clusterings we used Python, but we
1542 used the `mutual_info_score`, `adjusted_mutual_info_score`, and
1543 `normalized_mutual_info_score` functions from the `sklearn.metrics` part of
1544 the `sklearn` package.

1545 **3.3.5 Network analysis**

1546 **Correlation networks**

1547 In order to analyse functional networks created by the neurons in our ensemble, we mea-
1548 sured the total correlation between each pair of neurons. These measurements induced an

1549 undirected weighted graph/network between the neurons. The weight of each connection
1550 was equal to the total correlation between each pair of neurons.

1551 We followed the same procedure for total correlations 3.3.2, spike count correlations, and
1552 signal correlations 3.3.3.

1553 **Rectified correlations**

1554 At the time of writing, the community detection method outlined in (Humphries et al., 2019)
1555 could only be applied to networks with positively weighted connections. But many neuron
1556 pairs were negatively correlated. To apply the community detection method, we *rectified* the
1557 network, by setting all the negative weights to zero.

1558 We also looked for structure in the network created by negative correlations by reversing
1559 the signs of the correlations, and rectifying these correlations before applying our network
1560 analysis.

1561 Finally, we used the absolute value of the correlations as the weights for the graph/network.
1562 By doing this, we hoped to identify both correlated and anti-correlated functional communi-
1563 ties of neurons.

1564 **Sparsifying data networks**

1565 When creating our correlation networks, we wanted to exclude any correlations that could
1566 be judged to exist ‘by chance’. To do this, we measured the 5th and 95th percentile of
1567 the shuffled correlations (see section 3.3.2) for the given mouse and time bin width. We
1568 then set all the data correlations between these two values to 0. This excluded any ‘chance’
1569 correlations from our network, and created a sparser network. This allowed us to make use
1570 of the ‘sparse weighted configuration model’ as described in section 3.3.5.

1571 **Communities**

1572 Given some network represented by an adjacency matrix \mathbf{A} , a community within that net-
1573 work is defined as a collection of nodes where the number of connections within these nodes
1574 is higher than the expected number of connections between these nodes. In order to quan-
1575 tify the ‘expected’ number of connections, we need a model of expected networks. This is
1576 analogous to a ‘null model’ in traditional hypothesis testing. We test the hypothesis that our
1577 data network departs from the null network model to a statistically significant degree. For
1578 undirected unweighted networks, the canonical model of a null network is the configuration

3.3. Methods

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

1581 **Weighted configuration model**

1582 The *weighted configuration model* is a canonical null network model for weighted networks.
1583 Given some data network, the weighted configuration model null network will preserve the
1584 degree sequence and weight sequence of each node in the data network. But the edges will
1585 be distributed randomly (Fosdick et al., 2016). Any structure in the data network beyond
1586 its degree sequence and weight sequence will not be captured in the weighted configuration
1587 model. So, this model can be used in testing the hypothesis that this extra structure exists.

1588 **Sparse weighted configuration model**

1589 The *sparse weighted configuration model* is another null network model. Similar in nature to
1590 the weighted configuration model (see section 3.3.5), but the sparsity of the data network is
1591 preserved in the null network. This is achieved by sampling from a probability distribution
1592 for the creation or non-creation of each possible connection, then distributing the weight of
1593 the data network randomly in this sparse network (Humphries et al., 2019). This is the null
1594 network that we used when searching for additional structure in our data networks.

1595 **Spectral rejection**

1596 We made use of the spectral rejection algorithm as outlined in (Humphries et al., 2019). The
1597 spectral rejection algorithm is a method for finding structure in a network not captured by a
1598 supposed null model, if such structure exists.

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

$$\mathbf{B} = \mathbf{W} - \langle \mathbf{P} \rangle \quad (3.24)$$

1599 a common choice for $\langle \mathbf{P} \rangle$ in community detection is the ‘configuration model’ (Fosdick et
1600 al., 2016; Humphries, 2011). The matrix \mathbf{B} is often called the configuration matrix, in this
1601 context we will use the term ‘deviation matrix’ as it captures the deviation of \mathbf{W} from the
1602 null model.

1603 To test for structure in the network represented by \mathbf{W} , we examine the eigenspectrum of \mathbf{B}
1604 and compare it to the eigenspectrum of our null model. Firstly, note that since our data model
1605 doesn't allow self loops, and is not directed, the matrix representing the network will be
1606 symmetric and positive semi-definite, and will therefore be invertible with real eigenvalues.
1607 We selected a null model with the same characteristics.

1608 To find the eigenspectrum of the null model, we generated N samples from our null
1609 model P_1, \dots, P_N , and we measured their deviation matrices B_1, \dots, B_N . We then calculated
1610 the eigenspectrum of each of those samples. We calculated the upper bound of the null model
1611 eigenspectrum by taking the mean of the largest eigenvalues of B_1, \dots, B_N . We calculated a
1612 lower bound on the null model eigenspectrum by taking the mean of the smallest eigenvalues
1613 of B_1, \dots, B_N .

1614 We then calculated the eigenspectrum of \mathbf{B} , our data network deviation matrix. If any of
1615 those eigenvalues lay outside of the upper or lower bounds of the null model eigenspectrum,
1616 this is evidence of additional structure not captured by the null model. If we chose the sparse
1617 weighted configuration model (see section 3.3.5) as our null network model, then eigenvalues
1618 lying below the lower bound indicate k -partite structure in the network. For example, if one
1619 eigenvalue lay below the lower bound, this would indicate some bipartite structure in the data
1620 network. If any eigenvalues lay above the upper bound of the null model eigenspectrum, this
1621 is evidence of community structure in the data network. For example, one eigenvalue of \mathbf{B}
1622 lying above the upper bound of the null model eigenspectrum indicates the presence of two
1623 communities in the network (Humphries, 2011).

1624 **Node rejection**

1625 If there are d data eigenvalues lying outside of the null network eigenspectrum, the d eigen-
1626 vectors corresponding to these eigenvalues will form a vector space. If we project the nodes
1627 of our network into this vector space, by projecting either rows or columns of the data ma-
1628 trix, we can see how strongly each node contributes to the vector space. Nodes that contribute
1629 strongly to the additional structure will project far away from the origin, nodes that do not
1630 contribute to the additional structure will project close to the origin. We want to use this
1631 information to discard those nodes that do not contribute.

1632 We can test whether a node projects *far* away from the origin or *close* to the origin
1633 using the eigenvalues and eigenvectors of B_1, \dots, B_N . The j th eigenvector and eigenvalue
1634 of B_i gives a value for a null network's projection into the j th dimension of the additional
1635 structure vector space. The matrices B_1, \dots, B_N give N projections into that dimension.

3.3. Methods

1636 These projections are a distribution of the null networks' projections. If the data node's
1637 projection exceeds that of the null network projections this node is judged to project *far* from
1638 the origin, and therefore contribute to the additional structure. Otherwise, the node is judged
1639 to project *close* to the origin, and is therefore rejected (Humphries et al., 2019).

1640 **Community detection**

1641 Another application for this d dimensional space is community detection. We first project
1642 all of the nodes into this d -dimensional space, then perform the clustering in this space. The
1643 clustering and community detection procedure is described in (Humphries, 2011).

1644 In practice, the procedure is carried out n times (we chose $n = 100$ times), this returns n
1645 clusterings. We resolve these n clusterings to one final clustering using *consensus clustering*.
1646 We used the consensus clustering method that uses an explicit null model for the consensus
1647 matrix, as outlined in (Humphries et al., 2019).

1648 **3.3.6 Clustering Comparison**

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

$$C_k \cap C_l = \emptyset \quad (3.25)$$

$$\bigcup_{k=1}^K C_k = D \quad (3.26)$$

1649 If we consider two clusterings, \mathcal{C} with clusters C_1, C_2, \dots, C_K and \mathcal{C}' with clusters
1650 C'_1, C'_2, \dots, C'_K . There are a number of measurements we can use to compare \mathcal{C} and \mathcal{C}' . In
1651 the following, the number of elements in D is denoted by n , and the number of elements in
1652 cluster C_k is n_k .

1653 **Adjusted Rand Index**

1654 The *adjusted Rand Index* is a normalised similarity measure for clusterings based on pair
1655 counting.

1656 If we consider the clusterings \mathcal{C} and \mathcal{C}' , and denote

1657 • the number of pairs in the same cluster in \mathcal{C} and \mathcal{C}' by N_{11}

1658 • the number of pairs in different clusters in \mathcal{C} and \mathcal{C}' by N_{00}

1659 • the number of pairs in the same cluster in \mathcal{C} and different clusters in \mathcal{C}' by N_{10}

1660 • the number of pairs in different clusters in \mathcal{C} and the same cluster in \mathcal{C}' by N_{01}

then the *Rand Index* is defined as

$$RI = \frac{N_{11} + N_{00}}{N_{11} + N_{00} + N_{10} + N_{01}} = \frac{N_{11} + N_{00}}{\binom{n}{2}} \quad (3.27)$$

1661 The Rand Index is 1 when the clusterings are identical, and 0 when the clusterings are com-
1662 pletely different.

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

This is defined as

$$ARI = \frac{2(N_{00}N_{11} - N_{01}N_{10})}{(N_{00} + N_{01})(N_{01} + N_{11}) + (N_{00} + N_{10})(N_{10} + N_{11})} \quad (3.28)$$

1663 The adjusted Rand Index is 1 when the clusterings are identical, and 0 when the Rand Index
1664 is equal to its expected value.

1665 When the number of clusters in each clustering is different, the concept of a pair of
1666 being in the same cluster in both clusterings becomes difficult to define. To address this, two
1667 clusterings are drawn randomly with $N_{\mathcal{C}}$ and $N_{\mathcal{C}'}$ number of clusters respectively, and a fixed
1668 number of elements in each cluster corresponding to the number of elements in each cluster
1669 in \mathcal{C} and \mathcal{C}' . Then the adjusted Rand Index is the normalised difference between the Rand
1670 Index of \mathcal{C} and \mathcal{C}' and the mean value of the Rand Index measured using many pairs of these
1671 ‘random’ clusterings.

1672 Clusterings as random variables

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

$$P(K = k) = \frac{n_k}{n} \quad (3.29)$$

1673 this defines a probability distribution, which makes the clustering a random variable. Any
1674 clustering can be considered as a random variable this way.

3.3. Methods

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

$$H(\mathcal{C}) = - \sum_{k=1}^K \frac{n_k}{n} \log \frac{n_k}{n} \quad (3.30)$$

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

$$P(K = k, K' = k') = \frac{|C_k \cap C'_{k'}|}{n} \quad (3.31)$$

1675 The joint distribution allows us to define the mutual information between two clusterings,

1676 $I(\mathcal{C}; \mathcal{C}')$ (Meilă, 2007).

1677 Information based similarity measures

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

$$NMI_{joint} = \frac{I(\mathcal{C}; \mathcal{C}')}{H(\mathcal{C}, \mathcal{C}')} \quad (3.32)$$

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

$$NMI_{sum} = \frac{2I(\mathcal{C}; \mathcal{C}')}{H(\mathcal{C}) + H(\mathcal{C}')} \quad (3.34)$$

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

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

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

$$AMI_{sum} = \frac{I(\mathcal{C}; \mathcal{C}') - E\{I(\mathcal{C}; \mathcal{C}')\}}{\frac{1}{2}[H(\mathcal{C}) + H(\mathcal{C}')] - E\{I(\mathcal{C}; \mathcal{C}')\}} \quad (3.37)$$

1678 The first term in the denominator, taking the average of the marginal entropies, can be re-

1679 placed by taking the maximum, minimum, or the geometric mean (Vinh, Epps, and Bailey,

1680 2010).

1681 **Information based metrics**

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

$$VI(\mathcal{C}; \mathcal{C}') \geq 0 \quad (3.38)$$

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

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

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

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

$$D_{max} = \max\{H(\mathcal{C}), H(\mathcal{C}')\} - I(\mathcal{C}; \mathcal{C}') \quad (3.42)$$

Both of these can be normalised

$$NVI(\mathcal{C}; \mathcal{C}') = 1 - \frac{I(\mathcal{C}; \mathcal{C}')}{H(\mathcal{C}, \mathcal{C}')} \quad (3.43)$$

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

1682 **Comparing detected communities and anatomical divisions**

1683 In order to quantify the difference or similarity between the communities detected in our cor-
 1684 relation network and the anatomical classification of the cells in that network, we considered
 1685 the communities and the anatomical regions as clusters in two different clusterings, \mathcal{C}_{comm}
 1686 and \mathcal{C}_{anat} , respectively. We then measured the similarity between the clusterings using the
 1687 mutual information, the adjusted mutual information, and the normalised mutual informa-
 1688 tion. We measured the difference between, or the distance between, the clusterings using the
 1689 variation of information, the normalised variation of information, and the normalised infor-
 1690 mation distance. We also measured the difference between the clusterings using the adjusted
 1691 Rand Index, just to use a non-information based measure.

1692 We took all of these measures for communities detected using different time bin widths.
 1693 This gave us an idea of the effect of time bin width on correlation networks in neural ensem-
 1694 bles relative to anatomical regions within those ensembles.

3.4. Results

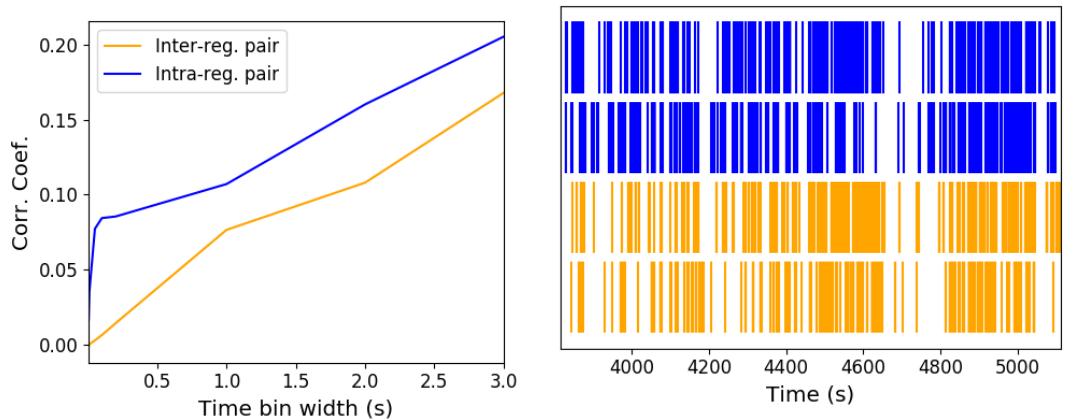
3.4 Results

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

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

3.4.1 Average correlation size increases with increasing time bin width

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



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

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

1713 We also separated the positively correlated pairs from the negatively correlated pairs
1714 using the mean correlation of each pair across all bin widths (see section 3.3.2). We found
1715 that the positively correlated pairs become more positively correlated with increasing time bin
1716 width, and the negatively correlated pairs become more negatively correlated with increasing
1717 time bin width (see figures 3.4b and 3.4c).

1718 In figure 3.3a we plot correlations from two example pairs, one pair from within a region,
1719 and one pair between regions. It can be seen that the correlation coefficient increases with
1720 bin width. The correlations can be observed by eye in the raster plot for these cells in figure
1721 3.3b.

1722 When taking the mean across all pairs, the positively correlated pairs dominate in terms
1723 of both number of pairs, and amplitude of correlations. Therefore the mean across all pairs
1724 is positive.

1725 These results were observed in each of the three mouse subjects from which we had data.

1726 **3.4.2 Goodness-of-fit for Poisson and Gaussian distributions across increasing
1727 time bin widths**

1728 We wanted to investigate if the width of the time bin used to bin spike times into spike counts
1729 had an effect on the distribution of spike counts. We used the χ^2 statistic as a goodness-of-fit
1730 measure for Poisson and Gaussian (normal) distributions to the spike count of 100 randomly
1731 chosen neurons for a number of bin widths ranging from 0.01s to 4s. For the χ^2 statistic, the
1732 higher the value, the worse the fit.

1733 We expected a Poisson distribution to be a better fit for shorter time bin widths because
1734 spike counts must be non-negative, therefore any distribution of spike counts with mass dis-
1735 tributed at or close to 0 will be skewed. The distribution of spike counts is more likely to be
1736 distributed close to 0 when the time bin widths used to bin spike times into spike counts are
1737 small relative to the amount of time it takes for a neuron to fire an action potential ($\sim 1\text{ms}$ in
1738 the case of non-burst firing neurons).

1739 We expected a Gaussian distribution to be a better fit for longer time bin widths, because
1740 a Poisson distribution with a large rate is well approximated by a Gaussian distribution with
1741 mean and variance equal to the Poisson rate. Therefore, a Gaussian distribution would ap-
1742 proximate the mean of a collection of large spike counts, and have more flexibility than a
1743 Poisson distribution to fit the variance.

3.4. Results

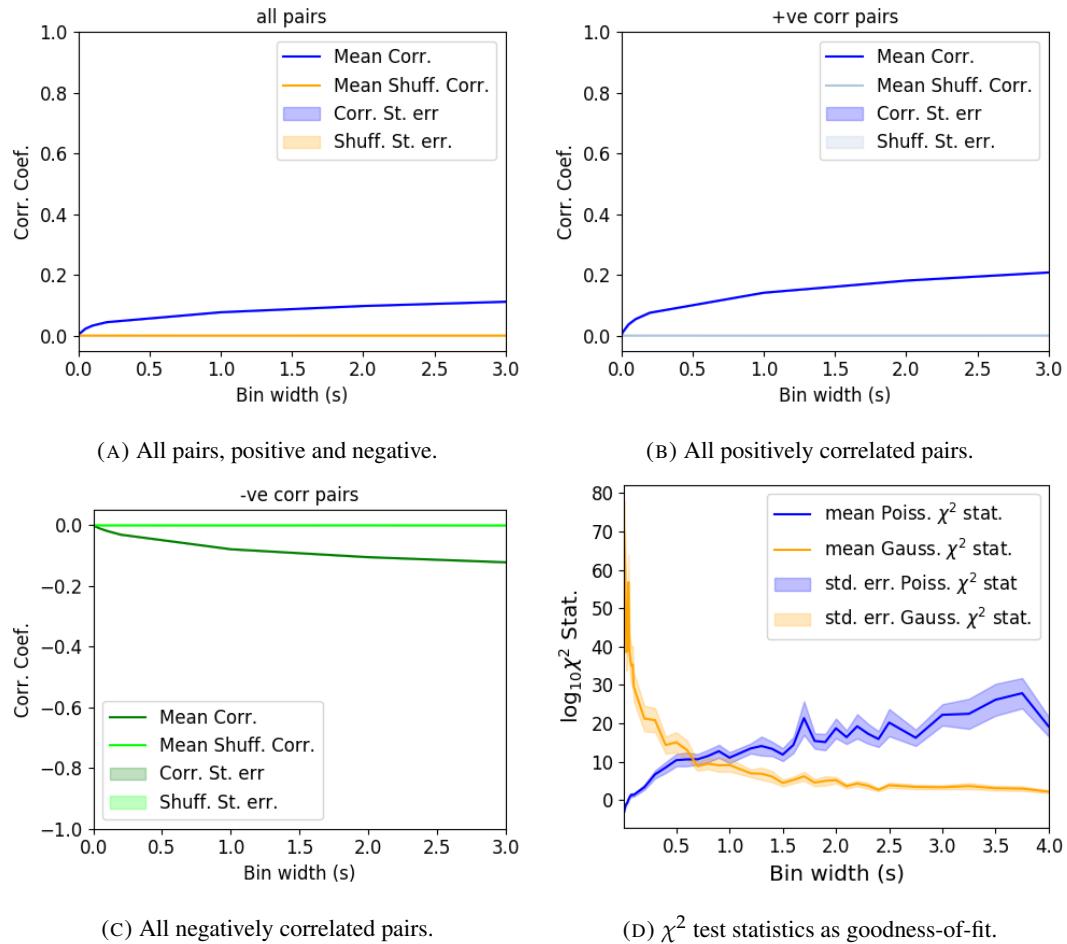


FIGURE 3.4: Mean correlation coefficients measured from pairs of 50 randomly chosen neurons. (A) All possible pairs, (B) positively correlated pairs, and (C) negatively correlated pairs. (D) Mean and standard error of χ^2 test statistics for Poisson and Gaussian distributions fitted to neuron spike counts.

1744 We found that that a Poisson distribution is the best fit for shorter time bins less than 0.7s
1745 in length. Then a Gaussian distribution is a better fit for time bins greater than 0.7s in length
1746 (see figure 3.4d).

1747 **3.4.3 Differences between and inter- and intra- regional correlations decrease
1748 with increasing bin width**

1749 We investigated the differences in distribution between inter-regional correlations, i.e. corre-
1750 lations between neurons in different brain regions, and intra-regional correlations, i.e. corre-
1751 lations between neurons in the same brain region.

1752 Firstly, we investigated these quantities for all possible pairs of ~ 500 neurons taken
1753 from across all the 9 brain regions from which we had data. We distributed these neurons as
1754 evenly as possible across all of the regions, so that cells from one region would not dominate
1755 our data. We observed that the mean intra-regional correlations were always higher than the
1756 mean inter-regional correlations for every value of time bin width used. We also observed
1757 that as the time bin width increased these mean correlations increased and the difference
1758 between the mean inter-regional and intra-regional correlations grew (see figure 3.5 (Left)).

1759 Stringer et al. (2019) had a similar finding using the same data. They used only one value
1760 for the time bin width, 1.2s. Using this time bin width to bin spike times and measure total
1761 correlations, they found that the mean ‘within-region’ correlations were always greater than
1762 the ‘out-of-region’ correlations (Stringer et al., 2019). The figure from their paper showing
1763 this result can be seen in figure 3.5 (Right).

1764 Examples of the correlations of one intra-regional pair and one inter-regional pair can be
1765 seen in figure 3.3.

1766 Secondly, we separated those pairs into intra-regional and inter-regional groups. We
1767 noted that the mean intra-regional correlations (coloured dots in figures 3.6a and 3.6b) for
1768 a given region tended to be higher than the mean inter-regional correlations (black dots in
1769 figures 3.6a and 3.6b) involving cells from that region. However, in contrast with our previous
1770 result, we noted that the difference between the mean intra-regional correlations and most
1771 highly correlated inter-regional correlations reduced as we increased the time bin width (see
1772 figures 3.6a and 3.6b). This shows that the mean correlations showin in figure 3.5 are not
1773 distributed evenly across all region pair combinations.

1774 Finally, to see these regional mean correlations in a bit more detail, to examine the indi-
1775 vidual pair combinations in particular, we displayed these data in a matrix of mean corre-
1776 lations (see figure 3.7), showing the mean intra-regional correlations on the main diagonal, and

3.4. Results

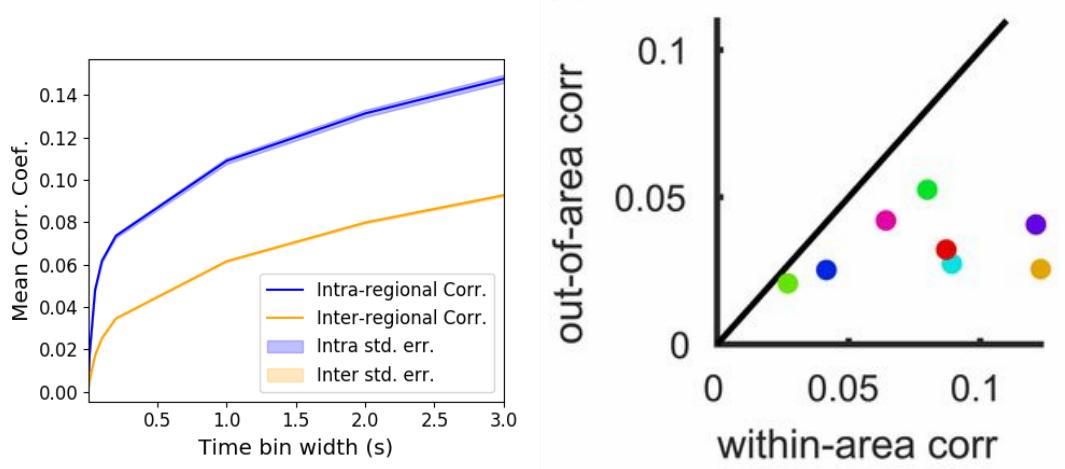


FIGURE 3.5: (Left) The mean intra-region and inter-region correlations using all possible pairs of ~ 500 neurons, spread across 9 different brain regions. (Right) Courtesy of Stringer et al. (2019), mean inter-regional (out-of-area) correlation coefficients vs mean intra-regional (within-area) correlation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

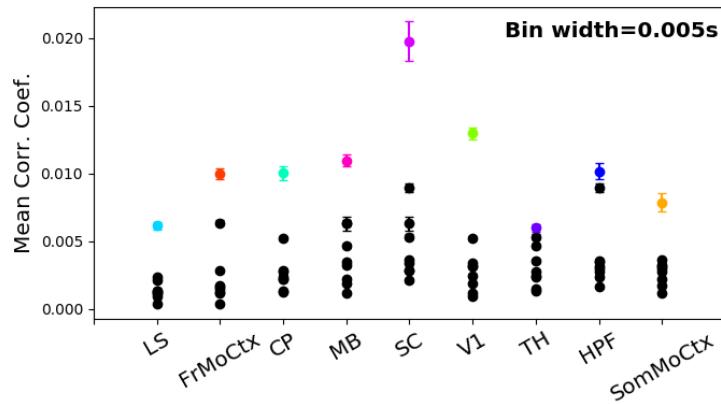
1777 the mean inter-regional correlations off diagonal. Comparing a version of this figure created
 1778 using a short time bin width of 5ms (figure 3.7a) and a version using a longer time bin width
 1779 of 1s (figure 3.7b) we observed that the mean intra-regional correlations are always relatively
 1780 high in comparison to the mean inter-regional correlations, but the mean correlations in some
 1781 inter-regional pairs are relatively much higher when using the longer time bin width.

1782 This could indicate information being processed quickly at a local or within-region level,
 1783 and the local representations of this information spreading between regions at longer timescales.

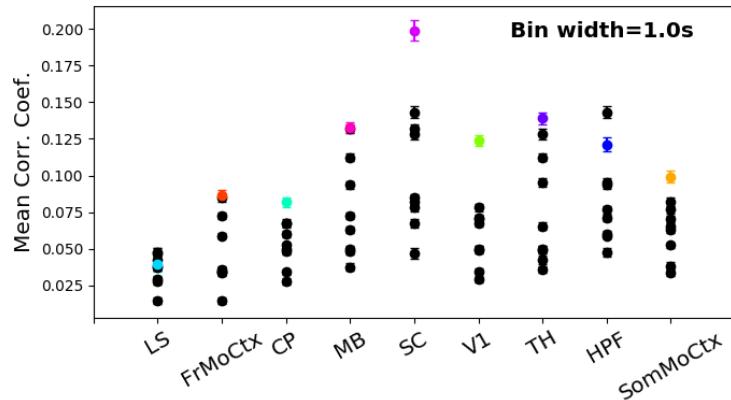
1784 These results were consistent across the three mouse subjects. But, the relative magni-
 1785 tudes of the mean intra-regional and inter-regional correlations were not consistent. For ex-
 1786 ample, the region with the highest mean intra-regional correlations when using 1s bin widths
 1787 for subject one is the superior colliculus (SC), but for subject two it is the midbrain (MB).

1788 **3.4.4 Connected and divided structure in correlation based networks reduces
 1789 in dimension with increasing bin width**

1790 We used the correlation measurements to create weighted undirected graphs/networks where
 1791 each node represents a neuron, and the weight of each edge is the pairwise correlation be-
 1792 tween those neurons represented by the nodes at either end of that edge. We aimed to find
 1793 communities of neurons within these networks, and compare the structure of these commu-
 1794 nities to the anatomical division of those neurons. The first step of this process involved
 1795 applying the ‘spectral rejection’ technique developed by Humphries et al (2019) (Humphries



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



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

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

3.4. Results

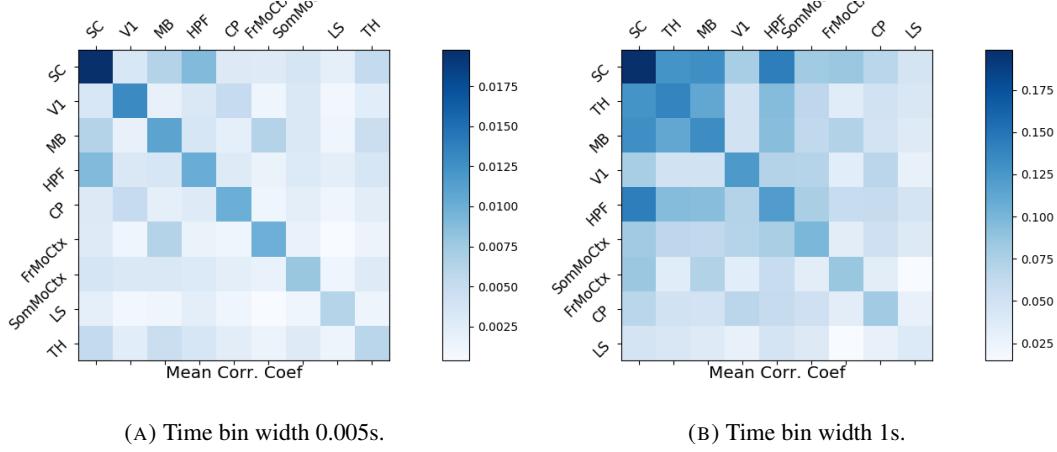


FIGURE 3.7: Mean inter-regional (main diagonal) and intra-regional (off diagonal) correlation coefficients. (A) Shows these measurements when spike times were binned using 5ms time bins. (B) Shows the same, using 1s time bins. Note that the matrices are ordered according to the main diagonal values,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

et al., 2019). This technique compares our data network to a chosen null network model, and finds any additional structure in the data network beyond that which is captured in the null network model (if there is any such structure).

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

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

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

We applied the spectral rejection method to our networks based on total correlations using different values for the time bin width. We observed that for smaller time bin widths, our data

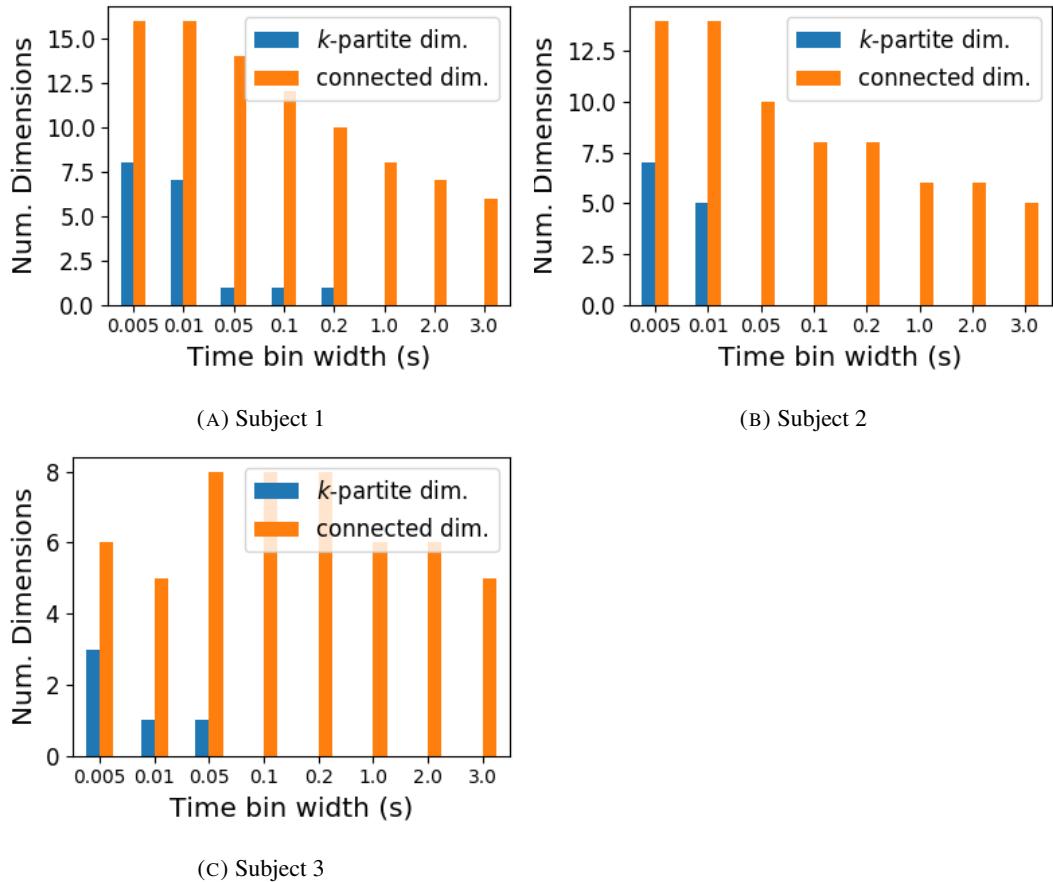


FIGURE 3.8: The number of dimensions in the k -partite and connected structure in the correlation based networks beyond the structure captured by a sparse weighted configuration null network model (see section 3.3.5), shown for different time bin widths. Note that the k -partite structure disappears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

3.4. Results

networks had both k -partite structure, and community structure. As the width of the time bin increased, we found that the k -partite structure disappeared from our data networks, and the dimension of the community structure reduced in two of the three mice from which we had data (see figure 3.8).

The reduction in dimensionality of the connected structure with larger bin widths could indicate information or activity being integrated through the merging of smaller functionaly communities over longer timescales.

The k -partite structure that we found when using small bin widths could be an indication of physical connections between neurons. This is supported by the fact that these k -partite communities are not found over longer timescales. The effect of physical connections through axodendritic or dendrodendritic synapses would only be noticeable at short timescales.

3.4.5 Detecting communities in correlation based networks

We applied the community detection procedure described in section 3.3.5 to our signal networks for our various time bin widths. We detected a greater number of smaller communities at shorter time bin widths, and a smaller number of larger communities for longer time bin widths (see figure 3.9). This was expected after the results found in section 3.4.4. We found more dimensions of additional structure at shorter time bin widths, therefore we found more communities at shorter time bin widths.

The number of communities that we detected was always greater than the dimensionality of the additional structure that we found by applying spectral rejection.

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

3.4.6 Functional communities resemble anatomical division at short timescales

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

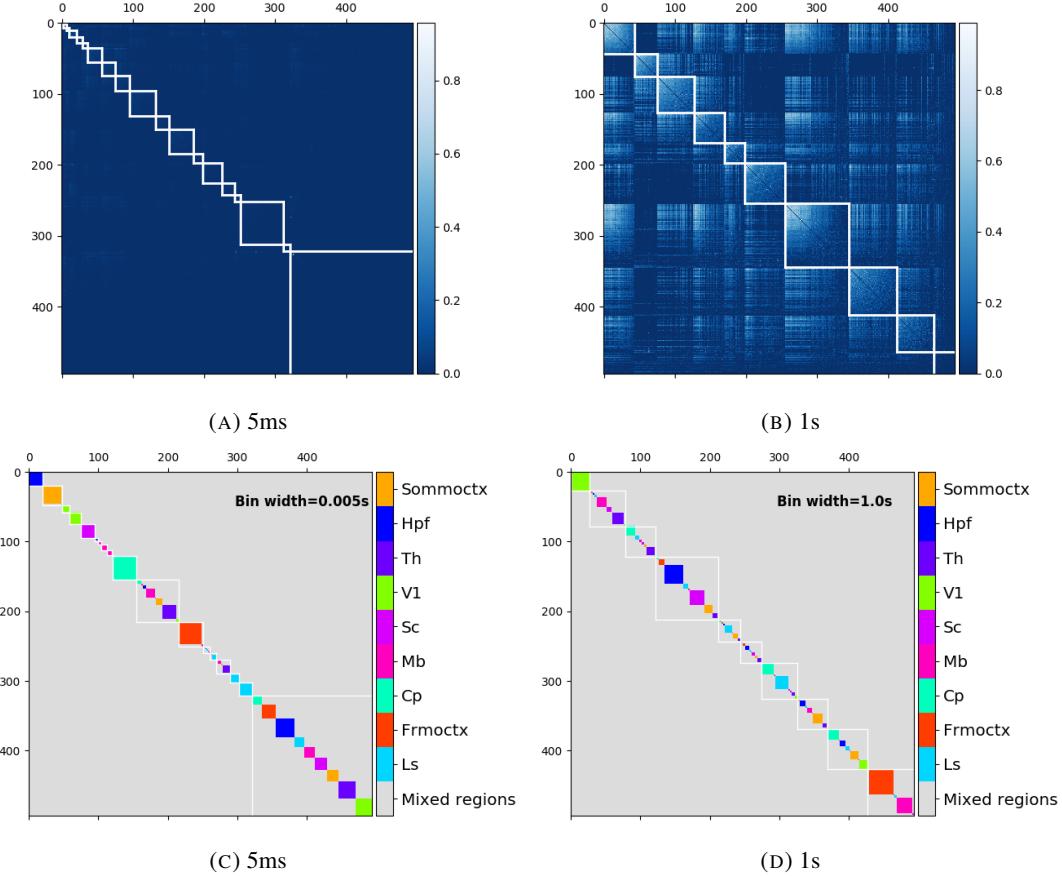


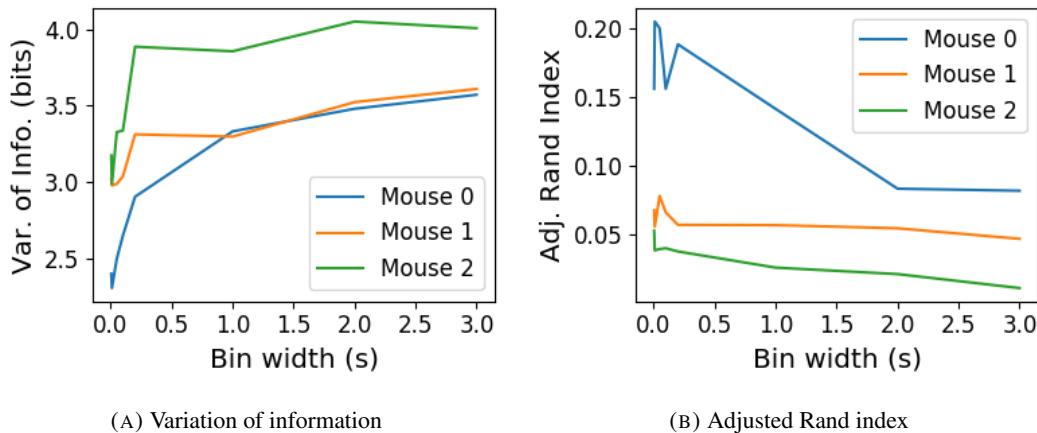
FIGURE 3.9: (A-B) Correlation matrices with detected communities indicated by white lines. Each off main diagonal entry in the matrix represents a pair of neurons. Those entries within a white square indicate that both of those neurons are in the same community as detected by our community detection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

3.4. Results

1849 We used two different types of measures for clustering comparison; information based
1850 measures (see section 3.3.6) and pair counting based measures (see section 3.3.6). We include
1851 one example of each in figure 3.10.

1852 The variation of information is the information based measure included in figure 3.10a.
1853 This measure forms a metric on the space of clusterings. The larger the value for the variation
1854 of information, the more different the clusterings.

1855 The adjusted Rand index is the pair counting based measure included in figure 3.10b. In
1856 contrast with the variation of information, the adjusted Rand index is a normalised similarity
1857 measure. The adjusted Rand index takes value 1 when the clusterings are identical, and takes
1858 value 0 when the clusterings are no more similar than chance.



(A) Variation of information

(B) Adjusted Rand index

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

1859 Both measures indicated that the detected communities and the anatomical division of
1860 the cells were more similar when we used shorter time bins widths (see figure 3.10). This
1861 indicates that correlated behaviour in neuronal ensembles is more restricted to individual
1862 brain regions at short timescales (< 250ms), and the correlated activity spreads out across
1863 brain regions over longer time scales.

3.4.7 Conditional correlations & signal correlations

1865 In light of the excellent research of Stringer et al (2019) showing that spontaneous behaviours
1866 can drive activity in neuronal ensembles across the visual cortex and midbrain (Stringer et
1867 al., 2019), we decided to control for the mouse’s behaviour when performing our analyses.
1868 It is possible that our community detection process may be detecting communities across

multiple brain regions at longer time scales due to aggregating neuronal activity driven by several spontaneous behaviours occurring during the time interval covered by a given time bin. A time bin of 1s, for example, could contain a spike count where those spikes were driven by different spontaneous behaviours. We aimed to investigate this possibility by applying our community detection analysis to conditional correlation measures.

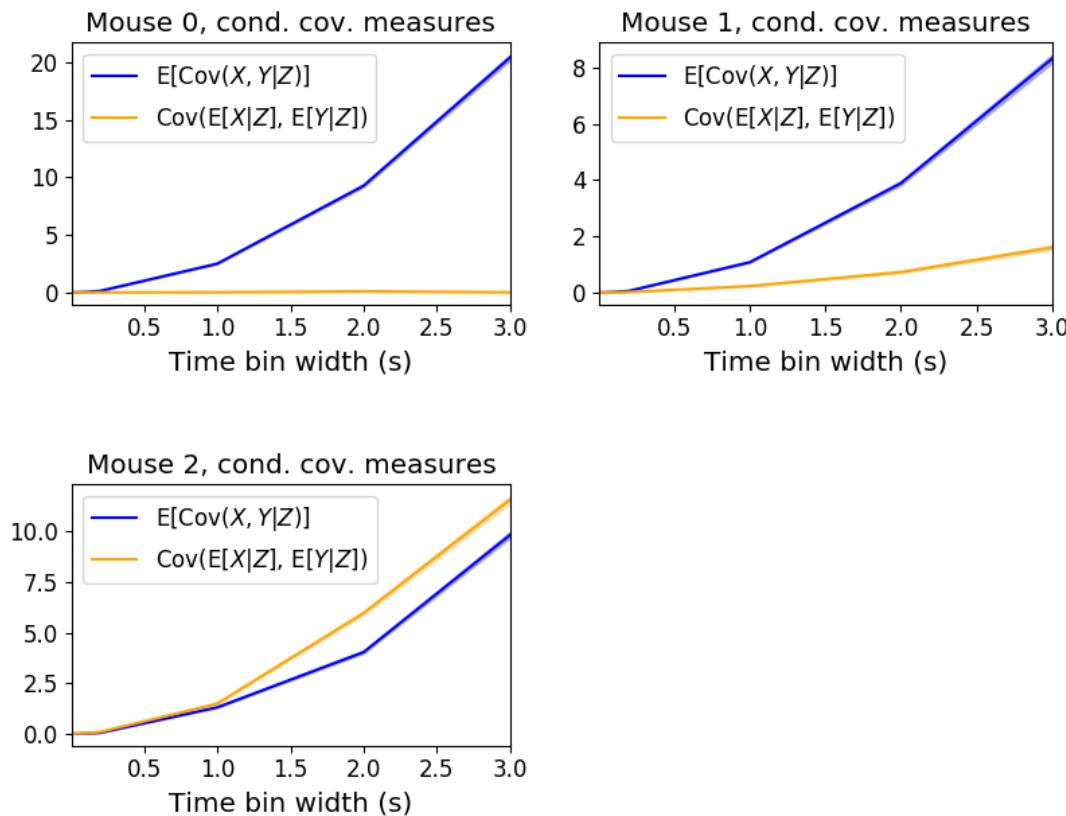


FIGURE 3.11: Comparing the components of the total covariance across different values for the time bin width. We observed a consistent increase in $E[\text{cov}(X, Y|Z)]$ as the time bin width increased. But we saw different trends for $\text{cov}(E[X|Z], E[Y|Z])$ for each mouse.

We used the top 500 principal components of a video of the mouse's face as a measure of the mouse's behaviour (see section 3.2.2). We modelled the spike counts as a linear combination of the principal components using linear regression with ElasticNet regularisation (see section 3.3.3). 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 3.3.3) to measure

3.4. Results

1881 $E[\text{cov}(X, Y|Z)]$. Here X and Y represent spike counts from individual cells, and Z is short-
 1882 hand for the 500 principal components mentioned above. The two components of the co-
 1883 variance, $\text{cov}(E[X|Z], E[Y|Z])$ and $E[\text{cov}(X, Y|Z)]$, represent a ‘signal covariance’ and ex-
 1884 pected value of a ‘spike count covariance’ respectively, analogous to the signal correlation
 1885 and spike count correlation (Cohen and Kohn, 2011).

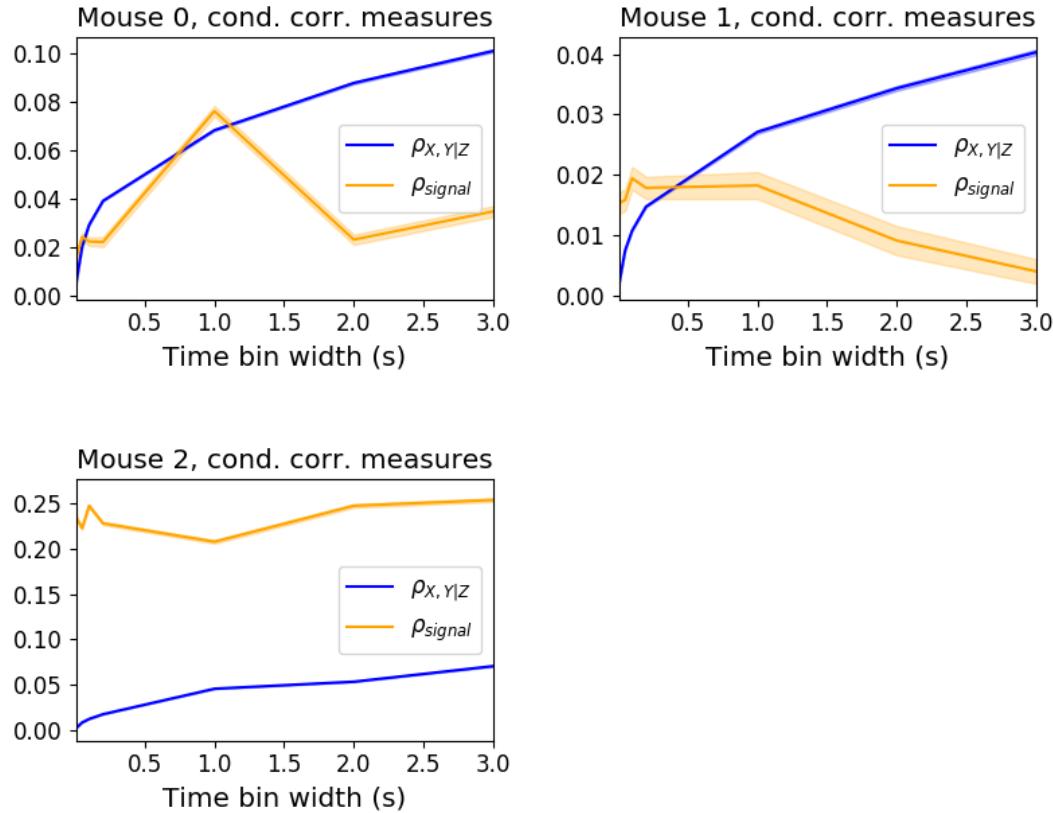


FIGURE 3.12: Comparing the components of the total covariance across different values for the time bin width. We saw a consistent increase in $\rho_{X,Y|Z}$ as the time bin width increased in all three subjects. But we saw different trends in ρ_{signal} for each of the subjects.

1886 We examined the means of these components for different values of the time bin width
 1887 (see figure 3.11). We observed a consistent increase in $E[\text{cov}(X, Y|Z)]$ as the time bin width
 1888 increased. But we saw different trends for $\text{cov}(E[X|Z], E[Y|Z])$ for each mouse.

1889 Using $\text{cov}(E[X|Z], E[Y|Z])$ we measured the signal correlation, ρ_{signal} , and using $E[\text{cov}(X, Y|Z)]$
 1890 we measured the event conditional correlation, $\rho_{X,Y|Z}$ (see section 3.3.3 for more details).
 1891 We saw a consistent increase in $\rho_{X,Y|Z}$ as the time bin width increased, this corresponds to
 1892 the result for $E[\text{cov}(X, Y|Z)]$. We observed different trends for ρ_{signal} for each mouse, this
 1893 corresponds to the result for $\text{cov}(E[X|Z], E[Y|Z])$.

1894 We applied our network noise rejection and community detection process to networks

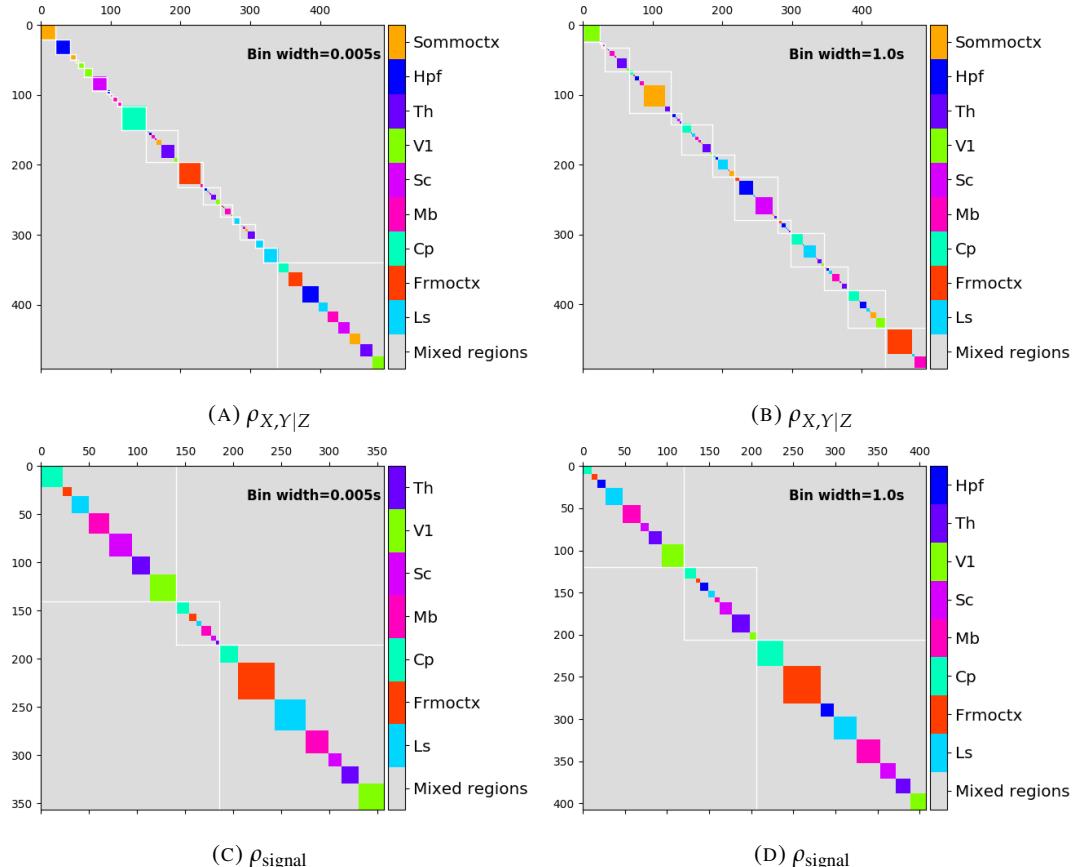


FIGURE 3.13: Matrices showing the regional membership of pairs by colour, and the communities in which those pairs lie. (A-B) Detected communities and regional membership matrix for network based on rectified spike count correlation $\rho_{X,Y|Z}$, using time bin widths of 0.005s and 1s respectively.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

3.4. Results

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

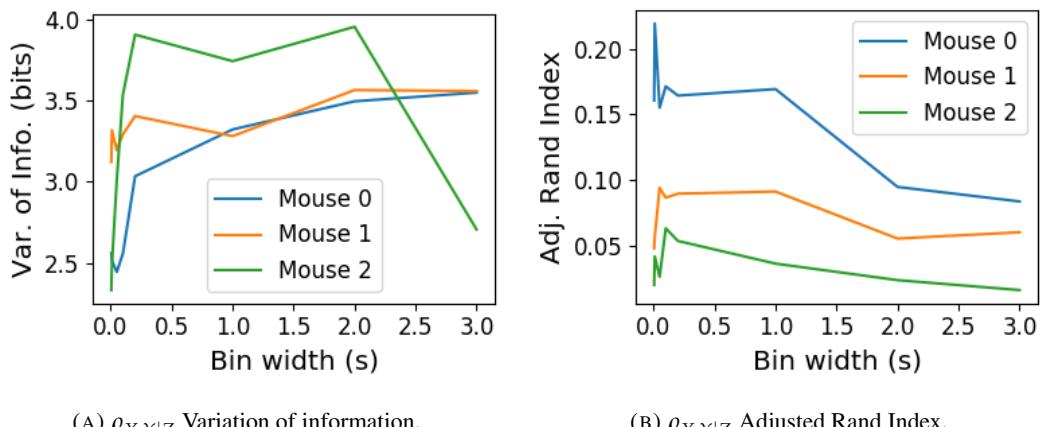


FIGURE 3.14: Distance and similarity measures between the anatomical division of the neurons, and the communities detected in the network based on the spike count correlations $\rho_{X,Y|Z}$. (A) The variation of information is a ‘distance’ measure between clusterings. The distance between the anatomical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

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

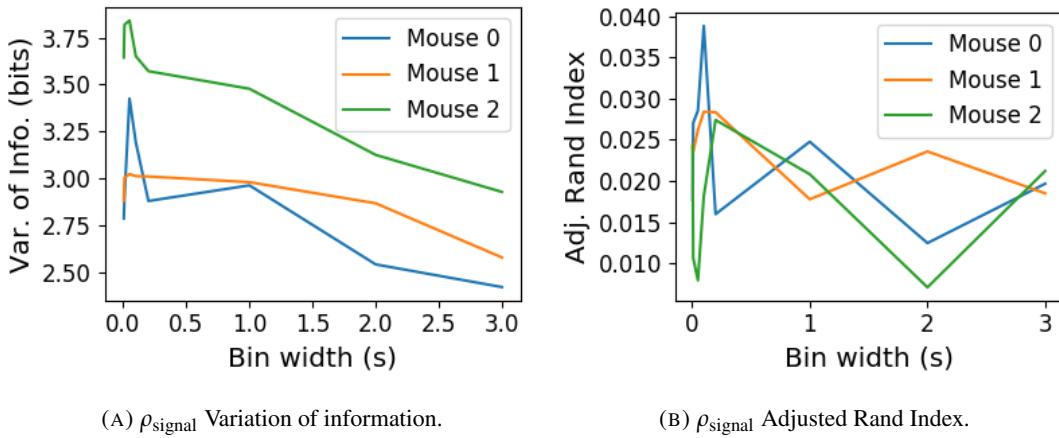
(A) ρ_{signal} Variation of information.(B) ρ_{signal} Adjusted Rand Index.

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

1914 detected rather than any relationship with anatomy.

1915 We also observed that the network noise rejection process rejected some of the cells
1916 when applied to the network based on the signal correlations. This means that those cells
1917 did not contribute to the additional structure of the network beyond that captured by the
1918 sparse weighted configuration model. This is why the matrices in figures 3.13c and 3.13d are
1919 smaller than their analogues in figures 3.13a and 3.13b.

1920 The communities detected in the signal correlation based networks indicate that there are
1921 groups of cells from different brain regions that react similarly to different activity states.
1922 These groups also exist at all timescales from milliseconds to seconds. This indicates that
1923 there are subsets of cells in each region that respond to activity states regardless of the
1924 timescale of these activities.

1925 3.4.8 Absolute correlations and negative rectified correlations

1926 At the moment, the network noise rejection protocol can only be applied to weighted undi-
1927 rected graphs with non-negative weights. This meant that we had to rectify our correlated
1928 networks before applying the network noise rejection and community detection process. We
1929 wanted to investigate what would happen if instead of rectifying the correlations, we used the
1930 absolute value, or reversed the signs of the correlations and then rectified.

3.4. Results

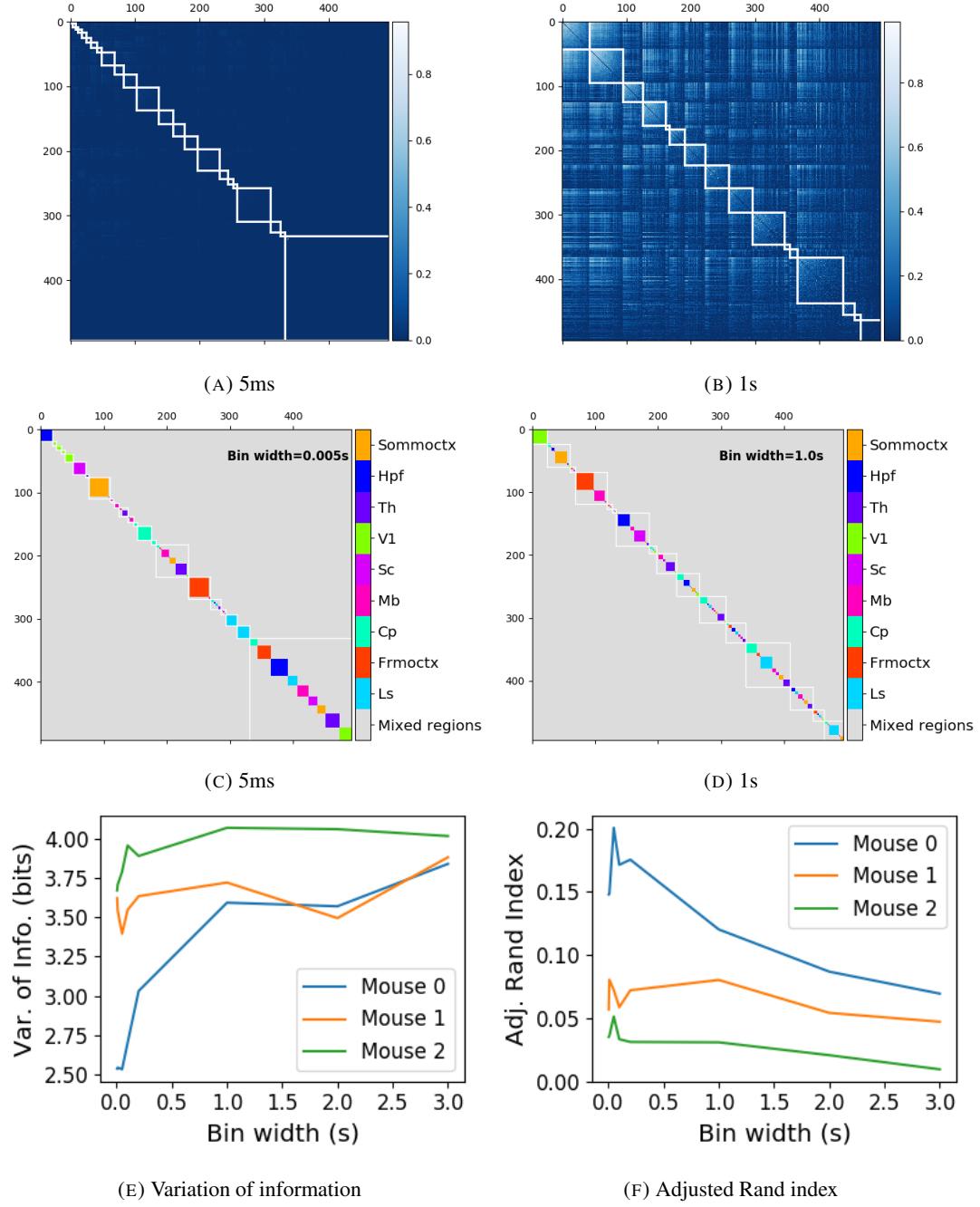


FIGURE 3.16: (A-B) Absolute correlation matrices with detected communities indicated by white lines. These communities are based on the absolute value of the total correlation between each pair of cells. Those entries within a white square indicate that both of those neurons are in the same community.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

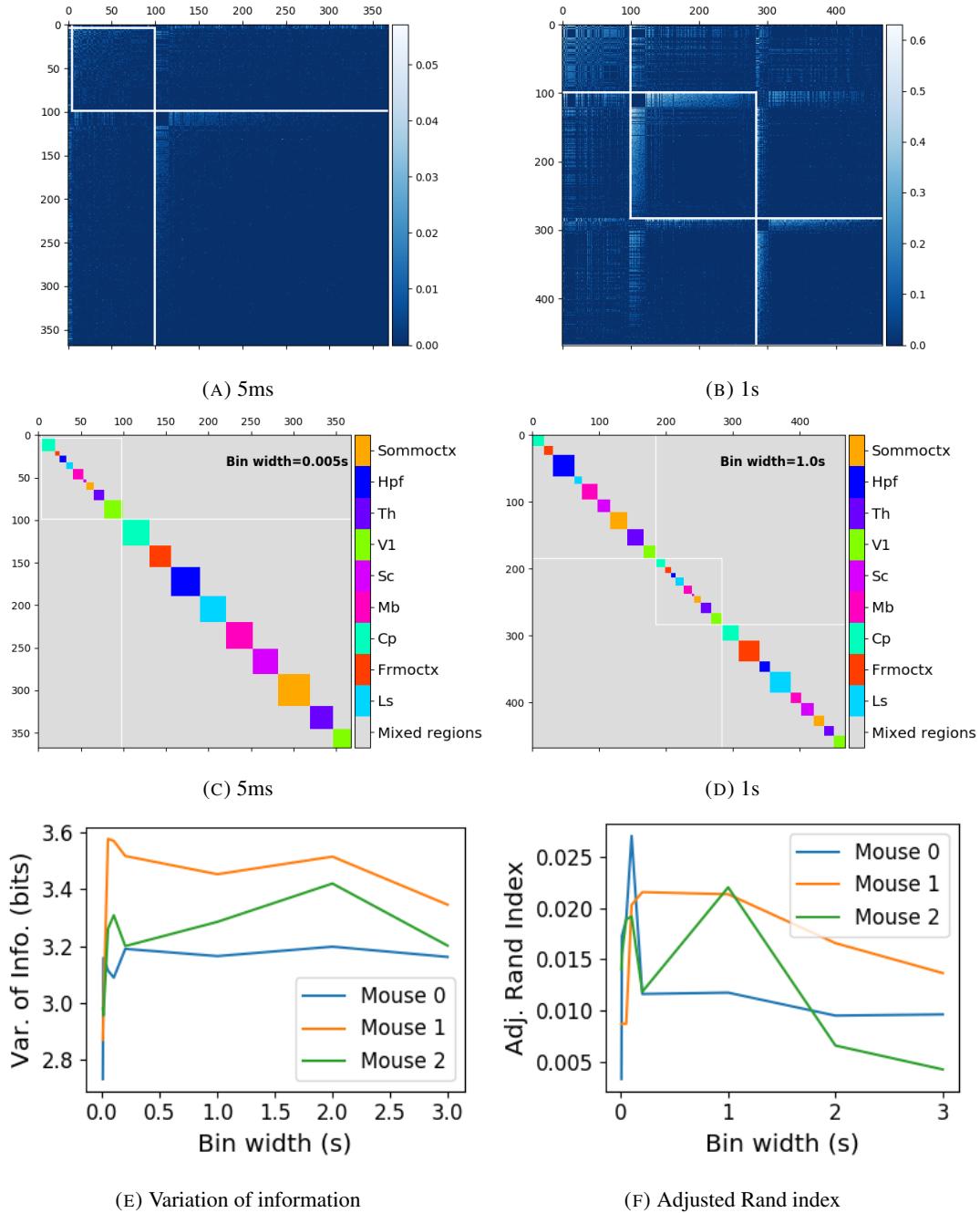


FIGURE 3.17: (A-B) Sign reversed rectified correlation matrices with detected communities indicated by white lines. Those entries within a white square indicate that both of those neurons are in the same community. Matrices shown are for 5ms and 1s time bin widths respectively. Main diagonal entries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

3.5. Discussion

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

When we used the sign reversed rectified correlated networks, we tended to find fewer communities. Each community contained cells from many different anatomical regions, at both long and short bin widths (see figures 3.17a, 3.17b, 3.17c, 3.17d). The communities bore little relation to the anatomical distribution of the cells, this can be seen in figure 3.17f, the values close to zero indicate that the similarity between the two clusterings are around chance level. This indicates that there was not much structure in the negatively correlated networks beyond that captured by the sparse weighted configuration model.

3.5 Discussion

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

A number of studies have found that the timescale of correlated behaviour induced by a stimulus can be modulated by the stimulus structure and behavioural context. For example, the spike train correlations between cells in weakly electric fish are modulated by the spatial extent of the stimulus (Litwin-Kumar, Chacron, and Doiron, 2012), and neurons in the marmoset primary auditory cortex modulate their spike timing (and therefore correlation) in

1963 response to stimulus features without modulating their firing rate (deCharms and Merzenich,
1964 1996). Furthermore, the width of the time bins over which spike counts are measured has
1965 been shown to have an effect on the magnitude of those correlations (Cohen and Kohn, 2011).
1966 Despite this, very little research has been done comparing correlation measures from the same
1967 dataset at different timescales. We investigated this by varying the time bin width used to bin
1968 spike times into spike counts from as short as 5ms up to 3s.

1969 In order to further investigate the effect of these correlations at different timescales, we
1970 regarded our neuronal ensemble as a weighted undirected graph, where each neuron is rep-
1971 resented by a node, and the weight on each edge is the correlation between the neurons
1972 connected by that edge. We then applied a novel clustering method from network science
1973 (Humphries et al., 2019) to identify communities in these networks. Communities in a net-
1974 work graph refer to sets of nodes that are more strongly connected to each other than the
1975 nodes outside of their set. Another way to put this is to say that the nodes in a community
1976 are more strongly connected than *expected*. What connection strength might be expected is
1977 defined by a null network model. We chose a null network model that matched the sparsity
1978 and total strength of our correlation based data networks. So, if two cells were in the same
1979 community, those cells were more correlated than would be expected given the correlation
1980 strength of their ensemble.

1981 These networks, and the community detection process, were completely agnostic of the
1982 anatomical division of the cells in our ensemble. When we compared the detected commu-
1983 nities with the anatomical division of the cells using distance and similarity measures for
1984 clusterings, we found that the detected communities were more similar to the anatomical
1985 division at shorter timescales. That is, when we used a wider time bin to count spikes, and
1986 computed pairwise correlations with these spike counts, the correlated communities tended to
1987 exist within anatomical regions at shorter timescales, and tended to span anatomical regions
1988 at longer timescales. This could reflect localised functional correlations at short time scales
1989 rippling outwards across brain regions at longer timescales. The brain may be processing
1990 some information quickly locally, and carrying out further, perhaps more detailed, represen-
1991 tation over a longer timescale across many regions using the representations that were just
1992 built locally.

1993 These changes in communities across timescales could also be driven by the anatomy
1994 of the individual cells. For example, it may simply take longer to transmit action potentials
1995 over longer distances, hence correlated activity over longer timescales will exist between

3.5. Discussion

1996 anatomical regions, rather than within. However, the switch to almost exclusively multi-
1997 regional functional networks at 1s bin widths, rather than a mixture of multi-region, and
1998 single-region suggests that the inter-regional correlations either overpower, or inhibit the
1999 local correlations. So there may be more at play than just timescales.

2000 We acknowledged that the region spanning correlated communities that we detected at
2001 longer time scales could exist due to collating activity driven by distinct spontaneous activ-
2002 ities. In order to account for this, we modelled the spike counts as a linear function of the
2003 top 500 principal components of a video of the mouse's face filmed simultaneously with the
2004 electrophysiological readings. We applied our network noise rejection and community de-
2005 tection process to the weighted undirected networks formed by the spike count correlations
2006 (or noise correlations) and the signal correlations that we calculated using our model. For the
2007 spike count correlation networks, we found much the same results as for the total correlations
2008 as described above. For the signal correlations, the communities detected in these networks
2009 bore little relation to the anatomical division of the cells. Recent findings have shown that
2010 behavioural data accounts for correlations in many brain regions that would otherwise be
2011 dismissed as noise (Stringer et al., 2019), our finding to shows that these correlations are still
2012 governed by the timescale division between local communication and across-region commu-
2013 nication.

2014 There is a lot of room for further investigation based on this research. For a start, the
2015 data that we used here were collected from nine different regions in the mouse brain, but
2016 none of these regions were part of the somatosensory cortex. Given that a mouse experiences
2017 so much of its environment through its sense of smell, some data from this region would be
2018 interesting to investigate. On the same theme, the mice in the experiment from which the
2019 data were collected were headfixed and placed on a rotating ball, but were otherwise behav-
2020 ing spontaneously. Had these mice been exposed to a visual, aural, or olfactory stimulus,
2021 we could have examined the responses of the cells in the brain regions corresponding to vi-
2022 sion, hearing, and olfaction, and compared these responses to the responses from the other
2023 brain regions. Furthermore, we could have investigated the interaction between the sets of
2024 responses.

2025 Another space for further investigation is the community detection. The algorithm that we
2026 used here never detects overlapping communities. But functional communities could indeed
2027 have overlaps. Clustering methods that detect overlapping clusters do exist (Baadel, Thabtah,
2028 and Lu, 2016). Applying one of those algorithms could yield some interesting results. Also,
2029 the community detection algorithm that we used here cannot process graphs with negative

2030 weights, this forced us to separate positive and negative correlations before applying our
2031 network noise rejection and community detections process, or use the absolute value of our
2032 correlations. A community detection algorithm that can work on weighted undirected graphs
2033 with negative weights could yield some interesting results here.

2034 **Chapter 4**

2035 **A simple two parameter distribution
2036 for modelling neuronal activity and
2037 capturing neuronal association**

2038 *Abstract*

2039 Recent developments in electrophysiological technology have lead to an increase in the size
2040 of electrophysiology datasets. Consequently, there is a requirement for new analysis tech-
2041 niques that can make use of these new datasets, while remaining easy to use in practice. In
2042 this work, we fit some one or two parameter probability distributions to spiking data collected
2043 from a mouse exposed to visual stimuli. We show that the Conway-Maxwell-binomial dis-
2044 tribution is a suitable model for the number of active neurons in a neuronal ensemble at any
2045 given moment. This distribution fits these data better than binomial or beta-binomial distribu-
2046 tions. It also captures the correlated activity in the primary visual cortex induced by stimulus
2047 onset more effectively than simply measuring the correlations, at short timescales (< 10ms).
2048 We also replicate the finding of Churchland et al (2010) relating to stimulus onset quenching
2049 neural variability in cortical areas, and we show a correspondence between this quenching
2050 and changes in one of the parameters of the fitted Conway-Maxwell-binomial distributions.

2051 **4.1 Introduction**

2052 Recent advances in electrophysiological technology, such as ‘Neuropixels’ probes (Jun et al.,
2053 2017) have allowed extracellular voltage measurements to be collected from larger numbers
2054 of cells than traditional methods, in multiple brain regions simultaneously, and routinely.
2055 These larger datasets require innovative methods to extract information from the data in a
2056 reasonable amount of time, ‘reasonable’ being subjective in this case.

2057 Theoretically, all the information at any given moment in an electrophysiological dataset
2058 with n neurons could be captured by calculating the probability distribution for every possi-
2059 ble spiking pattern. This would require defining a random variable with 2^n possible values, a
2060 task that quickly becomes impossible as n increases. Attempts at approximating this random
2061 variable often involve measuring pairwise or higher order correlations (Schneidman et al.,
2062 2006; Flach, 2013; Ganmor, Segev, and Schneidman, 2011). But pairwise correlations may
2063 not be enough to characterise instantaneous neural activity (Tkačik et al., 2014). Further-
2064 more, these kinds of models tend to ignore the temporal structure of neuronal data, in favour
2065 of smaller model size, and scalability.

2066 Higher order correlations would be helpful here, but defining and quantifying these cor-
2067 relations can be tricky (Staude, Grün, and Rotter, 2010). If we use the interaction parameters
2068 arising from the exponential family model as measures of higher order correlations, mea-
2069 suring these correlations becomes computationally impractical quite quickly (the number
2070 of ‘three neuron correlations’ to measure scales with $\binom{n}{3}$). In this work, we dispense with
2071 measuring correlations directly, and we attempt to characterise correlated behaviour using a
2072 parameter in statistical model.

2073 In this work, we examined the ability of simple distributions to model the number of
2074 active (spiking) neurons in a neuronal ensemble at any given timepoint. We compared a
2075 little-known distribution named the Conway-Maxwell-binomial distribution to the binomial
2076 distribution and the beta-binomial distribution. The binomial distribution is a probability dis-
2077 tribution over the number of successes in a sequence of independent and identical Bernoulli
2078 trials. The beta-binomial distribution is similar, but allows for a bit more flexibility while still
2079 being a model for heterogeneity. Similar to the binomial and beta-binomial, the Conway-
2080 Maxwell-binomial distribution is a probability distribution over the number of successes in a
2081 series of Bernoulli trials, but allows over- and under-dispersion relative to the binomial dis-
2082 tribution. This distribution should therefore be a good candidate for our purposes. We found
2083 that Conway-Maxwell-binomial distribution was usually the best candidate of the three that

4.2. Data

2084 we examined.

2085 We also observed some interesting changes in the number of active neurons in the primary
2086 visual cortex and hippocampus at stimulus onset and some changes in this activity in the
2087 thalamus which were sustained for the full duration of the stimulus presentation. This let us
2088 know that there were some responses to model.

2089 We found that fitting a Conway-Maxwell-binomial distribution was a better method of
2090 capturing association between neurons than measuring the spike count correlation for the
2091 short time bins that we used (< 10ms).

2092 Finally, we also wanted to investigate parallels between the parameters of the Conway-
2093 Maxwell-binomial distribution and quantities that have been established as relevant to sen-
2094 sory processing. So, we replicated the findings made by Churchland et al. (2010) relating
2095 to a reduction in neural variability at stimulus onset in the macaque cortical regions, but for
2096 data taken from the mouse primary visual cortex. We compared these findings to the values
2097 of the fitted Conway-Maxwell-binomial distribution parameters.

2098 4.2 Data

2099 We used data collected by Nick Steinmetz and his lab ‘CortexLab at UCL’ (Steinmetz, Caran-
2100 dini, and Harris, 2019). The data can be found online ¹ and are free to use for research
2101 purposes.

2102 Two ‘Phase3’ Neuropixels (Jun et al., 2017) electrode arrays were inserted into the brain
2103 of an awake, head-fixed mouse for about an hour and a half. These electrode arrays recorded
2104 384 channels of neural data each at 30kHz and less than $7\mu\text{V}$ RMS noise levels. The sites
2105 are densely spaced in a ‘continuous tetrode’-like arrangement, and a whole array records
2106 from a 3.8mm span of the brain. One array recorded from visual cortex, hippocampus, and
2107 thalamus, the other array recorded from motor cortex and striatum. The data were spike-
2108 sorted automatically by Kilosort and manually by Nick Steinmetz using Phy. In total 831
2109 well-isolated individual neurons were identified.

2110 4.2.1 Experimental protocol

2111 The mouse was shown a visual stimulus on three monitors placed around the mouse at right
2112 angles to each other, covering about ± 135 degrees azimuth and ± 35 degrees elevation.

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

2113 The stimulus consisted of sine-wave modulated full-field drifting gratings of 16 drift di-
2114 rections ($0^\circ, 22.5^\circ, \dots, 337.5^\circ$) with 2Hz temporal frequency and 0.08 cycles/degree spatial
2115 frequency displayed for 2 seconds plus a blank condition. Each of these 17 conditions were
2116 presented 10 times in a random order across 170 different trials. There were therefore 160
2117 trials with a drifting-grating visual stimulus present, and 10 trials with a blank stimulus.

2118 **4.3 Methods**

2119 **4.3.1 Binning data**

2120 We converted the spike times for each cell into spike counts by putting the spike times into
2121 time bins of a given ‘width’ (in milliseconds). We used time bins of 1ms, 5ms, and 10ms.
2122 We used different time bin widths to assess the impact of choosing a bin width.

2123 **4.3.2 Number of *active* neurons**

2124 To count the number of active neurons in each neuronal ensemble, we split the time interval
2125 for each trial into bins of a given width. We counted the number of spikes fired by each cell
2126 in each bin. If a cell fired *at least* one spike in a given bin, we regarded that cell as active in
2127 that bin. We recorded the number of active cells in every bin, and for the purposes of further
2128 analysis, we recorded each cell’s individual spike counts.

2129 It should be noted that when we used a bin width of 1ms, the maximum number of
2130 spikes in any bin was 1. For the wider time bins, some bins had spike counts greater than
2131 1. Consequently when using a bin width of 1ms, the number of active neurons and the total
2132 spike count of a given bin were identical. But for wider bin widths, the total spike count was
2133 greater than the number of active neurons.

2134 So for the 1ms bin width, the activity of a neuron and the number of spikes fired by that
2135 neuron in any bin can be modelled as a Bernoulli variable. But for wider time bins, only the
2136 activity can be modelled in this way.

2137 **4.3.3 Moving windows for measurements**

2138 When taking measurements (e.g. moving average over the number of active neurons) or
2139 fitting distributions (eg. the beta binomial distribution) we slid a window containing a certain
2140 number of bins across the data, and made our measurements at each window position. For
2141 example, when analysing 1ms bin data, we used a window containing 100 bins, and we slid

4.3. Methods

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

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

2142 the window across the time interval for each trial moving 10 bins at a time. So that for
 2143 3060ms of data, we made 296 measurements.

2144 For the 5ms bin width data, we used windows containing 40 bins, and slid the window 2
 2145 bins at a time when taking measurements.

2146 For the 10ms bin width data, we used windows containing 40 bins, and slid the window
 2147 1 bin at a time when taking measurements (see table 4.1 for concise details).

2148 By continuing to use windows containing 40 bins, we retained statistical power but sac-
 2149 rificed the number of measurements taken.

2150 There was an interval between each trial with a grey image in place of the moving bar
 2151 stimulus. This interval varied in time. But we included some of this interval when recording
 2152 the data for each trial. We started recording the number of active neurons, and the number
 2153 of spikes from each neuron from 530ms before each trial until 1030ms after each trial. This
 2154 way, we could see the change in our measurements at the onset of a stimulus and the end of
 2155 stimulus presentation.

2156 As mentioned in section 4.3.2, we recorded the number of active neurons in each bin, and
 2157 the spike count for each neuron in each bin. The measurements we took using these data in
 2158 each window were as follows:

2159 **Moving average** The average number of active cells in each window.

2160 **Moving variance** The variance of the number of active cells in each window.

2161 **Average correlation** We measured the correlation between the spike counts of each pair of
 2162 cells in the ensemble, and took the average of these measurements.

2163 **Binomial p** We fitted a binomial distribution to the data in each window and recorded the
 2164 fitted probability of success, p in each case.

2165 **Beta-binomial α, β** We fitted a beta-binomial distribution to the data in each window, and
 2166 recorded the values of the fitted shape parameters, α and β , of each distribution.

2167 **Conway-Maxwell-binomial distribution p, ν** We fitted a Conway-Maxwell-binomial dis-
2168 tribution to the data in each window, and recorded the fitted values of p and ν for each
2169 distribution.

2170 **Log-likelihoods** We also recorded the log-likelihood of each of the fitted distributions for
2171 each window.

2172 **4.3.4 Fano factor**

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

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

2173 We measured the Fano factor of the spike count of a given cell by measuring the mean and
2174 variance of the spike count across trials, and taking the ratio of those two quantities. When
2175 calculated in this way the Fano factor can be used as a measure of neural variability that
2176 controls for changes in the firing rate. This is similar to the calculation used in (Churchland
2177 et al., 2010).

2178 **4.3.5 Probability Distributions suitable for modelling ensemble activity**

2179 We present here three different probability distributions that could be suitable to model the
2180 number of active neurons in an ensemble. Each distribution has the set $\{0, \dots, n\}$ as its sup-
2181 port, where n is the number of neurons in the ensemble. These are simple distributions with
2182 either two or three parameters each. However, we regard n as known when using these dis-
2183 tributions for modelling, so in effect each distribution has either one or two free parameters.

2184 **Association**

2185 *Association* between random variables is similar to the correlation between random variables
2186 but is more general in concept. The correlation coefficient is a measure of association; and
2187 association doesn't necessarily have a mathematical definition like correlation does. Essen-
2188 tially, an association between two random variables is a dependency of any kind. Positively
2189 associated variables tend to take the same value, and negatively associated variables tend to
2190 take different values. In this research, we work with probability distributions of the num-
2191 ber of successes in a set of Bernoulli trials. These Bernoulli variables may or may not be
2192 associated.

4.3. Methods

2193 A probability distribution over the number of successes in n Bernoulli trials, where the
2194 Bernoulli variables may be associated, could constitute a good model for the number of active
2195 neurons in an ensemble of n neurons. As long as the observation period is divided into time
2196 bins short enough so that any neuron is unlikely to fire more than spike in any time bin.

2197 **Binomial distribution**

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

The probability mass function of the distribution is

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

2198 As a model for the activity of a neuronal ensemble, the main problem with the binomial
2199 distribution is that it treats each neuron, represented as a Bernoulli trial, as independent. It is
2200 well known that neurons are not independent, and that correlated behaviour between neurons
2201 is vital for representing sensory information (Cohen and Maunsell, 2009). The binomial dis-
2202 tribution falls short in this regard, but it is useful as performance benchmark when assessing
2203 the performance of other models.

2204 **Beta-binomial distribution**

2205 The beta distribution is the conjugate distribution of the binomial distribution. The beta-
2206 binomial distribution is the combination of the beta distribution and the binomial distribution,
2207 in that the probability of success for the binomial distribution is sampled from the beta dis-
2208 tribution. This allows the beta-binomial distribution to capture some over dispersion relative
2209 to the binomial distribution.

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

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

2210 where $B(\alpha, \beta)$ is the beta function.

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

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

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

2211 This reparametrisation is useful because π acts as a location parameter analogous to the p
2212 parameter of a binomial distribution. A value of $\rho > 0$ indicates over-dispersion relative to a
2213 binomial distribution.

2214 As a model for the activity of a neuronal ensemble, the beta-binomial distribution is
2215 more suitable than a binomial distribution because the over-dispersion of the beta-binomial
2216 distribution can be used to model positive association between the neurons. An extreme
2217 example of this over-dispersion/positive association can be seen in figure 4.1b. In this figure,
2218 the neurons are positively associated and so tend to take the same value, consequently the
2219 probability mass of the beta-binomial distribution builds up close to $k = 0$ and $k = n$. It is
2220 worth noting that the location parameter for each distribution has the same value, $p = \pi =$
2221 0.5.

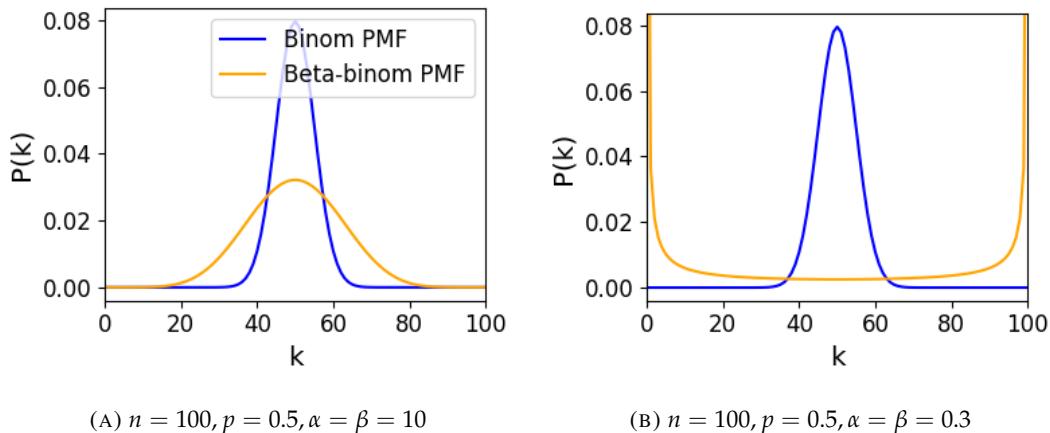


FIGURE 4.1: Figures showing the over-dispersion possible for a beta-binomial distribution relative to a binomial distribution. Parameters are shown in the captions.

2222 **Conway-Maxwell-binomial distribution**

2223 The Conway-Maxwell-binomial distribution (COMb distribution) is a three parameter generalisation of the binomial distribution that allows for over dispersion and under dispersion

4.3. Methods

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

The probability mass function of the COMb distribution is

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

where

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

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

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

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

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

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

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

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

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

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

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

2245 Since the COMb distribution has the potential to capture positive and negative associa-
2246 tions between the neurons/Bernoulli variables, it should be an excellent candidate for mod-
2247 elling the number of active neurons in a neuronal ensemble.

2248 We wrote a dedicated Python package to enable easy creation and fitting of COMb dis-
2249 tribution objects. The format of the package imitates the format of other distribution objects
2250 from the `scipy.stats` Python package. The COMb package can be found here:

2251 https://github.com/thomasjdelaney/Conway_Maxwell_Binomial_Distribution

2252 4.3.6 Fitting

2253 We fitted binomial, beta-binomial, and Conway-Maxwell-binomial (COMb) distributions to
2254 the neural activity in each of the overlapping windows covering each trial. To fit the distribu-
2255 tions we minimised the appropriate negative log likelihood function using the data from the
2256 window.

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

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

2257 We minimised the negative log likelihood function of the beta-binomial distribution nu-
2258 merically. We calculated the negative log likelihood for a sample directly, by taking the sum
2259 of the log of the probability mass function for each value in the sample. We minimised the
2260 negation of that function using the `minimise` function of the `scipy.optimize` Python
2261 package.

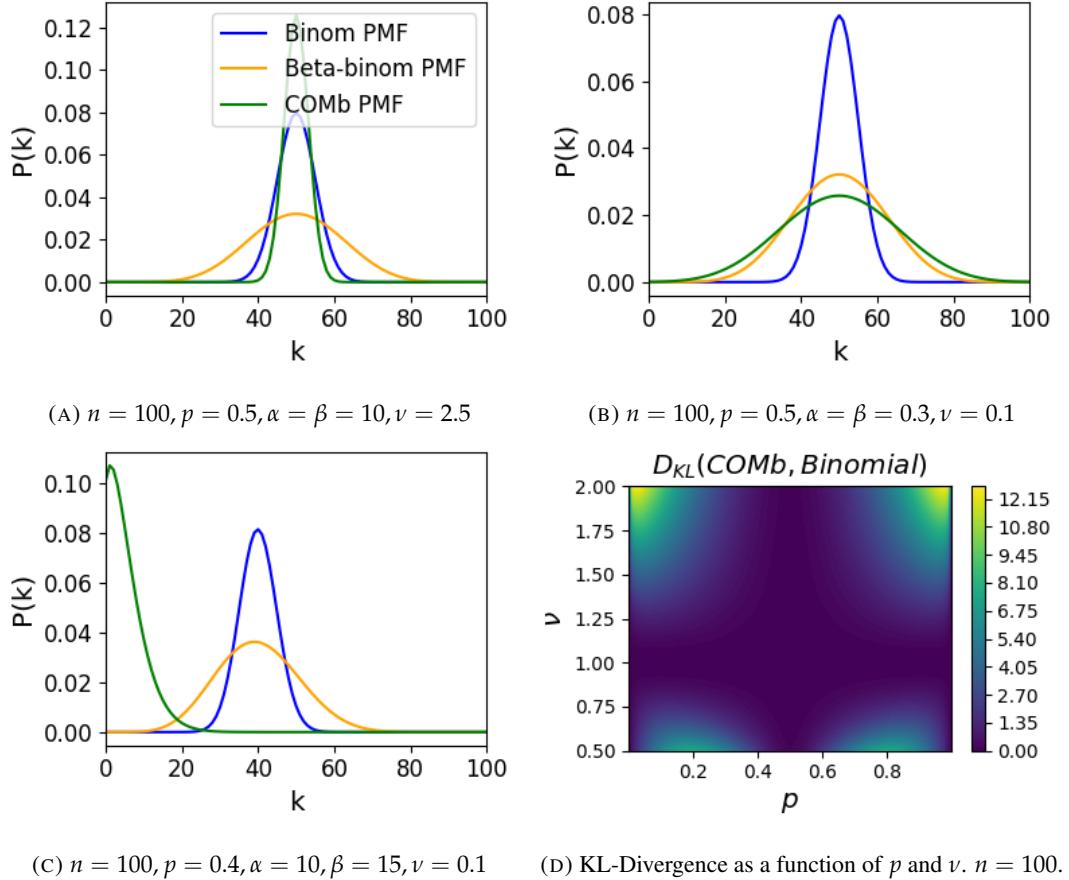


FIGURE 4.2: Figures showing (A) the under-dispersion and (B) over-dispersion permitted by the COMb distribution relative to a binomial distribution. (C) illustrates that the p parameter of the COMb distribution does not correspond to the mean of the distribution, as it does for the binomial and beta-binomial distributions. (D) shows a heatmap for the value of the Kullback-Liebler divergence between the COMb distribution and the standard binomial distribution with same value for n , as a function of p and ν . The point of this figure is to give the reader a sense of how the values of p and ν influence the difference between the COMb distribution and the binomial distribution. The divergence is smallest when $\nu \approx 1$, or when $p \approx 0.5$. When $\nu = 1$, the PMF for the COMb distribution is the same as the PMF for the binomial distribution. When $p = 0.5$ the mass of the distribution is centred around $n/2$ for both the COMb and the binomial distribution. The difference between the two distributions is controlled by the ν parameter. The further the p and ν parameters are from 0.5 and 1 respectively, the greater the difference between the COMb distribution and the binomial distribution. Parameters for all figures are shown in the captions.

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

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

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

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

2262 We minimised the negation of this function using numerical methods. More specifically, we
2263 used the `minimise` function of the `scipy.optimize` Python package.

2264 **4.3.7 Goodness-of-fit**

2265 After fitting, we measured the goodness-of-fit of each model/distribution with their log like-
2266 lihood. We calculated this directly using the `logpmf` functions of the distribution objects in
2267 Python.

2268 **4.4 Results**

2269 We defined a neuron as *active* in a time bin if it fires at least one spike during the time interval
2270 covered by that bin. We measured the number of active neurons in the primary visual cortex
2271 of a mouse in 1ms bins across 160 trials of a moving bar visual stimulus. We then slid a
2272 100ms window across these 1ms bins taking measurements, and fitting distributions along
2273 the way. We did the same for neurons in the thalamus, hippocampus, striatum, and motor
2274 cortex. We repeated the analysis for 5ms time bins with 40 bin windows, and 10ms time bins
2275 with 40 bin windows.

2276 **4.4.1 Increases in mean number of active neurons and variance in number of
2277 active neurons at stimulus onset in some regions**

2278 We measured the average number of active neurons, and the variance of the number of active
2279 neurons in a 100ms sliding window starting 500ms before stimulus onset until 1000ms after
2280 stimulus onset. We found differences in the response across regions. There were no observed
2281 changes in response to the stimulus in the motor cortex or the striatum. The changes in the
2282 other regions are detailed below.

4.4. Results

2283 Primary visual cortex

2284 We found a transient increase in both the average and variance of the number of active neu-
2285 rons at stimulus onset, followed by a fall to pre-stimulus levels, followed by another transient
2286 increase (see figure 4.3). The oscillation in both of these measurements appear to reflect the
2287 frequency of the stimulus (see Data section 4.2.1), and it is known that stimulus structure can
2288 influence response structure(Litwin-Kumar, Chacron, and Doiron, 2012). We see a similar
2289 but lower amplitude oscillation at the end of the stimulus presentation.

2290 Hippocampus

2291 In the hippocampus we observed a transient increase in the average number of active neurons
2292 and in the variance of the number of active neurons at stimulus onset (see figure 4.4). The
2293 increase lasted about 125ms, and the subsequent fall to baseline took the a similar amount of
2294 time.

2295 Thalamus

2296 In the thalamus we observed a transient increase in the both the average and variance of
2297 the number of active neurons on stimulus onset, followed by a fall to pre-stimulus levels,
2298 followed by a sustained increase until the stimulus presentation ends.

2299 As one you might expect for a visual stimulus, the change in the average number of active
2300 neurons was greatest in the primary visual cortex. In this region, this quantity doubled on
2301 stimulus onset. In contrast, in the hippocampus and the thalamus, the average number of
2302 active neurons only increased by a fraction of the unstimulated baseline value. The duration
2303 of the response in V1 and the hippocampus at stimulus onset was 300 – 400ms, but the
2304 response in the thalamus appeared to last for the duration of stimulus presentation. The V1
2305 also showed a change in the average number of active neurons at stimulus end. The change
2306 was similar to that observed at stimulus onset, but smaller in magnitude (see figures 4.3, 4.4,
2307 and 4.5)

2308 4.4.2 Conway-Maxwell-binomial distribution is usually a better fit than bino- 2309 mial or beta-binomial

2310 Since the Conway-Maxwell-binomial distribution has not been fitted to neuronal data before,
2311 it is not clear that it would be a better fit than the binomial or beta-binomial distributions.
2312 In order to find out which parametric distribution was the best fit for the largest proportion

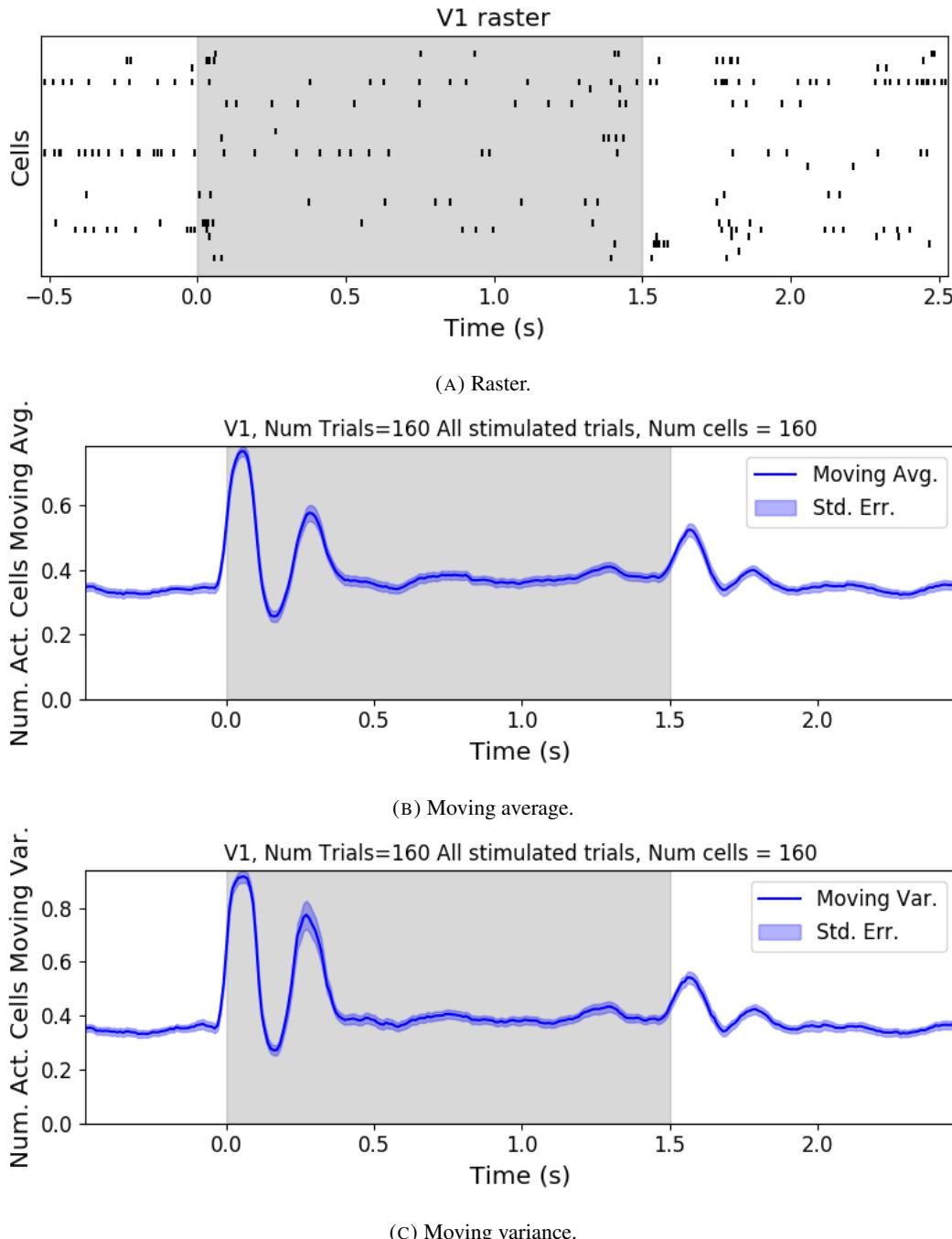


FIGURE 4.3: (A) Raster plot showing the spikes fired by 33 randomly chosen neurons in the primary visual cortex. (B-C) (B) average and (C) variance of the number of active neurons, measured using a sliding window 100ms wide, split into 100 bins. The midpoint of the time interval for each window is used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

4.4. Results

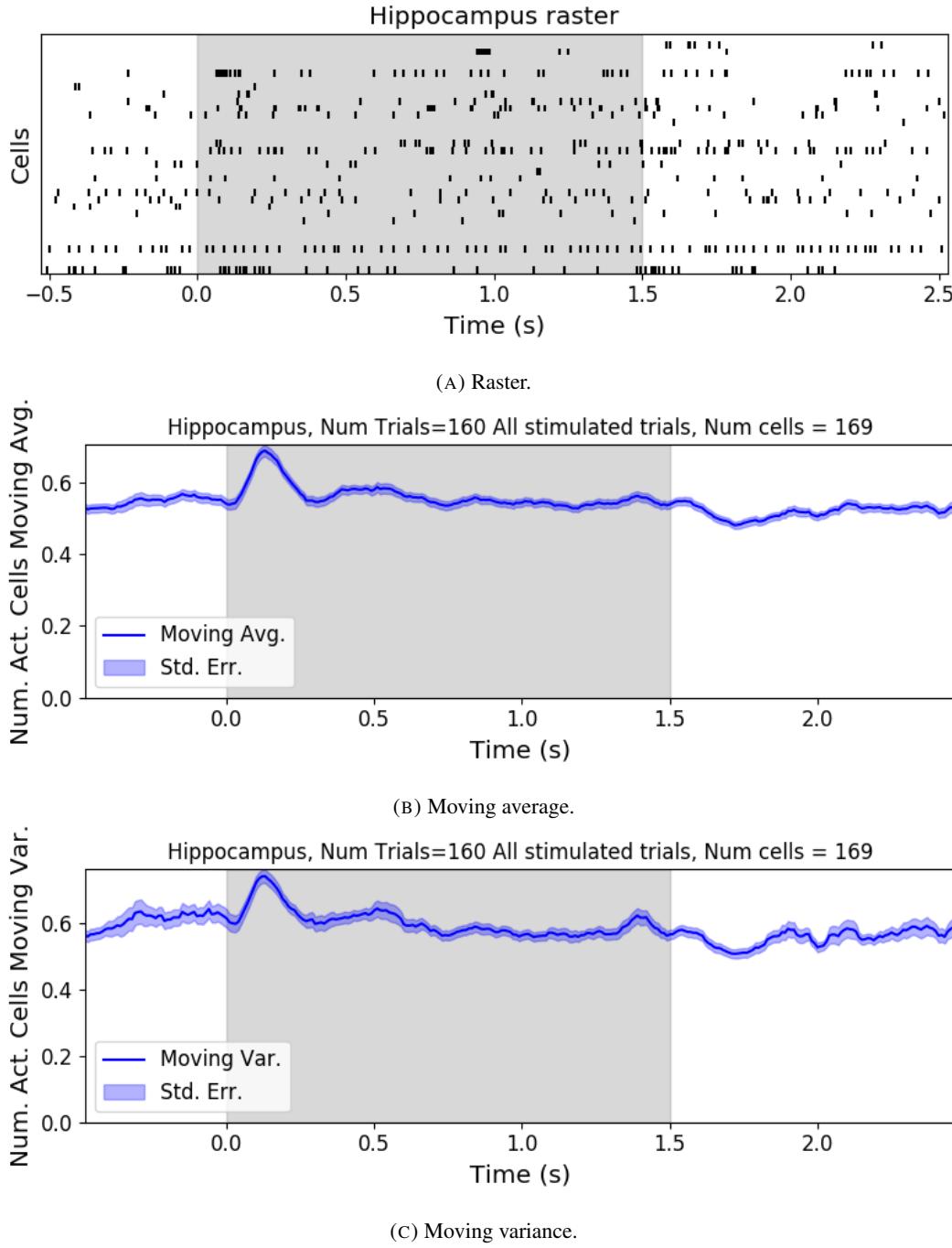


FIGURE 4.4: (A) Raster plot showing the spikes fired by 33 randomly chosen neurons in the hippocampus. (B-C) (B) average and (C) variance of the number of active neurons, measured using a sliding window 100ms wide, split into 100 bins. The midpoint of the time interval for each window is used as the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

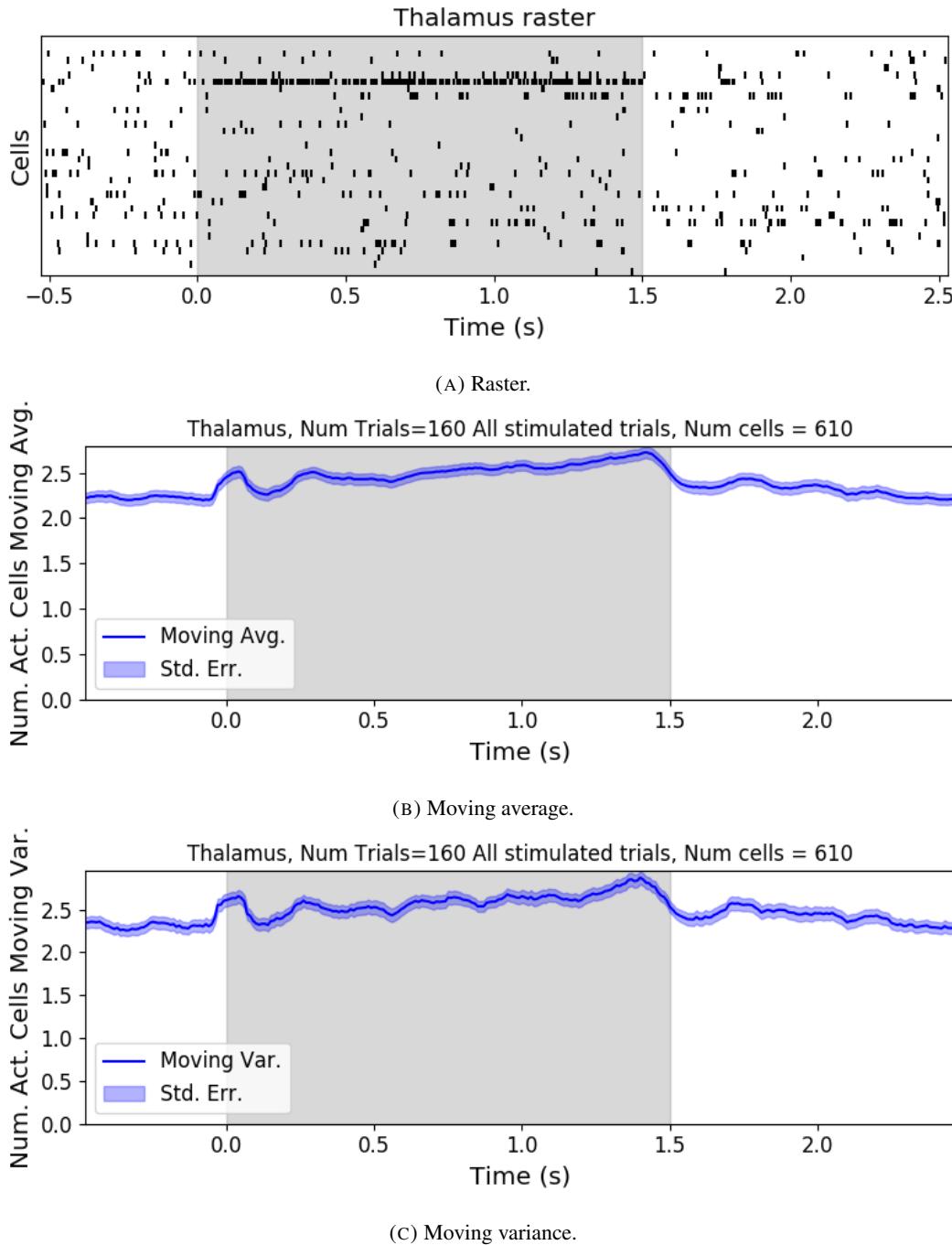
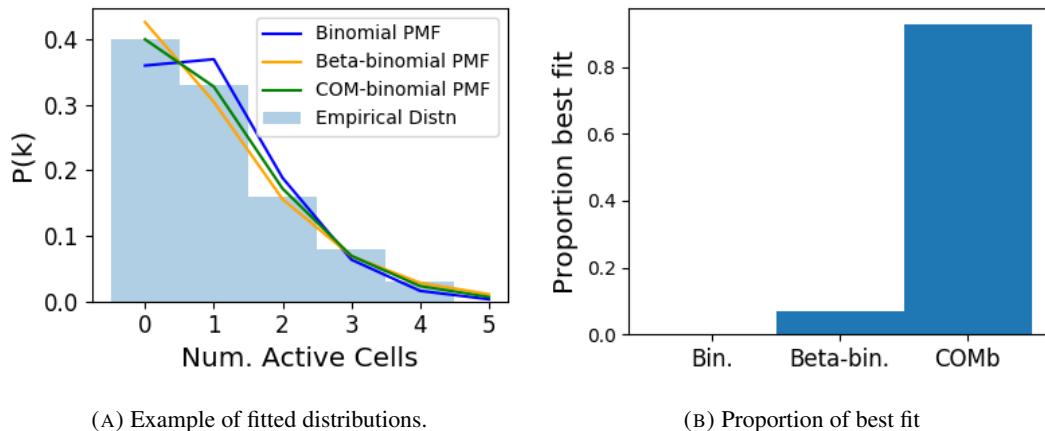


FIGURE 4.5: (A) Raster plot showing the spikes fired by 33 randomly chosen neurons in the thalamus. (B-C) (B) average and (C) variance of the number of active neurons, measured using a sliding window 100ms wide, split into 100 bins. The midpoint of the time interval for each window is used as the timepoint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

4.4. Results

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

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



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

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

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

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

2330 **4.4.3 Relative goodness-of-fit for binomial, beta-binomial, and COMb distri-**
2331 **butions**

2332 In the previous section we showed that the COMb distribution was usually a better option
2333 than the binomial or beta-binomial distributions when attempting to fit a distribution to a
2334 sample of the number of active neurons. In this section, we aim to illustrate typically how
2335 much better the COMb distribution is.

2336 **Log likelihoods of distributions fitted to stimulated and unstimulated responses**

2337 We fitted binomial, beta-binomial, and COMb distributions to two windows in each of the 160
2338 trials with a visual stimulus. One of the windows was the last full window before stimulus
2339 onset. The other window was the first full window after stimulus onset. We measured the log
2340 likelihood for each fitted distribution. For the histograms of these log likelihood values for
2341 data from the primary visual cortex using 1ms bin width, see figure 4.7.

2342 Comparing unstimulated to stimulated windows, we observed that the log likelihood val-
2343 ues were greater for the unstimulated windows for all three distributions. This shows that the
2344 distributions were fitting better to the number of active neurons in the unstimulated windows.
2345 This might be due to a greater diversity in the distributions of number of active neurons in
2346 the stimulated windows (see figure 4.3). We saw similar results for the primary visual cortex
2347 when using 5ms and 10ms bin widths. For other regions, the histograms for unstimulated
2348 and stimulated windows were more similar, covering similar ranges of log likelihoods.

2349 We observed a marginal increase in the log likelihoods from binomial to beta-binomial to
2350 COMb distributions for both unstimulated and stimulated windows. But, the distribution of
2351 the log likelihoods looked quite similar for all three distributions. So, the COMb distribution
2352 only fits a little bit better than the other two distributions. We observed similar results when
2353 using different time bin widths, and data from different brain regions.

4.4. Results

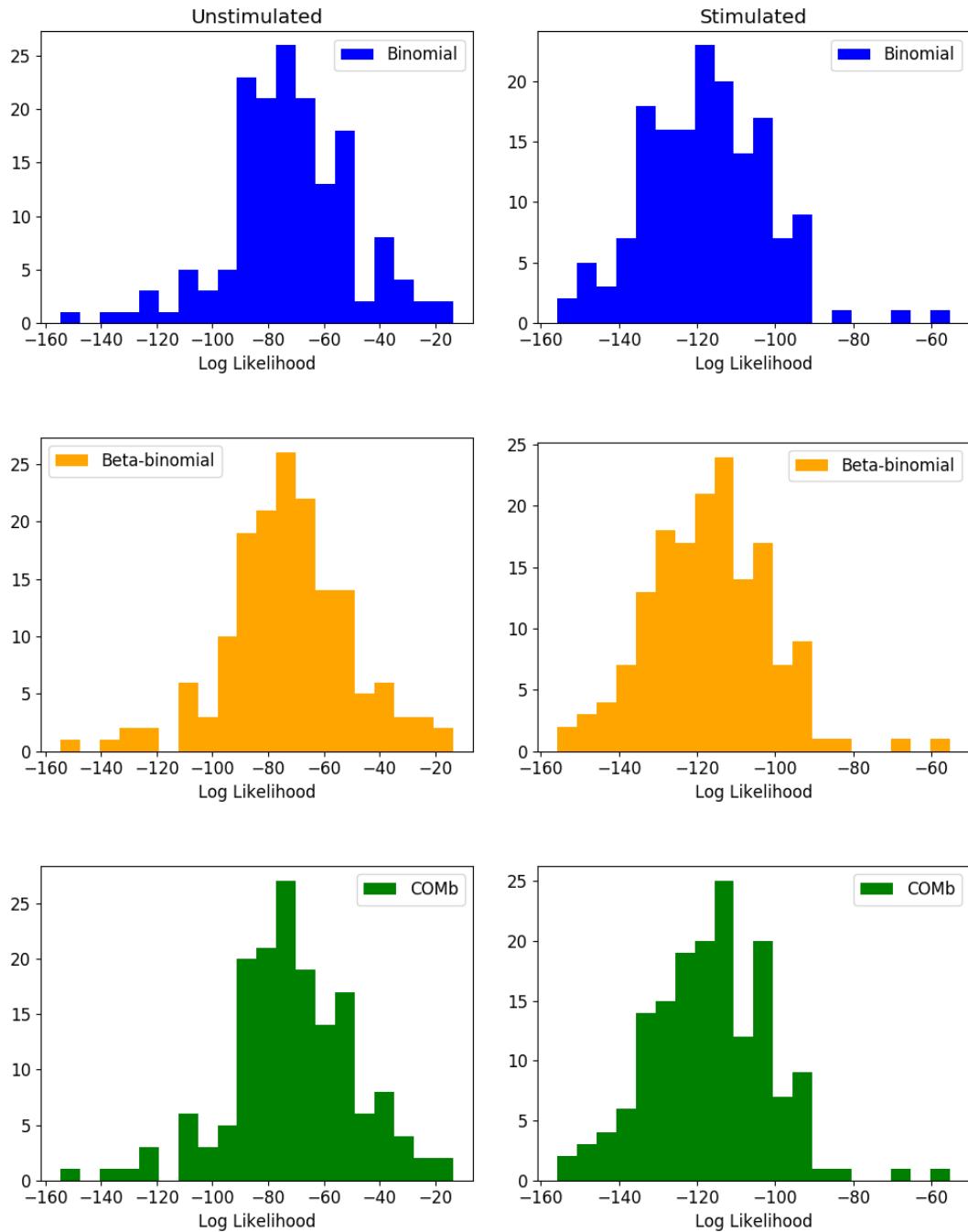


FIGURE 4.7: Number of active neurons data from the primary visual cortex, 1ms bin widths. (Left column) Histograms of log likelihoods for the binomial, beta-binomial, and COMb distributions fitted to windows where no visual stimulus was present. 160 trials. (Right column) Similar histograms for windows where a visual stimulus was present. 160 trials. In both cases, there are marginal increases in the log likelihoods from binomial to beta-binomial to COMb. The log likelihoods are larger for the unstimulated windows.

2354 **Distribution of fitted parameters for stimulated and unstimulated responses**

2355 We recorded the fitted parameters of each of the three distributions fitted to both the unstim-
2356 ulated and stimulated windows mentioned in section 4.4.3. We noticed an increase in the
2357 binomial distribution’s parameter for the stimulated windows (figure 4.8 top row). When
2358 we used wider bin widths, we noticed the same relative increase from unstimulated to stim-
2359 ulated windows, and the values over which the parameter was distributed increased. The
2360 relative increase from unstimulated to stimulated windows was also visible in data from the
2361 hippocampus, but not in other regions.

2362 For the beta-binomial distribution, we converted the fitted α and β parameters to π and
2363 ρ parameters (see Methods section 4.3.5) before examining their histograms. In this form,
2364 the $0 \leq \pi \leq 1$ parameter is a location parameter, and the $\rho > 0$ parameter is a shape
2365 parameter than encodes over-dispersion in the distribution relative to a binomial distribution.
2366 Comparing distribution of parameters fitted to the unstimulated windows to the distribution
2367 of those fitted to the stimulated windows, we observed slight increases in both the π and ρ
2368 paramters (figure 4.8 middle row). When we used a wider bin width, the paramter values were
2369 distributed across larger absolute values. The results comparing unstimulated to stimulated
2370 distributions were similar to the 1ms case. For the other regions, we observed similar results
2371 in the hippocampus. All the other regions show little difference between unstimulated and
2372 stimulated histograms in a given bin width. As we increased the size of the bin width used
2373 the absolute values across which the parameters were distributed increased.

2374 For the COMb distribution, the ν paramter was distributed around 1 for the unstimulated
2375 windows. For the stimulated windows ν was more tightly distributed and centred around
2376 0.75. This reflects a positive association between the neurons present immediately after stim-
2377 ulus onset. We also observed that the p parameter was distributed over slightly greater values
2378 for the stimulated windows as compared to the unstimulated (figure 4.8 bottom row). For
2379 increased bin widths, we observed that the ν parameter was distributed lower than for the
2380 1ms bin width case. The reduction in ν at for the stimulated windows was still present. We
2381 observed this drop in the ν parameter in the hippocampal data also, albeit to a lesser extent
2382 than in the primary visual cortex. We didn’t observe differences in the the data from the other
2383 regions.

4.4. Results

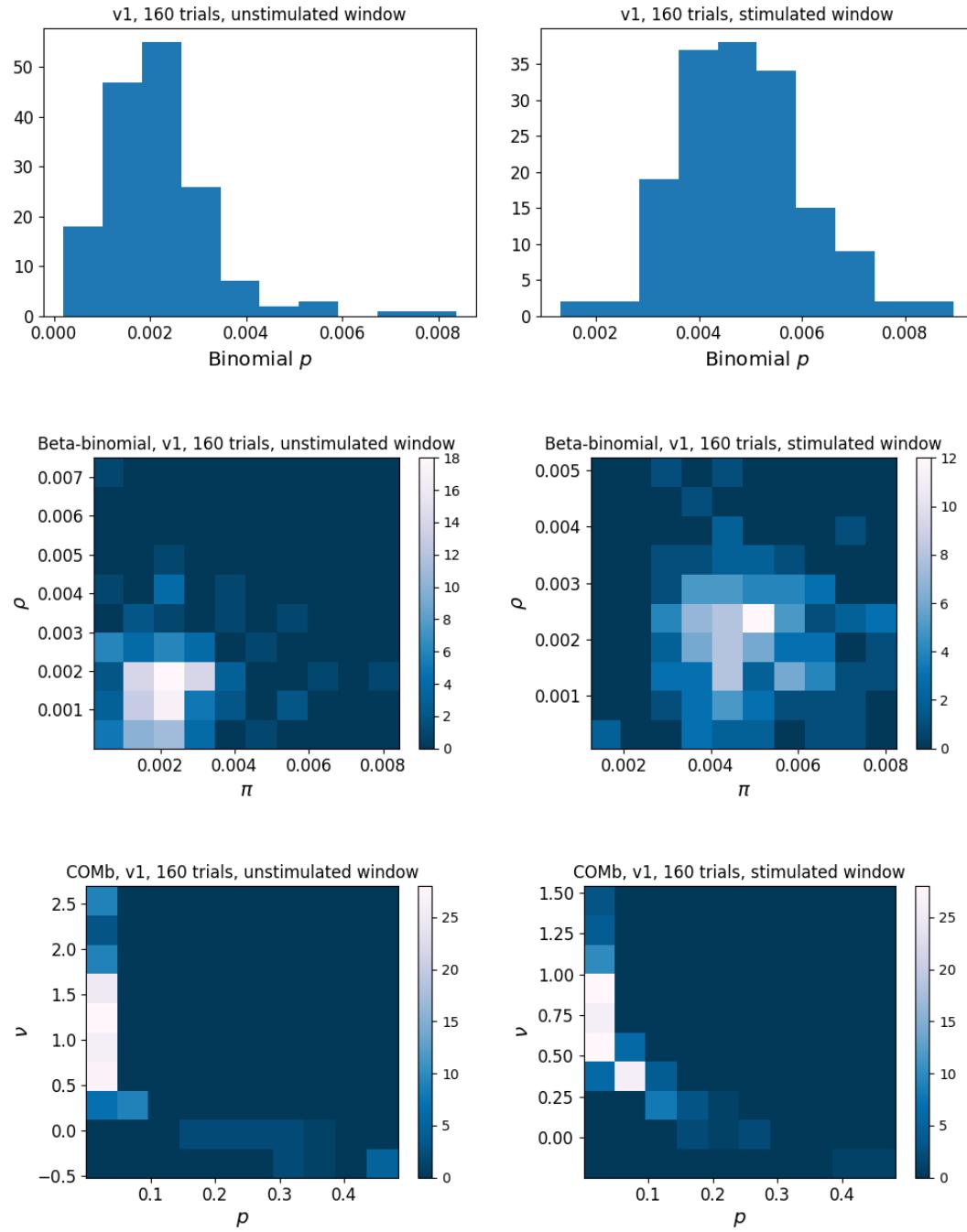


FIGURE 4.8: Histograms of fitted parameters for binomial, beta-binomial, and COMb distributions. Distributions fitted to data from the primary visual cortex, using 1ms bin widths. (Left column) The distributions were fitted a window before the onset of the visual stimulus. (Right column) The distributions were fitted to a window immediately after the onset of the visual stimulus.

2384 **Examples of empirical distributions and fitted distributions**

2385 In figure 4.9 there are some examples of fitted binomial, beta-binomial, and COMb distri-
2386 butions alongside the empirical distributions to which they are fitted. We can see that these
2387 fitted distributions over distributed their probability mass to $P(0)$ (or $P(0)$ and $P(1)$ for the
2388 thalamus) and underdistributed their probability mass elsewhere. Also, each of the three fit-
2389 ted distributions look similar in each example. This is in agreement with our observations in
2390 section 4.4.3.

2391 **4.4.4 Conway-Maxwell-binomial distribution captures changes in association
2392 at stimulus onset**

2393 We fit a Conway-Maxwell-binomial (COMb) distribution to the number of active neurons in
2394 the 1ms time bins in a 100ms sliding window. We also measured the correlation coefficient
2395 between the spike counts of all possible pairs of neurons, and took the average of these
2396 coefficients. We did this for all the trials with a visual stimulus. We observed a reduction in
2397 the COMb distribution's ν parameter at stimulus onset from around 1 to between 0 and 1 (see
2398 figure 4.10a). A value of ν less than 1 indicates positive association between the neurons (see
2399 section 4.3.5). We might expect to see this positive association reflected in the correlation
2400 coefficients, but this is not the case. We see no change in the time series of average correlation
2401 measures at stimulus onset.

2402 This may be due to the very short time bin we used in this case. We know that using small
2403 time bins can artificially reduce correlation measurements (Cohen and Kohn, 2011). In this
2404 case, fitting the COMb distribution may be a useful way to measure association in a neuronal
2405 ensemble over very short timescales (< 10ms).

2406 **4.4.5 Replicating stimulus related quenching of neural variability**

2407 Churchland et al. (2010) inspected the effect of a stimulus on neural variability. One of the
2408 measures of neural variability that they employed was the Fano factor of the spike counts of
2409 individual cells (see section 4.3.4). They found a reduction in neural variability as measured
2410 by the Fano factor in various cortical areas in a macaque at the onset of various visual stimuli,
2411 or a juice reward (Churchland et al., 2010).

2412 We measured the Fano factor of the spike count of each cell in each brain region, during
2413 each trial. We measured the mean and standard error of these Fano factors from 500ms before
2414 stimulus onset until 1000ms after stimulus end. For the primary visual cortex, we found a

4.4. Results

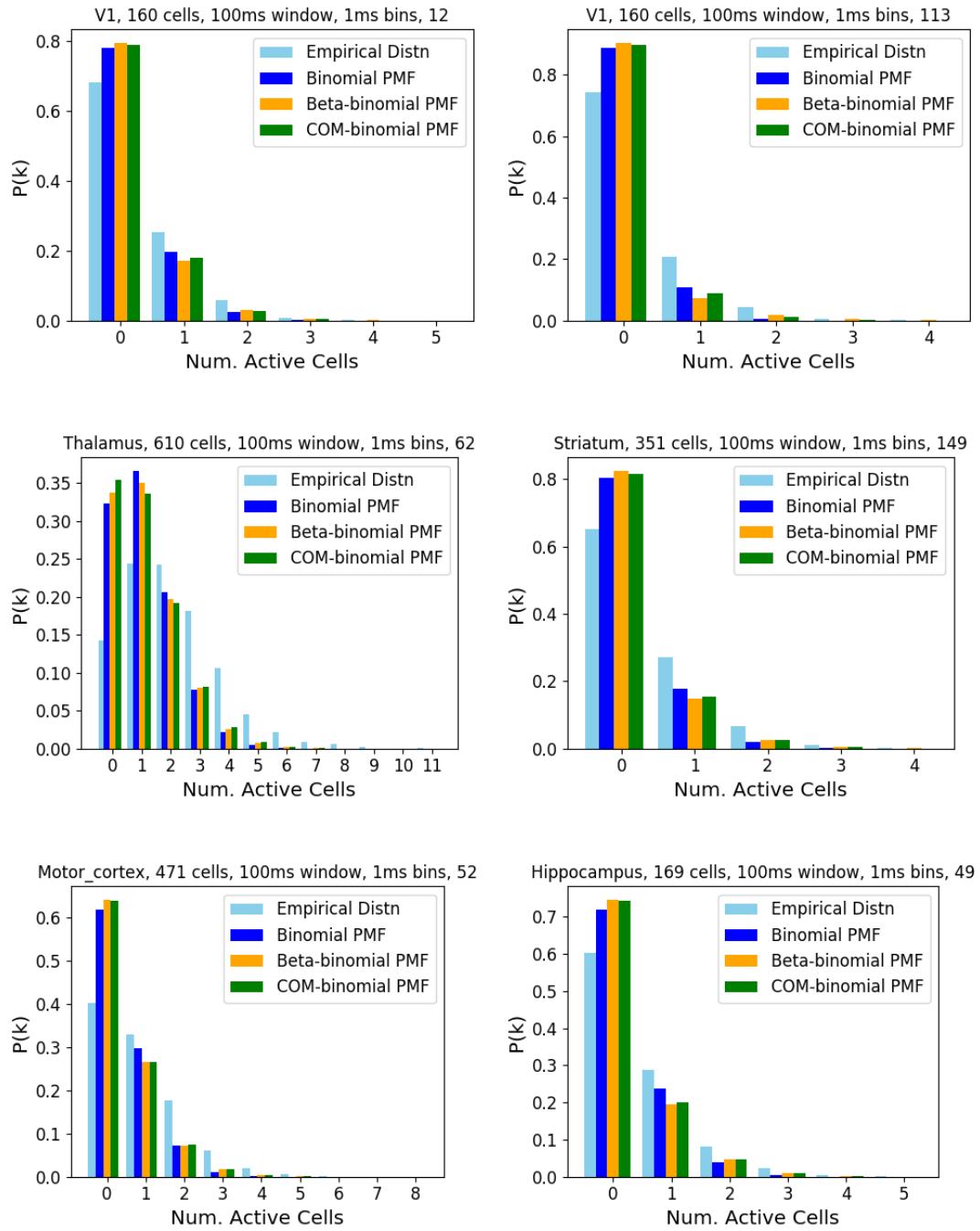


FIGURE 4.9: Examples of empirical and fitted distributions. At least one example from each of the 5 brain regions from which we had data.

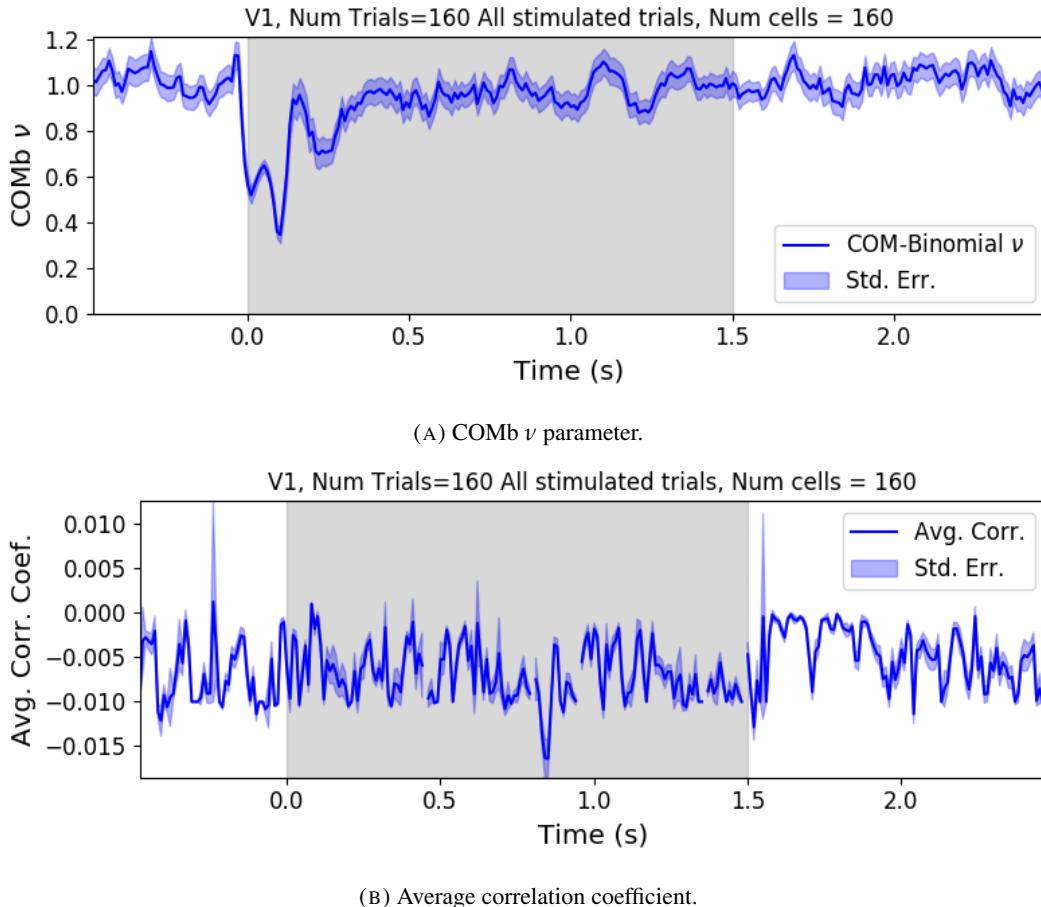


FIGURE 4.10: (A) We fit a Conway-Maxwell-binomial distribution to the number of active neurons in 1ms time bins of a 100ms sliding window. We did this for all trials with a visual stimulus and took the average across those trials. We see a transient drop in value for the distribution's ν parameter at stimulus onset. This shows an increase in positive 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. The took the average of those coefficients. We also did this for every visually stimulated trial, and took the average across trials. The increase in positive association is not reflected with an increase in average correlation.

2415 transient reduction in the Fano factor immediately after stimulus onset. We used a Mann-
 2416 Whitney U test to check that the Fano factors measured in a window starting at stimulus
 2417 onset and ending 100ms later were significantly lower than the factors measured in a window
 2418 ending at stimulus onset ($p < 0.001$, see figure 4.11a). We did not get this statistically
 2419 significant result in any other region.

2420 Our findings agree with those of Churchland et al. for the primary visual cortex. However
 2421 Churchland also found a reduction in the Fano factor in the dorsal premotor cortex (PMd) at
 2422 stimulus onset. Our measurements from the mouse motor cortex show no change at stimulus
 2423 onset (see figure 4.11b). This could indicate some difference in the functionality of the motor
 2424 cortex in a macaque and the motor cortex of a mouse.

4.4. Results

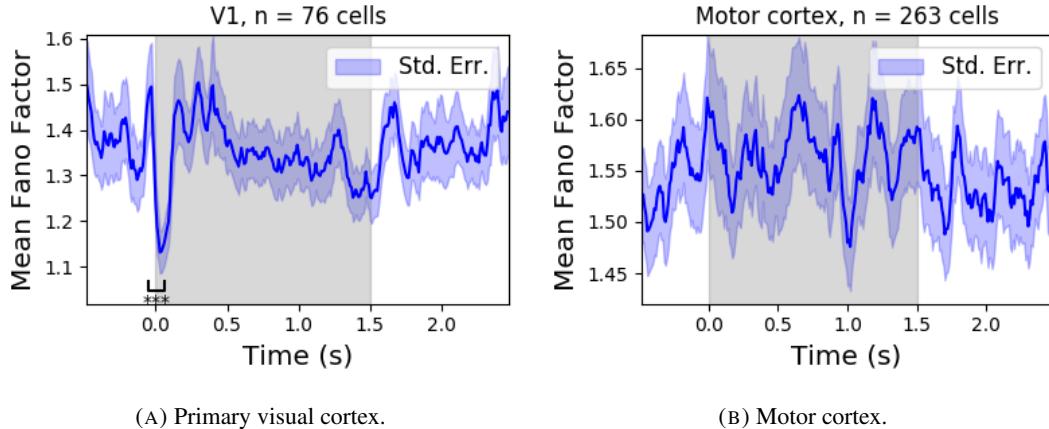


FIGURE 4.11: (A) The mean Fano factor of the spike counts of the cells in the primary visual cortex. Means were taken across cells first, then across trials. There was a significant decrease in the Fano factors immediately after stimulus onset. (B) The mean Fano factor of the spike counts of the cells in the motor cortex. No significant change in measurements at any point.

2425 Similar to these findings in the Fano factor, we found a reduction in the ν parameter of
 2426 the COMB distribution on stimulus onset in V1 (figure 4.10a) and in no other region from
 2427 which we had data. Specifically, the ν parameter reduced from around 1, to between 1 and 0.
 2428 This represents a change from no association between the neurons, to a positive association.
 2429 It is possible that this positive association may be responsible for the reduction in the Fano
 2430 factor.

2431 **4.4.6 Effects of greater bin widths**

2432 Using a greater bin width (10ms) affected the scale and shape of the most of the measure-
 2433 ments taken (described in section 4.3.3). The average correlation coefficient is the exception
 2434 to this. The 10ms bin width is still so small that the average correlation coefficient was also
 2435 small (similar scale as figure 4.10b).

2436 Using the greater bin width acted as a low pass filter on the other measurements taken
 2437 and the parameters of the fitted distributions. For example compare figure 4.10a to figure
 2438 4.12a. Note also that the ν parameter varies between 0.6 and 0.2 when using the 10ms bin
 2439 width. This indicates some positive association between the neurons at all times, even when
 2440 not stimulated or when adapted to the stimulus. This may be caused by the wider bin width
 2441 resulting in more neurons classified as active. The change in association at stimulus onset is
 2442 still captured by the change in the ν parameter.

2443 The mean number of active neurons was about 10 times greater for a 10ms bin width
 2444 compared to a 1ms bin width. The variance in the number of active neurons was also greater

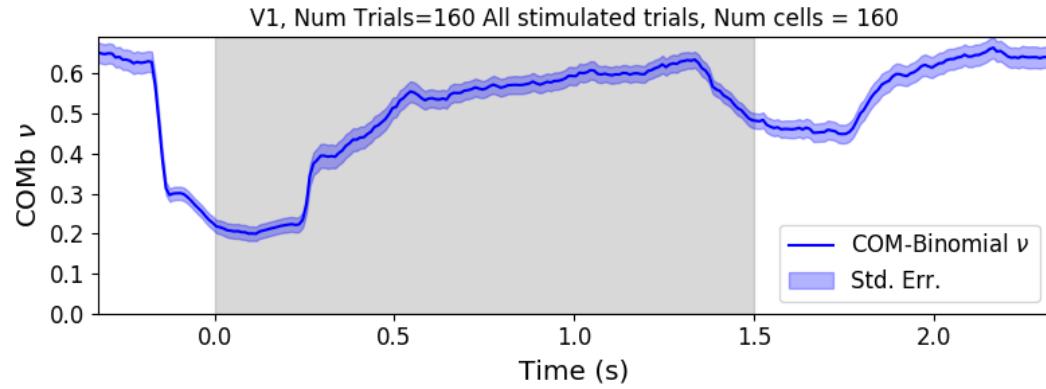
2445 for the wider bin width. This caused a change in the scale of the Fano factor (see figure 4.12b
2446 compared to figure 4.11a). Also, the reduction in the mean Fano factor at stimulus onset is
2447 not significant when using the 10ms bin width (Mann-Whitney U test, $p = 0.07$). This is
2448 likely due to greater variance in the Fano factors of the individual cells. Bear in mind that we
2449 are using the activity in each bin (either 0 or 1) rather than the actual spike counts in each bin.
2450 When using a 1ms bin width, there is no difference between the spike count and the activity
2451 but when using a wider bin width, there may be more than one spike per bin.

2452 4.5 Discussion

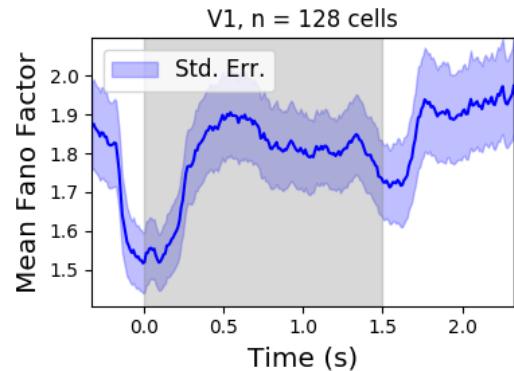
2453 Our aim in this research was to develop a new statistical method for analysing the activity
2454 of a neuronal ensemble at very short timescales. We wanted our method to use information
2455 taken from the whole ensemble, but we also wanted the method to be quick and easy to
2456 implement. It is likely that analysis methods with these characteristics will become valuable
2457 as electrophysiological datasets include readings from more cells over longer time periods. In
2458 this case, we used the number of active, or spiking, neurons in a very short time bin (< 10ms)
2459 as a measure of ensemble activity.

2460 First of all, we showed that there were changes in response that we could model at these
2461 very short time scales in some of the brain regions from which we had recordings. We ob-
2462 served changes in the average number of active neurons, and the variance of the number of
2463 active neurons in three different brain regions in response to visual stimuli. Since we know
2464 that correlated behaviour is associated with sensory perception (deCharms and Merzenich,
2465 1996), we might hope to measure the pairwise correlations within the neuronal population
2466 in order to further investigate these responses. But, using such short time bins can produce
2467 artificially small spike count correlation measurements (Cohen and Maunsell, 2009). Over-
2468 coming this limitation was one of our objectives for our new method. In order to do this, we
2469 abandoned the idea of measuring the correlations directly and embraced the concept of *asso-*
2470 *ciation*. In order to quantify the association between neurons, we used the Conway-Maxwell-
2471 binomial distribution to model the number of active (spiking) neurons in an ensemble as a
2472 sum of possibly associated Bernoulli random variables.

2473 We showed that the Conway-Maxwell-binomial distribution performed better than the
2474 more common options of the binomial and beta-binomial distributions. Furthermore, we
2475 showed that the positively associated behaviour between neurons in the primary visual cortex
2476 could be captured by fitting a Conway-Maxwell-binomial distribution, but was not captured



(A) Primary visual cortex ν parameter, bin width is 10ms.



(B) Primary visual cortex Fano factor, bin width is 10ms.

FIGURE 4.12: (A) The mean ν parameter of the COMb distribution fitted to activity in the primary visual cortex. Mean taken across all stimulated trials. A bin width of 10ms was used to classify cells as active or inactive. The change in association at stimulus onset is still captured. Some high frequency fluctuations are filtered out by using the wider bins (compare to figure 4.10a) (B) The mean Fano factor of the cells in the primary visual cortex. The change in the mean Fano factor at stimulus onset is not significant when using a bin width of 10ms.

2477 by the more standard approach of measuring the spike count correlation. The associated
2478 behaviour could not be measured using spike count correlations, because of the very short
2479 bins required to capture short timescale behaviour.

2480 We replicated a famous result from Churchland et al (2010) relating to the quenching of
2481 neural variability in cortical areas at stimulus onset, and in doing so, we established a corre-
2482 spondence between the association quantifying parameter of the Conway-Maxwell-binomial
2483 (COMb) distribution and the neural variability as measured by the Fano factor. We found a
2484 reduction in the ν parameter of the COMB distribution at stimulus onset, indicating a change
2485 from no association to positive association between neurons in V1. We found a corresponding
2486 reduction in the Fano factor of the individual cells in V1. The positive association between
2487 neurons induced by the stimulus would constrain the neurons to fire at the same time. The
2488 stimulus also induced a larger number of neurons to spike. These two actions combined could
2489 cause an increase in the firing rate of individual cells that is greater in magnitude than the in-
2490 crease in firing rate variability. If this is indeed the case, then the association as captured by
2491 the COMB distribution could be regarded as one of the ‘natural parameters’ of the ensemble
2492 response for short timescales. That is, a quantity that directly measures some aspect of the
2493 behaviour of the ensemble. In this case, it the correlated behaviour of the individual neurons
2494 is captured.

2495 This work could be just a first step in creating analysis methods based on the Conway-
2496 Maxwell-binomial distribution, or similar statistical models. One way to extend the method
2497 would be to pair it up with the ‘Population Tracking model’ (O’Donnell et al., 2017). This
2498 model attempts to characterise the interaction between an ensemble and each member of the
2499 ensemble by quantifying the probability of spiking for a given a cell, given the number of
2500 active cells in the whole population. Combining this model with the COMB distribution
2501 would give us a model that could accurately fit the number of active neurons at any moment,
2502 and that gives a probability of firing for each cell, and therefore probabilities for full spiking
2503 patterns, without adding a huge number of parameters to fit.

2504 A more complex way to extend the model would be to fit a Conway-Maxwell-binomial
2505 distribution to data recorded from multiple brain regions simultaneously, with a different fit
2506 for each region, then to analyse the temporal relationship between the fitted parameters of
2507 each region. If we analysed the time series of the COMB distribution parameters from the
2508 different regions, looking at cross-correlations between regions, this may give some results
2509 relating to the timescales in which information is processed in different brain regions.

2510 **Chapter 5**

2511 **Studies with practical limitations &**
2512 **negative results**

2513 *Abstract*

2514 Here I will present some details on research topics that I started, but that unfortunately did not
2515 lead anywhere useful. There are two pieces of research, based on two papers. Each paper is
2516 related to the overall theme of my PhD of analysing and modelling behaviours of populations
2517 of neurons. The first part is based on a model of parallel spike trains including higher order
2518 interactions by Shimazaki et al (2012). The second part is based on a multiscale model for
2519 making inferences on hierarchical data.

2520 **5.1 Dynamic state space model of pairwise and higher order neu-**
2521 **ronal correlations**

2522 In their paper Shimazaki et al (2012) aimed to model spike trains from populations of neurons
2523 in parallel, with pairwise and higher order dynamic interactions between the trains. They
2524 modelled the spike trains as multi-variate binary processes using a log-linear model, and they
2525 extracted spike interaction parameters using a Bayesian filter/EM-algorithm. They developed
2526 a goodness-of-fit measure for the model to test if including these higher order correlations
2527 is necessary for an accurate model. Their measure was based on the Bayes factor but they
2528 also assessed the suitability of higher order models using the AIC and BIC. So the increase
2529 in the number of parameters associated with fitting higher order interactions was taken into
2530 account. They tested the performance of the model on synthetic data with known higher
2531 order correlations. They used the model to look for higher order correlations in data from
2532 awake behaving animals. They use the model to demonstrate dynamic appearance of higher
2533 order correlations in the monkey motor cortex (Shimazaki et al., 2012).

2534 We used the available Python repository to implement the model, and we successfully
2535 worked through the tutorial provided. But we found that the model did not scale well to
2536 larger populations. We attempted to fit the model to a population of 10 neurons and found we
2537 didn't manage to finish the process. Since, our goal was to find a model to scale to hundreds
2538 or thousands of neurons, we decided that this model was no longer worth pursuing.

2539 **5.2 A multiscale model for hierarchical data applied to**
2540 **neuronal data**

2541 In their paper Kolaczyk et al (2001) developed a framework for a modelling hierarchically
2542 aggregated data, and making inferences based on a model arising from this framework. They
2543 assumed that a hierarchical aggregation existed on the data in question, where each element at
2544 each level of the hierarchy had some associated measurements, an associated mean process,
2545 which was the expected value of these measurements. They also assumed that the measure-
2546 ments of each parent were equal to the sum of the measurements from all of its children.
2547 They showed that these assumptions gave rise to a relationship between parent and child
2548 measurements across all levels of the hierarchy, where the product of the likelihood of the
2549 parameters of the lowest level of the hierarchy can be expressed as products of conditional
2550 likelihoods of the elements of higher levels of the hierarchy (Kolaczyk and Huang, 2010).

5.2. A multiscale model for hierarchical data applied to neuronal data

2551 We hoped that the hierarchical structure of the brain (regions to subregions to cells) and a
2552 high level activity measure (fMRI or EEG) could be combined with this model to infer lower
2553 level activity from a high level measure.

2554 They gave examples of these expressions for measurements sampled from Gaussian dis-
2555 tributions, and Poisson distributions, and showed the definitions of the hierarchical param-
2556 eters which reparametrise the distribution of these data taking the hierarchy into account.
2557 They go on to suggest prior distributions for this multiscale model, and integrate these priors
2558 to give posterior distributions for the measurements from each element at each level in the hi-
2559 erarchy, and expressions for the MAP estimated parameters of each the associated processes
2560 (Kolaczyk and Huang, 2010).

2561 We implemented their model in Python by creating some synthetic data from Poisson
2562 distributions, and defining a hierarchy by agglomerating these data. We calculated the MAP
2563 estimates using our knowledge of the hierarchy, and using the expressions given in the paper.
2564 We found that the MAP estimates were far less accurate than would be achieved by simply
2565 ignoring the hierarchy during estimation, and using a maximum likelihood approach. After
2566 that, we decided to move on.

2567 **Chapter 6**

2568 **Discussion**

2569 In this project, we attempted to address some of the challenges in data collection from
2570 large neuronal ensembles (specifically with calcium imaging) and some of the problems in
2571 analysing the data collected from large neuronal ensembles.

2572 Firstly, we investigated the relationship between cell biochemistry, action potentials and
2573 the fluorescence traces produced by fluorescent calcium indicators. We did this by building
2574 a biophysical model that takes in a spike train and returns the fluorescence trace that that
2575 spike would induce. The model included mechanics for the binding of calcium to fluorescent
2576 and endogenous mobile and immobile buffers, and the consequent changes in concentration
2577 of free and bounded calcium. The model consisted of 17 parameters, 13 of which were
2578 set according to data from the literature, and 4 of which were free parameters. We trained
2579 the model using simultaneously collected spiking and calcium imaging data (Berens et al.,
2580 2018). We fitted the model by matching the $\Delta F/F_0$ in response to an action potential, and
2581 by matching the power spectrum of the actual fluorescence trace. This meant that our model
2582 would include the correct amount of noise as well as return the correct change in amplitude
2583 in response to an action potential.

2584 Since our model produced fluorescence traces, we could apply spike inference algorithms
2585 to the modelled fluorescence traces that our model produced after training, and compare the
2586 performance of the algorithms on the modelled traces to their performance on the real traces.
2587 We used three spike inference algorithms, two of which were based on modelling the cal-
2588 cium trace as an autoregression (Friedrich and Paninski, 2016; Pnevmatikakis et al., 2016),
2589 and another inference algorithm that was a little more biologically inspired, but amounted to
2590 a very similar algorithm (Deneux et al., 2016). We compared the performance of the algo-
2591 rithms by using them to infer spikes from 20 real and modelled fluorescence traces induced
2592 by 20 corresponding real spike trains. We then used several binary classification measures
2593 (true positive rate, accuracy etc.) to asses the quality of the spike inference for the real and

2594 modelled fluorescence traces. We found that the spike inference algorithms performed sim-
2595 ilarly on real and modelled traces, showing that our model is capturing at least some of the
2596 characteristics of the real fluorescence traces.

2597 In order to investigate the effect of indicator characteristics on the modelled fluorescence
2598 trace and spike inference quality, we perturbed the indicator's affinity and dissociation rate
2599 in parallel, keeping the ratio of the two the same for all perturbations. We measured the SNR
2600 of the trace, and the true positive rate of the spike inference algorithms at each perturbed
2601 value pair. We found that perturbing the values lower caused in decrease in SNR and spike
2602 inference quality. This shows that our model could be used to test theoretical fluorescent cal-
2603 cium indicators without having to actually manufacture them. Experimental neuroscientists
2604 could also use our model to judge which indicator characteristics are most influential in their
2605 experimental context.

2606 We then investigated the effect of perturbing buffer concentration, and indicator concen-
2607 tration, on the signal-to-noise ratio of the modelled fluorescence trace and spike inference
2608 quality. This was a worthwhile experiment because endogenous buffer concentrations vary
2609 from cell to cell (Bartol et al., 2015; Maravall et al., 2000; Neher and Augustine, 1992), as
2610 does indicator expression (Chen et al., 2013). We found that extreme perturbations away
2611 from the indicator concentration taken from the literature lowered the SNR of the trace, and
2612 the spike inference quality. We also found that increases in the concentration of endogenous
2613 buffer above the value taken from the literature caused a decrease in the SNR and spike infer-
2614 ence quality. This reiterates that the indicator and endogenous buffers compete to bind with
2615 free calcium molecules, and this has an effect on fluorescence and consequently on spike
2616 inference.

2617 We then created some synthetic spike trains with controlled mean firing rates sampled
2618 the rates from an Ornstein-Uhlenbeck process. We found that the higher the firing rate, the
2619 lower the accuracy of the spike inference algorithms. But the mean firing could perhaps be
2620 inferred from the amplitude of the fluorescence traces. The higher firing rate, the higher the
2621 amplitude. Calibrating the model to facilitate and accurate measurement would require some
2622 kind of ground truth, but relative comparisons could be made without any other knowledge
2623 of the underlying spiking process.

2624 One obvious limitation to our model is the lack of binding mechanics for both the indi-
2625 cator and endogenous buffers. Greenberg et al included these mechanics in their successful
2626 spike inference model. We felt that the timescale of these binding mechanics was so small in
2627 comparison to the fluorescence dynamics that omitting them would make no difference. But

2628 it is possible that their inclusion would improve our model.

2629 After investigating the difficulties with inferring spiking data from calcium imaging data,
2630 we moved from data collection to analysis and we decided to implement a new network anal-
2631 ysis method on data from a neuronal ensemble. Using an electrophysiological dataset with
2632 spike sorted data from 9 different brain regions in 3 mice (Steinmetz, Carandini, and Harris,
2633 2019), we binned the spike times for each cell into spike counts for each cell and measured
2634 the correlation coefficients between these spike counts for a selection of cells evenly dis-
2635 tributed across the 9 regions. We repeated these measurements for time bin widths ranging
2636 from 5ms to 3s. We rectified these measurements and, for a given time bin width, used them
2637 as weights for a weighted undirected graph where each node represents a neuron, and the
2638 weight of each edge is the correlation between the neurons represented by the nodes on that
2639 edge. We applied a novel spectral analysis and community detection method (Humphries
2640 et al., 2019) to this network. This clustered the nodes in our ensemble into communities
2641 whose behaviour was more correlated than expected. Our measure of 'expected correlation
2642 strength' were based on a random network that matched our data network's sparsity and total
2643 weight. We compared the detected communities to the anatomical division of our cells using
2644 clustering comparison measures. We then conditioned the binned spike counts on the be-
2645 haviour of the mouse using the principal components of a video of the mouses face recorded
2646 simultaneously with the electrophysiology. We broke the total covariance down into 'spike
2647 count covariance' and 'signal covariance' components conditioning on the behavioural data
2648 and using the law of total covariance. We then repeated our analysis for spike count corre-
2649 lations, and signal correlation. Finally, since our community detection method was only valid
2650 on graphs with non-negative weights, we used different methods for creating a non-negative
2651 graph from our total correlations, and we repeated our analysis on those graphs.

2652 Our first finding was that the time bin width used to bin spike times into spike counts had
2653 a effect on the mean magnitude of the correlations measured. The wider the bin, the higher
2654 the correlations. Not only that, we separated the pairs into positively and negative correlated
2655 pairs, and we found that positively correlated pairs have greater correlation coefficients when
2656 using a wider bin, and negatively correlated pairs have more negative correlation coefficients
2657 when using a wider bin. We also found that the width of the bin used had an effect on the
2658 distribution of the spike counts. For smaller bin widths, the distribution of spike counts was
2659 better represented by a skewed distribution like the Poisson distribution. For wider bins, the
2660 spike counts were better represented by a Gaussian distribution.

2661 Next we investigated the differences between correlations within regions and between

regions. When we divided the pairs according to those two groups, we found that the mean within-region correlations were higher at every bin width, and the difference between the two means grew with increasing bin width. When we split the pairs of cells according to their regions, we found that the mean within-region correlations in any given region were usually greater than the mean between-region correlations for any region pair involving that region. The difference between the mean within-region correlation and the highest between-region correlations involving that region grew smaller with increasing bin width. To investigate this further, we plotted these mean correlations in matrices. Although the mean within-region correlations were usually the highest value in their row or column, as the bin width increased, the mean between-region correlations grew in magnitude relative to the within-region figure.

Next we chose a null network model, and we used the ‘Network Noise Rejection’ process (Humphries et al., 2019) to check for additional structure in our correlation based data network that was not captured by the null model. We found additional structure for any bin width that we used. We also found that the dimensionality of the additional structure reduced as we increased the bin width. This could mean that the processes or representations that take place over longer timescales within the brain also take place in a lower dimensional space.

We applied a community detection method (Humphries, 2011) to the signal correlation networks arising from the network noise rejection. We found that the number of communities detected decreased with increasing bin width. We also noticed that at shorter bin widths, the detected communities were more likely to consist of cells from one brain region only. We investigated this further by using clustering comparison methods to compare the detected communities with the anatomical division of the cells. We found that for short timescales $< 50\text{ms}$ correlated communities tended to exist within anatomical regions, and for longer timescales $> 100\text{ms}$, the correlated communities tended to exist across anatomical regions. This is broadly in agreement with a similar finding for EEG data from humans performing semantic or memory tasks (Stein and Sarnthein, 2000). Von Stein et al. (2000) found that visual processing taking place locally in the visual system was captured in the gamma frequency range (25 – 70Hz), while semantic processing was captured in the beta range (12 – 18Hz), and tasks involving mental imagery and working memory retention were captured in the theta and alpha ranges (4 – 8Hz, and 8 – 12Hz respectively).

We then conditioned our correlation measures on the the mouse’s behaviour. This allowed us to create spike count correlation (or noise correlation) networks, and signal correlation networks (Cohen and Kohn, 2011). We applied our analysis to these networks. For the

spike count correlation networks we found very similar results to the total correlation networks. More communities at smaller bin widths, and communities resembled the anatomical division at smaller bin widths. Given that recent findings show that behaviour can account for correlated spiking in many areas of the brain (Stringer et al., 2019), these results for the spike count correlation show that this correlated behaviour is still processed locally at short timescales, while processes and representations that take more time make use of correlated activity across multiple regions.

For the signal correlations, we still found additional structure in these networks. But we always detected a smaller number of communities. These communities also had no relation to the anatomical division of the cells. This result shows that there are groups of cells across multiple brain regions that are activated similarly by certain behaviours.

All of the networks so far were based on rectified correlation measures, because the network noise rejection and community detection processing is (currently) only valid for networks with non-negative weights. For the final part of our analysis, we tried different ways of transforming our total correlations into non-negative quantities before applying our analysis. First of all we took the absolute value of our correlation measures. Our results were very similar to those for the rectified correlations with the exception that we detected more communities consistently. It is possible that using this method detects both positively and negatively correlated communities.

We also tried reversing the sign of all the correlations, then rectifying the network. We hope that this would allow us to detect the negatively correlated communities. We did detect communities in these networks, but never more than three, and these communities bore no relationship with the anatomical distribution of the cells.

There is a lot of potential for network science applications in computational neuroscience. For example, some pairwise measure other than correlation coefficients could be used as the weights of the graph. The synaptic connections between cells can be isolated in-vitro (Okun et al., 2015). A map of these synaptic connections could be used as the basis for directed graphs. The analysis methods applicable to directed graphs could give insights about the formation of synaptic connections, or the dynamic changes in these connections over time. Other methods of community detection could be used on directed or undirected graphs. We used a ‘hard’ clustering method in our research, that is, each neuron could be a member of one cluster/community only. ‘Fuzzy-clustering’ methods do exist, where each element of the set to be clustered could be a member of more than one cluster (Baadel, Thabtah, and Lu, 2016).

Having spent much time investigating correlated behaviour using coefficients of spike counts, we decided to try another method for capturing correlated behaviour in neuronal ensembles. We used electrophysiological data taken from 5 brain regions of an awake mouse exposed to visual stimuli (Steinmetz et al., 2019). We modelled the number of active neurons in a given brain region as the number of successes in a collection of dependent Bernoulli random variables using the Conway-Maxwell-binomial distribution. To avoid violating the Bernoulli assumption, we binned the spike times using 1ms bins. The Conway-Maxwell-binomial distribution is a two parameter extension of the standard binomial distribution. The extra parameter allows the distribution to capture possible positive or negative association between the Bernoulli trials (Kadane, 2016). This means that we are assuming that all the neurons are dependent in the same way. This is not an accurate assumption, but it allows us model the data in a simple way.

First of all we established that there were changes in the number of active neurons in response to the visual stimuli. This was the case in the hippocampus, thalamus, and primary visual cortex. Each region had its own signature response. We measured the mean and variance of the number of active neurons in a sliding window starting before stimulus onset, and finishing after the end of stimulus presentation.

As well as the Conway-Maxwell-binomial distribution, we also fitted binomial, and beta-binomial distributions to the number of active neurons in a sliding window. We found that the Conway-Maxwell-binomial distribution was the best fit for over 90% of the samples. This means that the COMb distribution is capturing some dependency between the neurons, because the binomial distribution assumes independence. Also the COMb distribution captures this dependence more accurately than the beta-binomial distribution, which does have some capacity for over dispersion.

Next we showed that the Conway-Maxwell-binomial distribution captured the change in association at stimulus onset better than the correlation coefficient. The extremely small bin width artificially shrunk the correlation coefficient to the point where this measurement didn't detect any correlated activity. But the association parameter of the COMb distribution detected some positive association between the neurons at stimulus onset. So, for particularly short time bins, where neurons can be treated as Bernoulli random variables, the Conway-Maxwell-binomial distribution is a good way to capture correlated behaviour. There are other measurements for capturing association to which this distribution should be compared. Cross-correlograms could be used for some measure of synchrony, for example.

2762 Finally, we replicated a famous finding of Churchland et al. (2010) relating to the quenching
2763 of neural variability at stimulus onset, thereby finding a parallel between this reduction
2764 in the Fano factor and a reduction in the association parameter of the COMb distribution.

2765 We showed that computational neuroscientists can make progress by being inventive with
2766 their statistical models. A similar distribution to investigate would be the Conway-Maxwell-
2767 Poisson distribution. This is similar to the standard Poisson distribution, but with an addi-
2768 tional parameter that allows for over- or under- dispersion relative to a Poisson distribution.
2769 This might be ideal for modelling firing rates of individual neurons. Some interaction be-
2770 tween the fitted parameters could capture the association between neurons.

2771 There is one technology that has the potential to take over from both electrophysiology
2772 and calcium imaging. The technique of voltage imaging has become more useful in recent
2773 years. The aim for neuroscience would be to develop a voltage imaging dye or protein that
2774 images the membrane potential of a neuron with enough spatial and temporal resolution to de-
2775 tect action potentials. The voltage imaging dyes that have been developed so far do not have
2776 high enough spatial resolution to single out individual cells in-vivo using staining (Bando
2777 et al., 2019). But, genetically encoded voltage indicators have been developed that have high
2778 enough resolution to indicate individual spikes and subthreshold activity from small numbers
2779 of cells in the striatum, hippocampus, and cortex of awake behaving mice (Piatkevich et al.,
2780 2019). These indicators have the potential to take over from calcium imaging, and if imaging
2781 deep within the brain becomes possible, electrophysiology could also be replaced. This is
2782 speculation, but the potential is there.

²⁷⁸³ Bibliography

- 2784 Allen, William E. et al. (2019). “Thirst regulates motivated behavior through modulation of
2785 brainwide neural population dynamics”. In: *Science* 364.6437.
- 2786 Baadel, S., F. Thabtah, and J. Lu (2016). “Overlapping clustering: A review”. In: *2016 SAI
2787 Computing Conference (SAI)*, pp. 233–237.
- 2788 Baldassano, Christopher et al. (2017). “Discovering Event Structure in Continuous Narrative
2789 Perception and Memory”. In: *Neuron* 95.3, 709 –721.e5.
- 2790 Bando, Yuki et al. (2019). “Genetic voltage indicators”. In: *BMC Biology* 17.1, p. 71.
- 2791 Bartol, Thomas M. et al. (2015). “Computational reconstitution of spine calcium transients
2792 from individual proteins”. In: *Frontiers in Synaptic Neuroscience* 7, p. 17.
- 2793 Berens, Philipp et al. (May 2018). “Community-based benchmarking improves spike rate in-
2794 ference from two-photon calcium imaging data”. In: *PLOS Computational Biology* 14.5,
2795 pp. 1–13.
- 2796 Bezanson, Jeff et al. (Sept. 2012). “Julia: A Fast Dynamic Language for Technical Comput-
2797 ing”. In: *MIT*.
- 2798 Brini, Marisa et al. (2014). “Neuronal calcium signaling: function and dysfunction”. In: *Cel-
2799 lular and Molecular Life Sciences* 71.15, pp. 2787–2814.
- 2800 Buccino, Alessio P. et al. (2019). “SpikeInterface, a unified framework for spike sorting”. In:
2801 *bioRxiv*.
- 2802 Chen, Tsai-Wen et al. (July 2013). “Ultrasensitive fluorescent proteins for imaging neuronal
2803 activity”. In: *Nature* 499, 295–300.
- 2804 Churchland, Mark M. et al. (2010). “Stimulus onset quenches neural variability: a widespread
2805 cortical phenomenon”. eng. In: *Nature neuroscience* 13.3. 20173745[pmid], pp. 369–378.
- 2806 Cohen, Marlene R. and Adam Kohn (2011). “Measuring and interpreting neuronal corre-
2807 lations”. In: *Nature Neuroscience* 14.7, pp. 811–819.
- 2808 Cohen, Marlene R. and John H. R. Maunsell (2009). “Attention improves performance pri-
2809 marily by reducing interneuronal correlations”. eng. In: *Nature neuroscience* 12.12. 19915566[pmid],
2810 pp. 1594–1600.

- 2811 Dana, Hod et al. (Sept. 2014). “Thy1-GCaMP6 Transgenic Mice for Neuronal Population
2812 Imaging In Vivo”. In: *PloS one* 9, e108697.
- 2813 deCharms, R. Christopher and Michael M. Merzenich (1996). “Primary cortical represen-
2814 tation of sounds by the coordination of action-potential timing”. In: *Nature* 381.6583,
2815 pp. 610–613.
- 2816 Deneux, Thomas et al. (July 2016). “Accurate spike estimation from noisy calcium signals
2817 for ultrafast three-dimensional imaging of large neuronal populations in vivo”. In: *Nature*
2818 *Communications* 7.1.
- 2819 Dombeck, Daniel A. et al. (2010). “Functional imaging of hippocampal place cells at cellular
2820 resolution during virtual navigation”. In: *Nature Neuroscience* 13.11, pp. 1433–1440.
- 2821 Éltes, Tímea et al. (2019). “Improved spike inference accuracy by estimating the peak am-
2822 plitude of unitary [Ca₂₊] transients in weakly GCaMP6f-expressing hippocampal pyra-
2823 midal cells”. In: *The Journal of Physiology* 597.11, pp. 2925–2947.
- 2824 Faas, Guido C. et al. (2011). “Calmodulin as a direct detector of Ca₂₊ signals”. In: *Nature*
2825 *Neuroscience* 14.3, pp. 301–304.
- 2826 Fiala, John C. and Kristen M. Harris (1999). *Dendrite Structure*.
- 2827 Flach, Boris (Sept. 2013). “A Class of Random Fields on Complete Graphs with Tractable
2828 Partition Function”. In: *IEEE transactions on pattern analysis and machine intelligence*
2829 35, pp. 2304–6.
- 2830 Forney, G. D. (1973). “The viterbi algorithm”. In: *Proceedings of the IEEE* 61.3, pp. 268–
2831 278.
- 2832 Fosdick, Bailey et al. (Aug. 2016). “Configuring Random Graph Models with Fixed Degree
2833 Sequences”. In: *SIAM Review* 60.
- 2834 Friedrich, Johannes and Liam Paninski (2016). “Fast Active Set Methods for Online Spike In-
2835 ference from Calcium Imaging”. In: *Advances in Neural Information Processing Systems*
2836 29. Ed. by D. D. Lee et al. Curran Associates, Inc., pp. 1984–1992.
- 2837 Ganmor, Elad, Ronen Segev, and Elad Schneidman (2011). “Sparse low-order interaction
2838 network underlies a highly correlated and learnable neural population code”. In: *Pro-
2839 ceedings of the National Academy of Sciences* 108.23, pp. 9679–9684.
- 2840 Girard, P., J. M. Hupé, and J. Bullier (2001). “Feedforward and Feedback Connections Be-
2841 tween Areas V1 and V2 of the Monkey Have Similar Rapid Conduction Velocities”. In:
2842 *Journal of Neurophysiology* 85.3. PMID: 11248002, pp. 1328–1331.
- 2843 Greenberg, David et al. (Nov. 2018). “Accurate action potential inference from a calcium
2844 sensor protein through biophysical modeling:” in:

Bibliography

- 2845 Gründemann, Jan et al. (2019). “Amygdala ensembles encode behavioral states”. In: *Science*
2846 364.6437.
- 2847 Hodgkin, A. L. and A. F. Huxley (1939). “Action Potentials Recorded from Inside a Nerve
2848 Fibre”. In: *Nature* 144.3651, pp. 710–711.
- 2849 Humphries, Mark et al. (Jan. 2019). “Spectral rejection for testing hypotheses of structure in
2850 networks”. In:
- 2851 Humphries, Mark D. (2011). “Spike-Train Communities: Finding Groups of Similar Spike
2852 Trains”. In: *Journal of Neuroscience* 31.6, pp. 2321–2336.
- 2853 Jun, James J. et al. (2017). “Fully integrated silicon probes for high-density recording of
2854 neural activity”. In: *Nature* 551.7679, pp. 232–236.
- 2855 Kadane, Joseph B. (June 2016). “Sums of Possibly Associated Bernoulli Variables: The
2856 Conway–Maxwell-Binomial Distribution”. In: *Bayesian Anal.* 11.2, pp. 403–420.
- 2857 Kilhoffer, Marie-Claude et al. (Sept. 1992). “Use of Engineered Proteins With Internal Tryp-
2858 tophean Reporter Groups and Perturbation Techniques to Probe the Mechanism of Ligand-
2859 Protein Interactions: Investigation of the Mechanism of Calcium Binding to Calmodulin”.
2860 In: *Biochemistry* 31.34, pp. 8098–8106.
- 2861 Koch, Christoff (1999). *Biophysics of Computation: Information Processing in Single Neu-*
2862 *rons*. Oxford University Press.
- 2863 Kolaczyk, Eric and Haiying Huang (Sept. 2010). “Multiscale Statistical Models for Hierar-
2864 chical Spatial Aggregation”. In: *Geographical Analysis* 33, pp. 95 –118.
- 2865 Lee, Suk-HO et al. (July 2000). “Differences in Ca²⁺ buffering properties between excitatory
2866 and inhibitory hippocampal neurons from the rat”. In: *The Journal of Physiology* 525.
- 2867 Litwin-Kumar, Ashok, Maurice Chacron, and Brent Doiron (Sept. 2012). “The Spatial Struc-
2868 ture of Stimuli Shapes the Timescale of Correlations in Population Spiking Activity”. In:
2869 *PLoS computational biology* 8, e1002667.
- 2870 Maravall, M et al. (May 2000). “Estimating intracellular calcium concentrations and buffer-
2871 ing without wavelength ratioing”. In: *Biophysical Journal* 78.5, pp. 2655–2667.
- 2872 Maugis, Pa (Jan. 2014). “Event Conditional Correlation: Or How Non-Linear Linear Depen-
2873 dence Can Be”. In:
- 2874 Meilă, Marina (2007). “Comparing clusterings—an information based distance”. In: *Journal*
2875 *of Multivariate Analysis* 98.5, pp. 873 –895.
- 2876 Murray, John D. et al. (2014). “A hierarchy of intrinsic timescales across primate cortex”. In:
2877 *Nature Neuroscience* 17.12, pp. 1661–1663.

- 2878 Neher, E. and G. J. Augustine (1992). “Calcium gradients and buffers in bovine chromaffin
2879 cells”. eng. In: *The Journal of physiology* 450. 1331424[pmid], pp. 273–301.
- 2880 O’Donnell, Cian et al. (Jan. 2017). “The population tracking model: a simple, scalable statis-
2881 tical model for neural population data”. English. In: *Neural Computation* 29.1, pp. 50–
2882 93.
- 2883 Okun, Michael et al. (2015). “Diverse coupling of neurons to populations in sensory cortex”.
2884 In: *Nature* 521.7553, pp. 511–515.
- 2885 Ouzounov, Dimitre G. et al. (2017). “In vivo three-photon imaging of activity of GCaMP6-
2886 labeled neurons deep in intact mouse brain”. eng. In: *Nature methods* 14.4. 28218900[pmid],
2887 pp. 388–390.
- 2888 Patterson, Carlyn A. et al. (2014). “Similar adaptation effects in primary visual cortex and
2889 area MT of the macaque monkey under matched stimulus conditions”. In: *Journal of
2890 Neurophysiology* 111.6. PMID: 24371295, pp. 1203–1213.
- 2891 Peron, Simon P. et al. (2015). “A Cellular Resolution Map of Barrel Cortex Activity during
2892 Tactile Behavior”. In: *Neuron* 86.3, pp. 783–799.
- 2893 Piatkevich, Kiryl D. et al. (2019). “Population imaging of neural activity in awake behaving
2894 mice”. In: *Nature* 574.7778, pp. 413–417.
- 2895 Pnevmatikakis, E.A. et al. (Jan. 2016). “Simultaneous Denoising, Deconvolution, and Demix-
2896 ing of Calcium Imaging Data”. In: *Neuron* 89.2, pp. 285–299.
- 2897 Pnevmatikakis, Eftychios et al. (Nov. 2013). “Bayesian spike inference from calcium imaging
2898 data”. In: *Conference Record - Asilomar Conference on Signals, Systems and Computers*.
- 2899 Pnevmatikakis, Eftychios et al. (Sept. 2014). “A structured matrix factorization framework
2900 for large scale calcium imaging data analysis”. In:
- 2901 Schneidman, Elad et al. (2006). “Weak pairwise correlations imply strongly correlated net-
2902 work states in a neural population”. eng. In: *Nature* 440.7087. 16625187[pmid], pp. 1007–
2903 1012.
- 2904 Shannon, C. E. (1948). “A Mathematical Theory of Communication”. In: *Bell System Tech-
2905 nical Journal* 27.3, pp. 379–423.
- 2906 Shimazaki, Hideaki et al. (2012). “State-space analysis of time-varying higher-order spike
2907 correlation for multiple neural spike train data”. eng. In: *PLoS computational biology*
2908 8.3. 22412358[pmid], e1002385–e1002385.
- 2909 Staude, Benjamin, Sonja Grün, and Stefan Rotter (2010). “Higher-Order Correlations and
2910 Cumulants”. In: *Analysis of Parallel Spike Trains*. Ed. by Sonja Grün and Stefan Rotter.
2911 Boston, MA: Springer US, pp. 253–280.

Bibliography

- 2912 Stein, Astrid von and Johannes Sarnthein (2000). “Different frequencies for different scales
2913 of cortical integration: from local gamma to long range alpha/theta synchronization”. In:
2914 *International Journal of Psychophysiology* 38.3, pp. 301 –313.
- 2915 Steinmetz, Nick, Matteo Carandini, and Kenneth D. Harris (2019). ”*Single Phase3*” and
2916 ”*Dual Phase3*” *Neuropixels Datasets*.
- 2917 Steinmetz, Nick et al. (Mar. 2019). “Eight-probe Neuropixels recordings during spontaneous
2918 behaviors”. In:
- 2919 Stevenson, Ian H. and Konrad P. Kording (2011). “How advances in neural recording affect
2920 data analysis”. In: *Nature Neuroscience* 14.2, pp. 139–142.
- 2921 Stringer, Carsen et al. (2019). “Spontaneous behaviors drive multidimensional, brainwide
2922 activity”. In: *Science* 364.6437.
- 2923 Tada, Mayumi et al. (2014). “A highly sensitive fluorescent indicator dye for calcium imaging
2924 of neural activity in vitro and in vivo”. In: *European Journal of Neuroscience* 39.11,
2925 pp. 1720–1728.
- 2926 Tian, Lin et al. (2009). “Imaging neural activity in worms, flies and mice with improved
2927 GCaMP calcium indicators”. eng. In: *Nature methods* 6.12. 19898485[pmid], pp. 875–
2928 881.
- 2929 Tkačik, Gašper et al. (2014). “Searching for collective behavior in a large network of sen-
2930 sory neurons”. eng. In: *PLoS computational biology* 10.1. 24391485[pmid], e1003408–
2931 e1003408.
- 2932 Treves, Alessandro and Stefano Panzeri (1995). “The Upward Bias in Measures of Informa-
2933 tion Derived from Limited Data Samples”. In: *Neural Computation* 7.2, pp. 399–407.
- 2934 Vinh, Nguyen Xuan, Julien Epps, and James Bailey (Dec. 2010). “Information Theoretic
2935 Measures for Clusterings Comparison: Variants, Properties, Normalization and Correc-
2936 tion for Chance”. In: *J. Mach. Learn. Res.* 11, 2837–2854.
- 2937 Vogelstein, Joshua T. et al. (Oct. 2010). “Fast nonnegative deconvolution for spike train infer-
2938 ence from population calcium imaging”. In: *Journal of neurophysiology* 104.6, 295–300.
- 2939 Wierzyński, ”Casimir M. et al. (2009). ””State-Dependent Spike-Timing Relationships be-
2940 tween Hippocampal and Prefrontal Circuits during Sleep””. In: ”*Neuron*” ”61”. ”4”, ”587
2941 –596”.
- 2942 Zariwala, Hatim A. et al. (2012). “A Cre-dependent GCaMP3 reporter mouse for neuronal
2943 imaging in vivo”. eng. In: *The Journal of neuroscience : the official journal of the Society
2944 for Neuroscience* 32.9. 22378886[pmid], pp. 3131–3141.

- 2945 Zou, Hui and Trevor Hastie (2005). “Regularization and variable selection via the Elastic
2946 Net”. In: *Journal of the Royal Statistical Society, Series B* 67, pp. 301–320.