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Detecting connectivity changes in neuronal networks

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HIGHLIGHTS

- The Cox method can be used to estimate connectivity in networks of neurons.
- We evaluate sensitivity and specificity of the method for general computational neural models.
- A variation of the method is developed to track significant changes in network connectivity.
- The method is demonstrated on a network of cultured mammalian spinal cord cells with MEA measurements.

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ABSTRACT

We develop a method from semiparametric statistics (Cox, 1972) for the purpose of tracking links and connection strengths over time in a neuronal network from spike train data. We consider application of the method as implemented in Masud and Borisyuk (2011), and evaluate its use on data generated independently of the Cox model hypothesis, in particular from a spiking model of Izhikevich in four different dynamical regimes. Then, we show how the Cox method can be used to determine statistically significant changes in network connectivity over time. Our methodology is demonstrated using spike trains from multi-electrode array measurements of networks of cultured mammalian spinal cord cells.

1. Introduction

Studying the dynamics of a neuronal network in the laboratory requires a reliable method for determining network topology and connection strengths. Ideally, such a method would use readily available data types, such as spike train recordings. The method should be robust across various neuron models and heterogeneity of neurons and connection modalities. Because we wish to measure changes on the finest possible time scale, the method should be very sensitive with low data requirements. Finally, the method should have high specificity, meaning it should not return many false positives.

Methods for detecting network links from complex time series have been the focus of several recent studies. The analysis of interactions between nonlinear processes was pursued in Dahlhaus et al. (1997) and Rosenblum and Pikovsky (2001), and ideas from compressed sensing were introduced in Napoletani and Sauer (2008). The concept of Granger causality (Granger, 1969) has been exploited by calculating partial directed coherence in Sommerlade et al. (2012).

However, in many experimental situations, accurate recording of time series may be infeasible. When using spike sorting from multi-electrode array (MEA) measurements, for example, the series of spike times may represent the effective totality of available information. Methods for detecting dynamical influences from spike trains begin with cross-correlation, which is limited to linear aspects of connectivity. Methods that involve higher moments, including coherence, joint densities and cumulant spectra were pursued in Brillinger (1975), Aertsen and Gerstein (1985) and Aertsen et al. (1989). An information theory approach was proposed in Garofalo et al. (2009). Maximum likelihood methods for neuronal interactions from spike trains (Chornoboy et al., 1988; Okatan et al., 2005; Brillinger, 1992; Stevenson et al., 2008, 2009) can more naturally take nonlinear effects into account.

Other researchers have developed methods for assessing similarity and correlations in spike trains (Sacerdote et al., 2012; Lyttle and Fellous, 2011). Interesting recent approaches for the inference of functional connectivity between neurons in a network using nonparametric statistics include Ostojic et al. (2009) and Eldawlatly et al. (2008).

Masud and Borisyuk (2011) develop a hazard model for neuronal interactions and apply the Cox method to find connections. Hazard models comprise a class of survival models, in which the quantity of interest, in this case the connection strength in a

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network, is associated with the length of time before an event, such as a spike. In a proportional hazard model, the association is assumed proportional. The Cox method (Cox, 1972; Borisyuk et al., 1985) is a semiparametric technique that does not require specification of a neuron model, but only a model for interaction between neurons, and allows a single technique to handle mixed or unknown neuron models. Moreover, because the interaction between neurons is modeled as directional, the Cox method detects causality. Since the Cox method estimates the parameters for all possible sources simultaneously for each target neuron, causality errors such as common cause and transitive connections are dramatically reduced.

A key strength of the Cox method is that it provides an estimate for the covariance matrix of the estimated parameters, which leads to a *statistical* test for connectivity. This is of great importance, as many tests for connectivity only give relative indicators of connectivity, and an *ad hoc* cut-off level must be chosen. One important contribution that we make in this article is to carry out a verification of the Cox method statistics and evaluation of sensitivity and data requirements of the test. We make these analyses both within the context of the proportional hazard model assumptions and for some more general neural models, which do not satisfy the hazard model assumptions.

A second goal of this article is to address the problem of network nonstationarity. Previous applications of the Cox method were focused on finding static network structure. For many purposes, it may be even more important to study changes in the network connectivity, for example when evaluating the effects of perturbing the system electrically or pharmacologically. Static applications of the Cox method cannot be compared statistically across time segments, due to lack of control on false negatives, a weakness that is not well appreciated. To overcome this challenge, we introduce a new statistical test within the Cox method framework that can detect nonstationarity of network connections from multivariate spike trains. Design of experiments on structure and function of neural networks in general will be contingent on statistical tests of stationarity, such as the one developed here.

In Section 2, we carry out testing in a general setting to verify that the specificity of the test is at least equal to the desired confidence level. In addition, we add implementation details that increase the computational robustness of the method. We also point out that specificity only controls errors at the individual connection level, whereas it is natural to apply the Cox method to find all the connections in a network. We show that such repeated testing requires adjusting the testing procedure carefully to control the Familywise Error Rate (FWER).

The success of the Cox method will be determined by its robustness to the assumptions of the hazard model. These assumptions may not be satisfied in actual neuron interactions. In Section 3, we investigate the performance of the method on model data with varying faithfulness to the underlying hazard model assumptions, to test its applicability to real data. In particular we use the Izhikevich model, which exhibits a diverse collection of observed neuronal network behaviors, including bursting and chattering modes, to produce a wide variety of spike trains, that are not explicitly connected to the hazard model assumption. Surprisingly, we find that the method is very effective on data generated by Izhikevich neurons, which gives us confidence that it can work in laboratory applications.

Finally, the real-world significance of the Cox method hinges on its ability to detect changes in a network using spike trains. Network changes may be structural, involving the creation or elimination of connections, or changes may occur in the strength of connections. In Section 4, we show how the Cox method can be used to detect these types of changes using data sets recorded from spontaneously active *in vitro* cultures taken from embryonic mice and plated on micro-electrode arrays. Section 5 is a discussion of results and future outlook.

2. Semiparametric statistics: the Cox method

The Cox method, as illustrated by Masud and Borisyuk (2011), is a statistical test for determining connectivity in a neuronal network. As a semiparametric test, it does not require models for the individual neurons but does require a model for neuron interactions, which is given by the proportional hazard model. When spikes are generated in a manner consistent with this assumption, the Cox method is a powerful test for direct connectivity. As we will show in Section 3.2, the Cox method can also be successfully applied to complex models such as the Izhikevich model which are designed to replicate observed neuronal network behaviors, and whose consistency with the proportional hazard model assumption is questionable.

For each target neuron the Cox method simultaneously estimates a set of connection parameters β_i that quantify the strength of influence of the ith potential source neuron. In this section we describe the model assumptions on the β_i, and explain the procedure for maximum likelihood estimation of these parameters. Essentially, the spike times of each neuron are assumed to follow a renewal process, with the influence of the source neurons given by the aforementioned proportional hazard model.

2.1. Proportional hazard model

To describe the model, let $\varphi_A(t)$ be the intrinsic hazard function for neuron A, denoting the expected spike rate at time t among all interspike intervals X of the spike train of A of length t or more:

$$\varphi_A(t) = \lim_{\Delta t \to 0} \frac{\Pr(t \le X < t + \Delta t | t \le X)}{\Delta t},$$

where Pr denotes probability. This renewal process is modulated with possible influence from the n neurons in the network by defining the modified hazard function

$$\varphi(t) = \varphi_A(U_A(t)) \exp\left\{ \sum_{i=1}^{n} \beta_i Z_{B_i}(t) \right\} \tag{1}$$

where $U_A(t)$ is the time since the previous spike of A, β_i is the connection strength parameter of neuron B_i to A, and Z_{B_i} is an influence function describing how B_i affects A.

The choice of $Z_B(t)$ corresponds to a model of neuron interactions, and Masud and Borisyuk (2011) propose several possibilities, the most general of which can be written

$$Z_B(t) = \sum_{j=1}^{k} \frac{g_m}{\tau_s - \tau_r} (e^{-U_B^j(t-\Delta)/\tau_s} - e^{-U_B^j(t-\Delta)/\tau_r}) \tag{2}$$

where g_m is a normalization constant, Δ corresponds to the propagation delay time, τ_s and τ_r are the characteristic times of decay and rise of postsynaptic potential, and $U_B^j(t-\Delta)$ is the time of the jth spike preceding time $t-\Delta$. These extra parameters can be estimated before the analysis. Only the general shape of the influence function is relevant to achieve good results. This is crucial because the true influence function will not be known in laboratory applications.

The Cox method parameters $\beta = \{\beta_i\}$ are estimated simultaneously by maximizing the log likelihood $L(\beta)$ of the modified hazard

function (1). Assuming n possible input neurons to the target and m spike times, the log likelihood is

$$L(\beta) = \sum_{i=1}^{n}\sum_{k=1}^{m}\beta_i Z_{B_i}(t_{kk}) - \sum_{k=1}^{m}\log\left(\sum_{l=k}^{m}\exp\sum_{i=1}^{n}\beta_i Z_{B_i}(t_{lk})\right) \quad (3)$$

where t_{lk} are a discrete set of times given by $t_{lk} = t_l + \delta_k$ where t_l is the left hand endpoint of the lth smallest interspike interval of the target neuron and δ_k is the length of the kth smallest interspike interval. Note that l is always greater than or equal to k so that t_{lk} always lies inside the lth smallest interspike interval, and t_{kk} is simply the right hand end-point of the kth smallest interspike interval.

The maximum likelihood estimate $\hat{\beta}$ of β is found by maximizing (3). Multivariate Newton's method is applied to solve for the zero of the gradient $\nabla L(\beta)$. We found numerical stability to be greatly enhanced in our application by applying a regularized Newton's iteration. Let $H(\beta^k)$ be the Jacobian of the gradient, or Hessian, at the kth iteration step. The formula for the Newton iteration is

$$\beta^{k+1} = \beta^k + H(\beta^k)^{-1}\nabla L(\beta^k).$$

To regularize the iteration, we replace this with the iteration of Levenberg–Marquardt,

$$\beta^{k+1} = \beta^k + ((H^k)^T H^k + \lambda\,\mathrm{diag}((H^k)^T H^k))^{-1}(H^k)^T \nabla L(\beta^k)$$

where $H^k \equiv H(\beta^k)$. The regularization parameter λ is initialized to 1 and is successively halved if there is improvement in convergence, i.e. $\|\nabla L(\beta_{k+1})\| < \|\nabla L(\beta_k)\|$, and doubled otherwise. The limit of this iteration is the estimated connection vector $\hat{\beta}$.

2.2. Confidence intervals and multiple hypothesis testing

One important use of the estimates of the connection strength vector β is to determine whether a link exists from the ith neuron to the target neuron, i.e. whether $\beta_i \neq 0$ for a given i. The advantage of the Cox method is that it is a statistical test. Namely, the inverse of the Hessian, $H(\hat{\beta})^{-1}$, provides an estimate of the covariance matrix of the best estimate $\hat{\beta}$. Let h_{ii} be the ith diagonal entry of $H(\hat{\beta})^{-1}$. The standard confidence interval for $\hat{\beta}_i$ with confidence level γ_0 is

$$[\hat{\beta}_i - \kappa_{(1-\gamma_0)/2}\sqrt{h_{ii}}, \hat{\beta}_i + \kappa_{(1-\gamma_0)/2}\sqrt{h_{ii}}]$$

where $\kappa_{(1-\gamma_0)/2}$ is the $(1-\gamma_0)/2$ quantile of the normal distribution. Thus if zero does not lie in the standard confidence interval for a given $\hat{\beta}_i$ then we conclude that the ith spike train does influence the target spike train.

This technique is equivalent to a 1-dimensional Wald test (see, for example, Fox, 1997) of the null hypothesis $H_{0,i} : \beta_i = 0$. At this point we want to repeat this test for each potential source to determine which sources are affecting the target. However, the β_i were determined collectively, not individually, so there may be correlations between these repeated tests. This is a subtle point, but due to the power and convenience of the Cox method, we feel that it is worth explaining carefully. One may be tempted to use a higher order Wald test, however we will show that this is invalid for determining individual connections. Instead, the correct method is to apply the 1-dimensional Wald test repeatedly but with a carefully modified confidence level. This approach will control the Family-wise Error Rate (FWER), which is the probability of one or more false positives.

First, consider the null hypothesis $H_0 : \beta = 0$. It says that all of the connection strengths are zero, meaning that none of the source neurons affects the target. It is valid to test this hypothesis by applying a multi-dimensional Wald test, computing $\hat{\beta}^T H(\hat{\beta})^{-1}\hat{\beta}$ and comparing to the Chi-squared statistic with confidence level γ and with n degrees of freedom. However, rejecting H_0 merely tells us that not all connections are zero; it gives us no information about the individual connections. In other words, this test would at most tell us that *some* β_i is nonzero, when in fact we are interested in which specific one(s) are nonzero. While this may be useful in some other contexts, the power of the Cox method is in determining individual connections.

The equation of the multidimensional Wald test, $\hat{\beta}^T H(\hat{\beta})^{-1}\hat{\beta} < \chi^2_{1-\gamma,n}$, defines an ellipsoidal region in $\mathbb{R}^n$ and it is tempting, but ultimately invalid, to attempt to use this ellipsoid region to draw conclusions about individual sources. For example, Masud and Borisyuk (2011) suggest projecting this region onto the coordinate axes to test individual connections; however, there is no proper statistical interpretation for such a procedure. Actually, we will show next that such a projection is equivalent to a modified confidence interval where $\kappa_{(1-\gamma_0)/2}$ is replaced by $\sqrt{\chi^2_{(1-\gamma,n)}}$. Without a probabilistic justification for such a modification of the confidence interval, such a procedure is unwarranted.

Let Σ be the covariance matrix of a mean zero n-dimensional Gaussian. Then the n-dimensional confidence region is given by the ellipsoid defined by $x^T\Sigma^{-1}x < \chi^2_{1-\gamma,n}$ where $\chi^2_{1-\gamma,n}$ is the Chi-squared statistic for confidence level γ with n degrees of freedom. The following Lemma shows that projecting this confidence region onto the ith coordinate axis gives the interval

$$[-\sqrt{\chi^2_{1-\gamma,n}}\sqrt{\Sigma_{ii}}, \sqrt{\chi^2_{1-\gamma,n}}\sqrt{\Sigma_{ii}}]$$

where Σ_{ii} is the ith diagonal element of the covariance matrix, the variance of the ith variable.

Lemma. *Consider the ellipsoid* $E = \{x : x^T A x = c^2\}$ *where* $B = A^{-1}$ *is a symmetric positive definite matrix. Then* $\max_{x\in E}\{x_i\} = c\sqrt{b_{ii}}$ *and* $\min_{x\in E}\{x_i\} = -c\sqrt{b_{ii}}$.

Proof. By relabeling the axes, we may assume $i = n$. Let $A = R^T R$ be the Cholesky factorization, where R is nonsingular and upper triangular. Define $y = Rx$ and $x = Sy$, where $S = R^{-1}$ is also upper triangular so that so $x_n = s_{nn}y_n$. Thus x_n is maximized when y_n is maximized. Moreover, since $y^T y = x^T A x = c^2$, y_n is maximized when $y = (0, 0, \ldots, 0, c)$. Finally, note that $B = A^{-1} = SS^T$, which is the product of an upper triangular matrix with a lower triangular matrix. This implies $b_{nn} = s^2_{nn}$ therefore $\max_{x\in E}\{x_n\} = s_{nn}c = \sqrt{b_{nn}}c$ and similarly $\min_{x\in E}\{x_n\} = -\sqrt{b_{nn}}c$. □

Lemma shows that projecting the high-dimensional confidence region onto a coordinate axis results in an arbitrary modification of the confidence interval. Note that the higher order tests are perfectly valid for testing joint hypotheses. However, we are usually interested in testing the individual hypotheses separately. This creates a problem when many individual hypotheses are tested: corrections must be made for multiple hypothesis testing.

Assume for example that our network contains 10 neurons so that, omitting self-connections, there are 90 potential neuron–neuron connections. If there are 20 true connections and we apply our test with 95% confidence *per connection* and correctly identify all 20 connections, we would expect on average one false positive (5% of the 20 connections). Since our eventual goal is to detect changes in a network, we will run the Cox method repeatedly on different time segments. If we have 100 time segments with 95% confidence per connection, then we can expect 100 false positives, which will significantly distort our results. Instead, we would like to implement the Cox test with a 95% confidence *per network*, i.e. a 95% probability that no false positives exist in the network.

Thus it is important to use a modified confidence level to control the Familywise Error Rate (Shaffer, 1995).

The Familywise Error Rate (FWER) is the probability of at least one false positive occurring in a set of tests. An easy method to control the FWER, which does not even require the tests to be independent, is the Bonferroni correction (Benjamini and Hochberg, 1995), which corrects the error rate by dividing by the number of tests. Note that the Bonferroni correction (unlike other methods of controlling FWER) does not require the individual tests to be independent. If the desired FWER for a set of n tests is γ, the individual tests should be conducted at confidence level

$$\gamma_0 = \frac{1-(1-\gamma)}{n}.$$

For example, if we want a 95% confidence level *per network*, where there are 90 candidate connections, then we should test with a 99.94% confidence level *per connection*. Fortunately, as we see in the next section, the Cox method is efficient enough to be able to meet this goal with a reasonable amount of spike train data.

3. Computational network models and results

In this section we will introduce two network models and evaluate the Cox method on each. The first model is a proportional hazard model, on which the Cox method is based. The second model is a network of Izhikevich neurons, which is capable of replicating multiple realistic network behaviors. Note that there is no *a priori* reason to expect the Izhikevich model to behave as a proportional hazard model, and it was chosen only because it gives the best tradeoff of computational efficiency and realistic network behavior as found in experiments. Before introducing these models, we first explain some considerations which apply to both.

Both of the computational models incorporate a matrix S representing the graph of true direct connection strengths β_{ij} from neuron i to neuron j. In each simulation, to match the experimental data in the section to follow, we used randomly generated networks of 10 neurons with 10 direct connections out of 90 possible (neurons could not connect to themselves). Connections can be excitatory or inhibitory with strengths that vary randomly around a prescribed average strength.

Each simulation was assigned an average connection strength and minimum number of spikes. Given these assignments, we randomly chose 10 of the potential 90 connections and generated a specific network that was assigned the prescribed connection strength. The simulated network was run until each neuron spiked the specified number of times. The spike trains were collected and analyzed by the Cox method, using only the prescribed number of spikes per target. For each average connection strength and prescribed number of spikes, the simulation was repeated 10 times with different randomly chosen connections.

We calculated the sensitivity and specificity of the two computational models, averaged over the 10 repeated realizations. The sensitivity is the number of correctly identified direct connections (true positives) divided by the total number of direct connections (10 in our case). The specificity counts true negatives as a percentage of all non-connections. The specificity should be the chosen confidence level: however, because the error distribution is only asymptotically normal, the Cox method is an asymptotic test. In the limit as the number of spikes per target becomes large we expect the specificity to approach the desired confidence level.

3.1. Proportional hazard model

The proportional hazard model is the archetype of a semiparametric model, and is exactly the assumption of our application of the Cox method. The spiking of each neuron is assumed to occur according to a renewal process, a generalization of the Poisson process where the waiting time is uncertain. For a network of $n+1$ neurons, the model is made up of $n+1$ renewal processes, one for each target neuron, which are arbitrary but interact in a prescribed manner. There are n^2+n parameters describing a directional strength of influence in the network.

Each renewal process has a hazard function, defined in terms of the probability density function as

$$\varphi(t) = \lim_{\Delta t \to 0} \frac{\Pr(t \le X < t+\Delta t | t \le X)}{\Delta t} \tag{4}$$

where X is the inter-spike interval. The hazard function can be written as in (1) and in the case of a neuronal network with $n+1$ neurons, the hazard function takes the form,

$$\varphi_j(t) = \varphi_{A_j}(U_{A_j}(t)) \exp\left\{ \sum_{i=1, i\neq j}^{n+1} \beta_{ij} Z_{B_i}(t) \right\}$$

where $(\beta_{1j}, \ldots, \beta_{n+1\,j})$ is the jth row of the connectivity matrix S. The influence function $Z_{B_i}(t)$ was of form (2) with parameters $\tau_S = 10$, $\tau_R = 0.1$, and $\delta = 0$.

This process was simulated by taking a fine discretization of time by $\Delta t = 10^{-4}$ s, evaluating each hazard function at each time step and assigning neuron spiking times according to the probability given by formula (4). Using the data from this simple simulation we were able to accurately recover the weights β_{ij} using the Cox method. This replicates the results of Masud and Borisyuk (2011), however, we were additionally interested in confirming that the correct statistics were achieved.

For each number of spikes per target, we repeated the simulation 10 times with randomly selected networks, each time producing 90 candidate connections. The results of the simulations are shown in Fig. 1. Fig. 1(a) shows that correct identification of the network connections increases with the number of spikes used, and asymptotically converges to 100% sensitivity. Secondly, of the 90 candidate connections, the number of correctly identified non-connections was divided by the total number of non-connections. This ratio is the specificity of the test, and should be at least the confidence level. The specificity was averaged across the 10 simulations at the 95% and 99% confidence levels and the results are shown in Fig. 1(b) with error bars indicating standard error. The results confirm that the test achieves the correct asymptotic statistics at both levels with as few as 256 spikes; compare results to the dotted lines indicating the specified confidence levels.

These results are as expected by construction of the Cox method on the proportional hazard model. Next we turn to a more representative and realistic neuron model.

3.2. Izhikevich model

The Izhikevich model (Izhikevich, 2003, 2006) is a mathematical model capable of generating a network of spiking neurons. It is able to reproduce the general behavior of more biophysically accurate Hodgkin–Huxley-type models, but it is simpler and thus more computationally efficient. The Izhikevich model is defined by a system of two differential equations of the following form:

$$\dot{v} = 0.04v^2 + 5v + 140 - u + I \tag{5}$$

$$\dot{u} = a(bv - u)$$

where v represents the membrane potential of the neuron and u represents a membrane recovery variable. Once a neuron spike

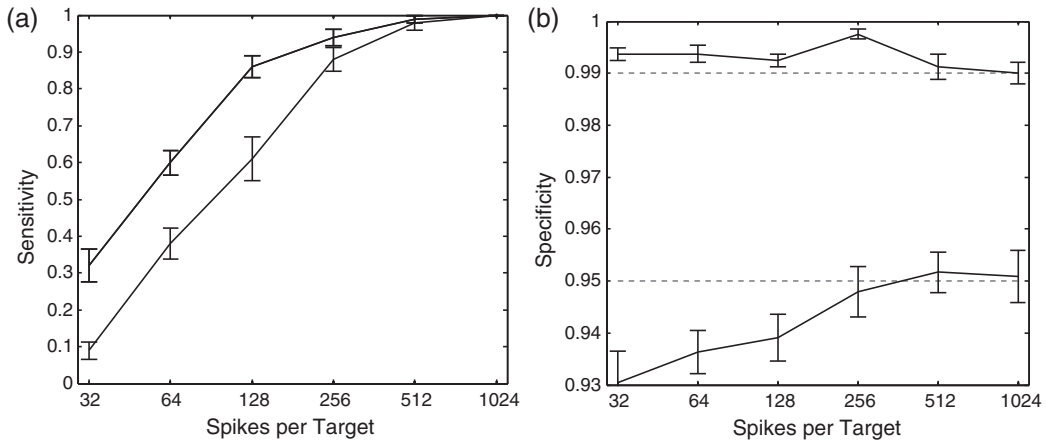

Fig. 1. Sensitivity and specificity for proportional hazard model. (a) Sensitivity (number of correctly identified connections divided by number of connections) as a function of number of spikes per target neuron, plotted for 95% confidence (upper curve) and 99% confidence (lower curve). (b) Specificity (number of correctly identified non-connections divided by number of non-connections) plotted for 95% confidence (lower curve) and 99% confidence (upper curve). Since the results for the three different connection strengths $E[\beta_{ij}]$ were similar, we averaged the results over the three connection strengths as well as the 10 realizations per number of spikes per target, for presentation in the graph. Error bars denote standard error.

reaches a peak of +30 mV, v and u are reset according to the following protocol:

$$\text{if } v \geq 30\,\text{mV then} \begin{cases} c \rightarrow v \\ u + d \rightarrow u \end{cases} . \tag{6}$$

The Izhikevich model dynamics varies with the settings of the four parameters, a setting the time scale of the recovery variable u, b describing the sensitivity of u to the subthreshold fluctuations of v, and c, d respectively determining the reset value of the voltage v and recovery variable u once a spike occurs. Various settings of these parameters cause the neurons in the generated network to exhibit a range of firing behaviors. The four different behaviors (regular spiking, fast spiking, intrinsically bursting and chattering) are shown in Fig. 2.

As in the previous section, we simulated networks of 10 neurons with 10 randomly generated, excitatory or inhibitory connections out of 90 possible. The connection strengths vary randomly around a prescribed average strength. For each of the different qualitative behaviors of the Izhikevich neurons, the simulation was repeated 10 times with different connectivities.

Surprisingly, the performance of the Cox method was consistent across all four behaviors. As with the proportional hazard model, for each behavior the test was repeated on 10 randomly selected networks for the 95% and 99% confidence levels. Fig. 3 plots the sensitivity and specificity of the Cox method statistical test for two of the four behaviors (regular spiking and chattering, corresponding to (a) and (d) in Fig. 2). Sensitivity is asymptotically satisfactory, and the expected confidence levels for specificity are clearly attained. Results for the other two behaviors are similar (not shown). The Izhikevich model is not known to obey a proportional hazard property, and the influence function was unknown, so the generic shape specified in (2) was used. Moreover, the same generic influence function $Z_{B_i}(t)$ with parameters $\tau_S = 10$, $\tau_R = 0.1$, and $\delta = 0$ was applied to each behavior of the Izhikevich model without any tuning of the parameters.

As can be expected, finding correct connections of the network (true positives) depends monotonically on the strength of the connection. Therefore the sensitivity increases with strength. We tested networks for Izhikevich neurons as described above, with connection strengths chosen randomly with means 3, 4, and 6, and show the results, averaged across all four spiking behaviors, in Fig. 4.

While this statistical agreement was expected for the proportional hazard model, where the modeling assumptions are exact and the influence function Z_{B_i} was known, it is a nontrivial fact for the Izhikevich model. This represents a compelling example where the hazard function is unknown, but where a statistically significant nonzero strength indicates a connection with the correct specificity. This demonstrates a key feature of the Cox method which makes it appropriate for laboratory applications, where the influence function is rarely known. We make this application to determine dynamic changes in networks from neuron cultures in the next section.

4. Detecting changes in neural cultures

In this section we illustrate the application of the Cox method to track network connections over time. We use neural cultures grown in the laboratory as an experimental test case. The existence of a statistical test for specificity in the Cox method, independent of arbitrary threshold selection, is an essential characteristic of the approach.

In vitro networks provide a reduced size and complexity and allow researchers to study the dynamics of a network in a dish, under controlled conditions. We plated cortical murine cells (E18) onto a micro-electrode array (MEA). The MEA records the spiking behavior of nearby plated neurons by measuring the change in voltage in the extracellular environment that occurs when a neuron spikes. They allow for the simultaneous recording of spike trains from a plated culture.

We analyzed recordings of several channels of a network of spinal cord cells plated on a commercially available MEA with 64 electrodes. The area of each electrode is approximately 400 μm^2. Neuronal-glial cultures from E18 mice (approximately 300,000 cells/dish) were plated and kept in neurobasal culture media supplemented with fetal bovine serum. Dishes were kept sterile, and handled in a biological hood when necessary. Cultures were visually checked under an inverted microscope for contamination and homogeneous cell coverage of the MEA. Cultures were kept in incubators with controlled humidity and temperature for up to 21 days before recording commenced.

Once the MEA was connected to a recording system (64 channels at 40 kHz per channel, bandpass filter from 0.5 Hz to 8 kHz, 2000× gain), temperature was adjusted to 37 °C through a heated base plate; no perfusion was applied. Thresholds for spike

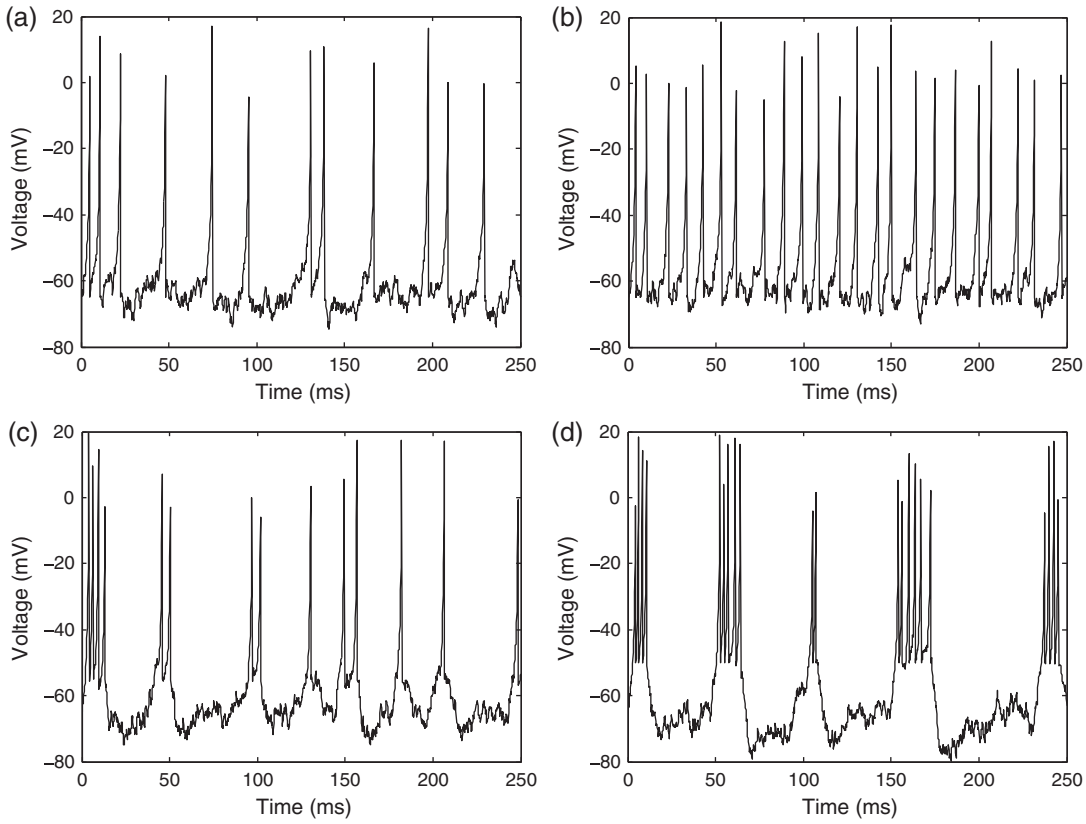

Fig. 2. Examples of neuron behavior from the Izhikevich model. (a) Regular spiking: $a = 0.02$, $b = 0.2$, $c = -65$, $d = 8$; (b) fast spiking: $a = 0.10$, $b = 0.2$, $c = -65$, $d = 2$; (c) intrinsically bursting: $a = 0.02$, $b = 0.2$, $c = -55$, $d = 4$ and (d) chattering: $a = 0.02$, $b = 0.2$, $c = -50$, $d = 2$.

detection were set to 15% of the amplitude of a typical spike. After a 1-h stabilization period, spontaneous activity from active channels was recorded for a 3-h period. When recording was completed, the culture was returned to the incubator for storage.

After the data collection process was completed, the raw data was sorted. Sorting was done offline to disregard noisy events and to separate individual units. The term "unit" is used to describe an individual neuron. Since each electrode records all activity within a small radius of its location, it can potentially record the activity of multiple neurons. Sorting the data allows us to distinguish multiple units recorded by an electrode so that they can be included individually in our analysis. The sorting was done based on examination of the waveforms of recorded spiking activity. Five spike waveforms from the same sorted unit are shown in Fig. 5.

4.1. Detecting structural network changes

In the experiment we used spike times recorded from 10 units of an *in vitro* network obtained from 9 active channels. A continuous 180-min recording was split into nine equal time segments of 20 min each, which was determined to ensure that each unit had at least 1000 spikes per segment. The Cox method was applied to each segment of data to produce an estimated connection strength β_{ij} for each of the 90 candidate connections. The results in Fig. 6 indicate the total number of segments in which each connection's strength was statistically different from zero. For example, the 9 occurring in the (1, 2) position indicates that the influence of unit 2 on unit 1 was statistically significant in each of the nine time segments; the 8 in the (2, 1) position indicates that the reverse influence is also significant in 8 of the 9 segments. On the other hand, comparison of the (2, 3) and (3, 2) positions shows that unit 2 drives 3 during seven of the nine time segments but that no reverse influence was detected. The entire test was conducted with a Familywise Error Rate of 5%, which corresponds to a confidence level of 99.994% per neuron using the Bonferroni correction for 810 tests (90 candidate connections for each of the 9 segments).

This test found many connections to be stable, either appearing in every segment or not appearing in any segments. Moreover, the strong control of the FWER indicates that each statistically significant connection should correspond to a real connection. However, the nature of the statistical test prevents us from concluding that the network structurally changed. This is because we do not have control of the false negatives, meaning that when a connection is not found to be statistically significant, that does not imply that it is not present. To overcome this challenge and determine whether the network is in fact changing with time, we introduce a new method in the next section which is sensitive to modifications in the strength of connections.

4.2. Detecting changes in connection strength

We now apply the Cox method across multiple data segments in order to test whether the strength of a connection has changed. Note that although the true influence function is unknown, if the influence function is stationary then the strength β_{ij} determined by the Cox method will be constant since we use the same generic influence function for each test. Thus if we split our data into multiple time segments, we can test whether the change of the β_{ij} coefficients is significantly different from zero, in which case we can conclude that the connection strength has changed. While a change in a connection may be due to nonstationarity of the unknown influence function, the β coefficients are always

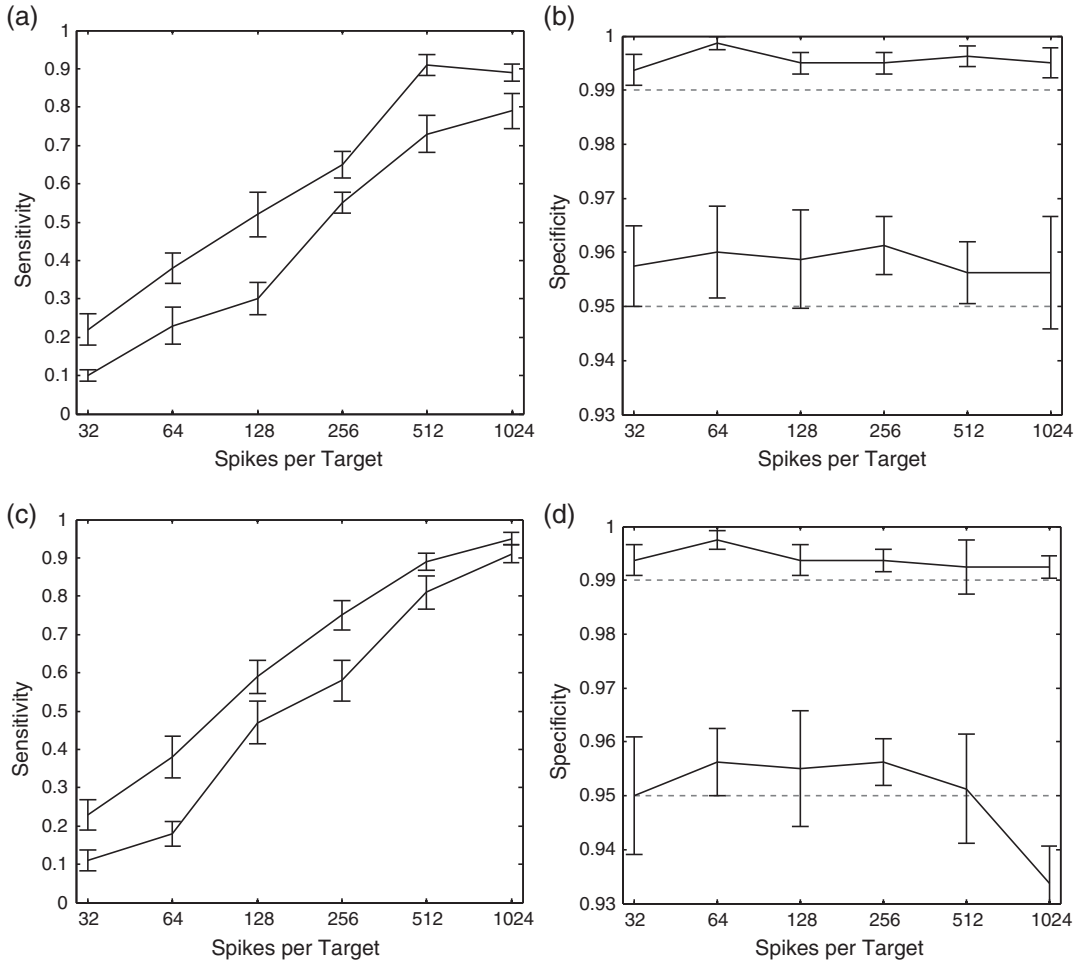

Fig. 3. Sensitivity and specificity for spiking and chattering behaviors of the Izhikevich model. (a) Sensitivity of regular spiking neuronal network for 95% confidence (upper curve) and 99% confidence (lower curve). (b) Specificity for regular spiking: 95% confidence (lower curve) and 99% confidence (upper curve). (c) Sensitivity of chattering neuronal network for 95% confidence (upper curve) and 99% confidence (lower curve). (d) Specificity for chattering: 95% confidence (lower curve) and 99% confidence (upper curve). Results are averaged across mean connection strength $E[\beta_{ij}] = 3$, 4 and 6 with 10 simulations per level; error bars denote standard error.

determined relative to the same generic influence function. Thus the strengths estimated by the Cox method can still be compared as long as the same generic influence function is used in each test.

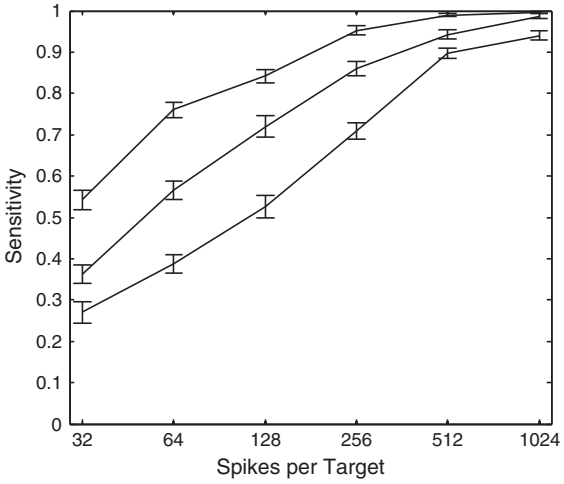

Fig. 4. Sensitivity (number of correctly identified connections divided by number of connections) increases as a function of the number of spikes per target neuron, for connection strengths 3 (lowest curve), 4 (middle curve), and 6 (highest curve). Results are averaged across Izhikevich model (all four behaviors).

We first apply this method to a network of simulated Izhikevich neurons to validate the use of the Cox method for detecting changes in connectivity strength. The network consists of 10 neurons with 10 connections (no self-connections) of varying strengths. In each simulation, we generated two time segments of data. The first segment consists of multivariate spike train series with a minimum of 500 spikes per neuron. Next we randomly select 5 of the 10 connections and either increase or decrease the connection strength by

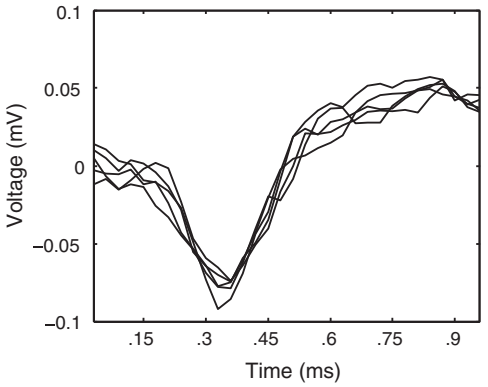

Fig. 5. Voltage trace of five waveforms. Based on the similarity of waveform shape, these were classified as belonging to the same unit.

	9	1	2	0	0	0	0	0	2
8		0	0	0	0	0	0	1	0
7	7		2	0	0	0	0	0	1
0	1	0		0	0	2	0	0	1
0	0	0	1		1	0	0	0	0
0	0	0	0	1		1	0	0	0
0	0	0	0	0	0		9	0	0
0	1	0	3	0	0	9		5	2
0	1	1	0	0	0	0	3		8
0	0	0	0	0	0	0	0	9	

Fig. 6. Summary connectivity matrix for the Bonferroni correction with 95% FWER. The (i, j) entry of the matrix corresponds to the number of time segments when there was a statistically significant influence on unit i by unit j. Results are from *in vitro* data recording split into nine equal time length segments (at least 1000 spikes per target per segment).

	7	0	3	0	0	0	0	0	5
6		0	0	0	0	0	0	2	0
3	1		2	0	0	0	0	0	2
0	0	0		0	0	1	0	0	1
0	0	0	2		2	0	2	0	0
0	0	0	0	1		1	0	0	0
0	0	0	0	0	0		0	0	0
0	0	1	3	0	2	2		1	3
0	1	0	0	0	0	0	1		0
0	0	0	0	0	0	0	0	0	

Fig. 8. Total number of statistically significant changes in strength across adjacent time segments for each connection.

a specified percentage. The choice of increase/decrease was made randomly. In the second segment we repeat the simulation with the modified connection strengths, again for a minimum of 500 spikes per neuron. For each percentage change in strength, we repeated the simulation 10 times and calculated the sensitivity and specificity.

In Fig. 7 we plot the results of the simulation. As the magnitude of the change in connection strength increases, there is a corresponding increase in sensitivity of detection of the change. Moreover, the test performs above the 95% confidence level (plotted as dashed line in Fig. 7(b)). We used a 95% confidence level with the Bonferroni correction to account for the 90 repeated tests, one for each potential connection change. The Bonferroni correction is conservative, accounting for the high specificity compared to the expected level. This validates our use of the Cox method across time segments to detect changes in network connection strength.

Next, we apply the same method to the neural culture data. Instead of the two time segments in the simulation, we have 9 separate time segments to analyze. We apply the above test based on the Cox method to each pair of adjacent time segments, and tabulate the statistically significant changes.

In Fig. 8, the changes between adjacent time segments are displayed. The results indicate the total number of statistically significant changes in strength that occurred across adjacent time segments out of a total of nine segments; thus the most possible changes is eight. The entire test was conducted with a FWER of 5% which corresponds to a confidence level of 99.993% per neuron using the Bonferroni correction for 720 tests (90 candidate connections for each of 8 adjacent pairs of time segments).

An evaluation of the stationarity of four different β coefficients is shown in Fig. 9. If a segment's midpoint does not fall within the range of the confidence interval of the previous segment, then a change is assumed. For example, Fig. 9(a) tracks change in the connection β_{12} from node 1 to node 2 over time. There are significant changes in the first seven transitions, corresponding to the 7 in the (1, 2) entry of Fig. 8. Fig. 8(b)–(d) corresponds to transition in the (1, 4), (1, 10), and (2, 1) connections, respectively. Fig. 9 shows that some of the changes in network connectivity are extreme, while others are relatively smooth. This comparison shows the wide variety of levels of stationarity in neural cultures. We find this to be a key issue for the evaluation of neural cultures and neural networks in general. Such statistical tools provide a crucial foundation for attempting analysis of medium- to long-term studies of such networks, when observations are limited to spike trains.

5. Discussion

The Cox method is a powerful technique for studying connectivity in networks from spike train observations. While the model

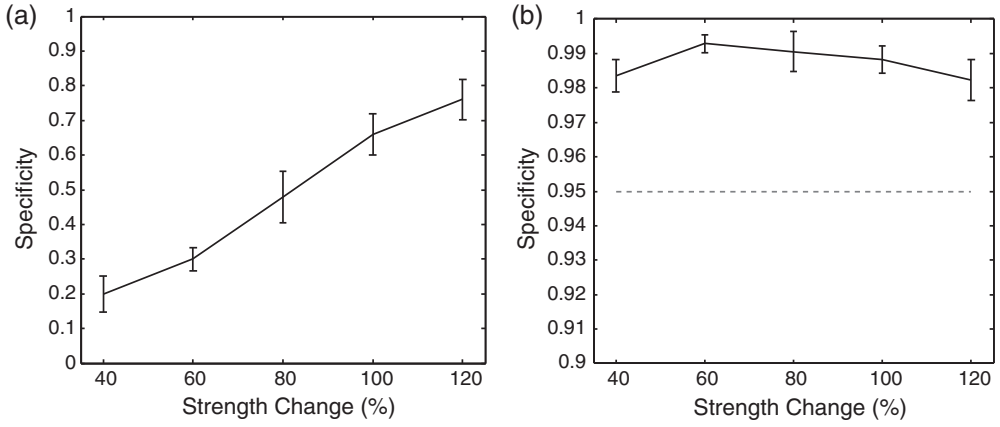

Fig. 7. Sensitivity and specificity of changes in connection strength across two adjacent time segments for a simulated network of Izhikevich neurons. Error bars display the standard error over 10 realizations. (a) Sensitivity and (b) specificity.

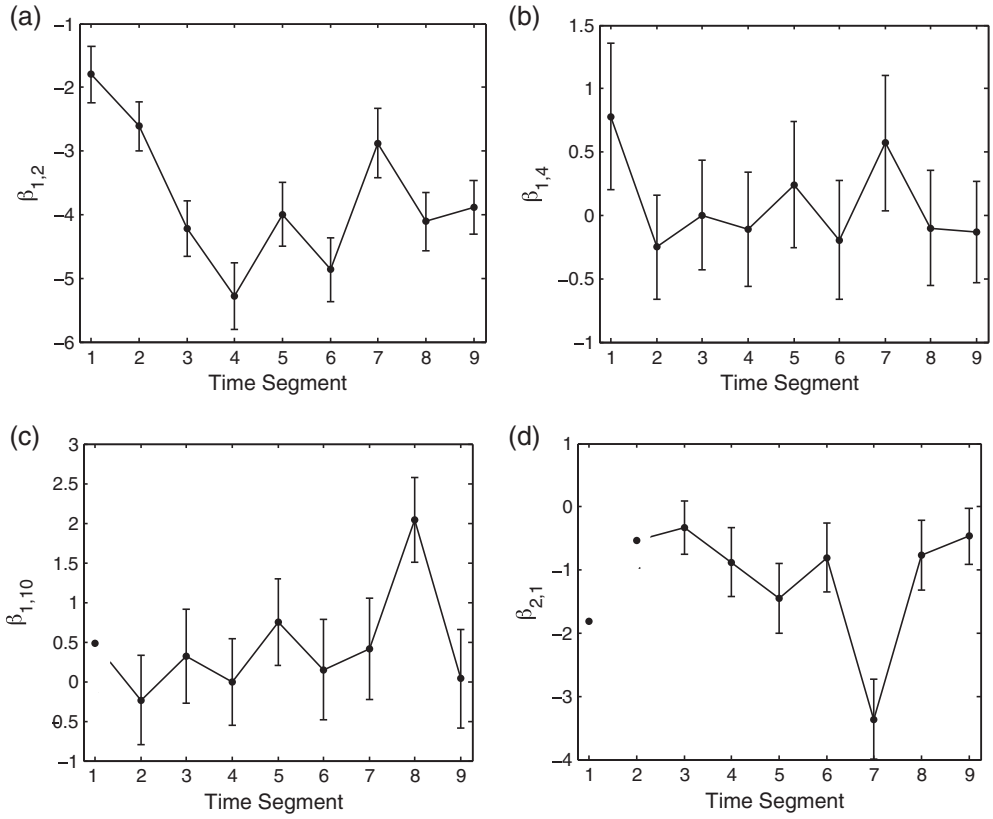

Fig. 9. (a–d) A selection of Cox β coefficients which have statistically significant changes across segments. Error bars indicate 99.993% confidence intervals. A connection is counted for each segment where the confidence interval does not contain zero. A change is counted for each segment where the midpoint lies outside the confidence interval of the previous segment.

seems to require knowledge of an influence function, which quantifies the interaction mechanism, we have shown that a generic influence function achieves the correct statistics when searching for connections. Indeed the Cox method achieved the correct statistics even for the physically realistic Izhikevich model. However, one difference between the computational model and the *in vitro* experiment is that *in vitro* we only have partial information as many neurons in the culture are unobserved. Thus the connections found *in vitro* may represent complex pathways in the unobserved network.

We have shown that the Cox method can also be used to detect changes in connections. Detecting the creation or elimination of connections is difficult because of the nature of the statistical test for connectivity. However, by modifying the test we are able to find statistically significant changes in the strength of connections. For neural cultures in the laboratory, we consider the stationarity or lack thereof to be a key issue and note that tools that can give a validated statistical test are sorely needed.

Applications of the results presented here range from cortical, hippocampal, and spinal cord networks kept *in vitro* for long term, to deep brain stimulation situations. Here we have demonstrated the case of a network of spinal cord neurons. We intend to expand these experimental results to other neuronal-glial cultures *in vitro*. One of our long term objectives is to demonstrate the steering of neural activity with electrical stimulation.

A potential clinical use of our implementation of the Cox method is in closed loop control implants for neurodisorders, such as in a deep brain stimulation scenario. Careful applications of this method could indicate network connections that are changing faster than others, representing the evolution of the disease or defect being monitored. Knowledge of these changes could improve the ability of the control system to apply an appropriate stimulation protocol, with the objective of counteracting symptoms or delaying the progression of the neurodisorder.

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