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MCMC for Ion-Channel Sojourn-Time Data: A Good Proposal

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Stochastic modeling of ion-channels has come a long way since the patch-clamp technique enabled the current flowing through a single channel to be recorded. But a kinetic model is only as good as the reliability of its rate constants, and obtaining good estimates of parameter uncertainty remains challenging. The article by Epstein et al. (1) in this issue of the *Biophysical Journal* presents an exciting, new methodology for making inferences about such parameters.

The gating mechanism of a single ion-channel is typically modeled as a finite-state continuous-time Markov chain, whose states are aggregated into two classes (either open or shut). The theory of such models of single-ion channels was largely worked out ~30 years ago (e.g., Colquhoun and Hawkes (2) and Fredkin et al. (3)). Estimation of rate constants of a model from single-channel recordings is more problematic. The aggregation of states means that distinct models of channel gating can yield probabilistically indistinguishable observable processes. Another problem is that single-channel current recordings are corrupted by noise and low-pass filtering, and are sampled at finite intervals. The sequence of open and closed sojourns of a channel is then reconstructed, often using some kind of threshold-crossing algorithm, which results in the loss of brief sojourns in either the open or shut classes of states. Failure to account correctly for such missed brief sojourns leads to biased estimates.

Two main approaches have been used for overcoming the problem of missed brief sojourns. One is to extend the theory of aggregated Markov processes to include missed brief sojourns, and incorporate it into maximum-likelihood (ML) fitting (Colquhoun et al. (4,5)). This approach underpins the methodology in Epstein et al. (1). The other is to base inference directly on the current record by explicitly incorporating a model for noise and low-pass filtering, as done, for example, by Fredkin and Rice (6) and Qin et al. (7).

These ML procedures yield estimates of the rate constants, together with associated standard errors. However, the latter assume that the estimates are approximately normally distributed, which may be unreliable for ion-channel data (Fredkin and Rice (6)). Also, whereas estimates of channel properties (such as the equilibrium probability that the channel is in an open state) are readily available using the estimates of the rate constants, standard errors for those estimates are not. Further, although problems identifying rate constants may be detected from correlations between their estimates, other more subtle problems, for example those involving nonlinear dependencies, are harder to unravel.

ML belongs to classical statistics, in which properties of estimators are derived from hypothetical independent repetitions of an experiment under the same conditions. An alternative conceptual approach to statistical inference is the Bayesian one, in which, before an experiment, an investigator expresses his/her beliefs about the unknown parameters as a (prior) probability distribution. The results of the experiment update the prior distribution (according to Bayes' theorem) to yield a posterior distribution of the unknown parameters. Despite some zealous advocates, Bayesian methods were not adopted widely, partly because the posterior distribution involves a normalizing constant, which in many applications is a high-dimensional intractable integral, and consequently (even numerically) unavailable. All that changed ~25 years ago, with the rapid explosion in the use of Markov chain Monte Carlo (MCMC) methods, which increased greatly the applicability of Bayesian inference to real-world problems, including many that were computationally inaccessible to classical methods.

The idea of MCMC methods is to construct a discrete-time Markov chain (not to be confused with the continuous-time Markov chain used to model the ion channel gating mechanism), whose equilibrium distribution is the posterior distribution of the unknown parameters (usually rate constants), θ

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say, in that ion-channel model. The discrete-time Markov chain is then simulated until it has reached equilibrium, after which further simulation of the chain gives a (usually correlated) sample from the posterior distribution for θ, thus enabling inferences to be made concerning the parameters that govern the ion-channel model. A general way to construct the discrete-time Markov chain is by the Metropolis-Hastings algorithm, in which given the current state (θ_n say) of the chain, first a new state θ', is proposed (i.e., simulated) from a specified proposal distribution, which typically depends on the current state θ_n. The proposed state is accepted (or rejected) according to a probability $p(\theta_n, \theta')$, which depends on both the current and proposed states. In practice, this involves generating a random number U that is uniformly distributed on the interval $(0, 1)$; if U is less than $p(\theta_n, \theta')$, then the proposed state is accepted and becomes the next state of the chain, i.e., $\theta_{n+1} = \theta'$, otherwise the next state is given by the current state, i.e., $\theta_{n+1} = \theta_n$. The process is then repeated, with the current state now being θ_{n+1}, and so on. The formula for the acceptance probability $p(\theta_n, \theta')$ involves the posterior probability densities of θ_n and θ', only through their ratio, so the normalizing constant disappears.

The Metropolis-Hastings algorithm provides a general way of constructing a discrete-time Markov chain with the required equilibrium distribution, but designing good MCMC samplers (i.e., choosing good proposal distributions) is often highly challenging. On the one hand, if proposals are too timid (i.e., very close to the current state), then their acceptance probability is high but the consequent jump of the Markov chain is small. On the other hand, if proposals are too bold then their acceptance probability is usually low and the Markov chain is likely to stay at its current state. Either way, the resulting MCMC sampler will have poor mixing properties, i.e., it will not sample the parameter space well. At best, this will result in a computationally inefficient sampler. More seriously, erroneous inferences may result if parts of the parameter space having relatively high posterior probability are not explored properly.

Although several MCMC samplers have been proposed for ion-channel inference directly from single-channel recordings, Epstein et al. (1) present the first MCMC sampler for inference based on reconstructed sequences of open and closed sojourn times, which accounts correctly for missed brief events. The authors use a two-step MCMC strategy. First they use a pilot MCMC sampler to locate approximately the mode (peak) of the posterior distribution and then they use a different MCMC sampler to estimate the posterior distribution. The output from the latter overcomes many of the shortcomings of ML-based estimation indicated above. Application to simulated data demonstrates that the methodology works for a model of the muscle nicotinic receptor with 10 free parameters. Application to experimental data from the same receptor channel demonstrates clear advantages of the procedure over ML estimation. Estimates of the marginal posterior distributions of individual rate constants show that whereas some are well approximated by the normal distributions predicted by asymptotic ML theory, others are not, with the uncertainty in their values being appreciably underestimated by the ML approach. Equally useful is that estimates of (observable and unobservable) channel properties now have measures of their posterior uncertainty.

As the authors state, their two-step MCMC strategy relies on the assumption that the posterior distribution is unimodal, and more discussion of what happens when that assumption is not met would be welcome. Also, how do these samplers perform for poorly resolved models (for example, those with flat ridges of posterior probability density, as happens when parameters of a model are close to being unidentifiable)? A very challenging, direction for future research is the use of MCMC as a tool for Bayesian discrimination between rival gating mechanisms for a channel, as in Hodgson and Green (8). That is all for the future. For the present, the MCMC methodology of Epstein et al. (1) is an important addition to the ion-channel toolbox; a good proposal, gladly accepted!

REFERENCES

1. Epstein, M., B. Calderhead, …, L. G. Sivilotti. 2016. Bayesian statistical inference in ion-channel models with exact missed event correction. *Biophys. J.* 111:333–348.
2. Colquhoun, D., and A. G. Hawkes. 1982. On the stochastic properties of bursts of single ion channel openings and of clusters of bursts. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 300:1–59.
3. Fredkin, D., M. Montal, and J. A. Rice. 1985. Identification of aggregated Markovian models: application to the nicotinic acetylcholine receptor. *In* Proceedings of the Berkeley Conference in Honor of Jerzy Neyman and Jack Kiefer, Vol. 1. L. L. Cam and R. Olshen, editors. Wadsworth, Belmont, CA. 269–289.
4. Colquhoun, D., A. G. Hawkes, and K. Srodzinski. 1996. Joint distributions of apparent open and shut times of single-ion channels and maximum likelihood fitting of mechanisms. *Philos. Trans. R. Soc. Lond. A.* 354:2555–2590.
5. Colquhoun, D., C. J. Hatton, and A. G. Hawkes. 2003. The quality of maximum likelihood estimates of ion channel rate constants. *J. Physiol.* 547:699–728.
6. Fredkin, D. R., and J. A. Rice. 1992. Maximum likelihood estimation and identification directly from single-channel recordings. *Proc. Biol. Sci.* 249:125–132.
7. Qin, F., A. Auerbach, and F. Sachs. 2000. A direct optimization approach to hidden Markov modeling for single channel kinetics. *Biophys. J.* 79:1915–1927.
8. Hodgson, M. E. A., and P. J. Green. 1999. Bayesian choice among Markov models of ion channels using Markov chain Monte Carlo. *Proc. R. Soc. Lond. A.* 455:3425–3448.