

RAPID COMMUNICATION

Characterizing Multiple Independent Behavioral Correlates of Cell Firing in Freely Moving Animals

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ABSTRACT: The heterogeneous sampling of behavioral states by freely moving animals hinders our ability to relate neuronal firing rates to behavioral variables by introducing dependencies between them. We specifically consider the animal's location and orientation, although our analyses may generalize to other behavioral variables, such as speed of movement. A maximum-likelihood approach is presented for producing estimates of the separate histograms relating firing rate to multiple independent causes. Examples show that the method can be used to avoid the artifactual behavioral correlates of place and head direction-cell firing produced by standard analyses; to characterize the independent influences of both location and orientation in a third cell type (Cacucci et al., 2004); and to demonstrate the location-independence of the directional firing of head-direction cells. © 2004 Wiley-Liss, Inc.

KEY WORDS: place; head direction; maximum likelihood; spatial, hippocampus

There are good examples of surprisingly pure firing-rate responses to an animal's location and orientation: "place cells" fire at a high rate whenever the animal is in a specific portion of its environment (the "place field") (O'Keefe and Dostrovsky, 1971; O'Keefe, 1976), while "head-direction cells" fire whenever the animal's head is pointing in a specific direction (Taube et al., 1990). In standard analyses, the experimenter collects the number of spikes fired by a putative single neuron and the "dwell time" spent by the animal corresponding to different intervals ("bins") in the range of the behavioral variable in question. A histogram of the firing rate (number of spikes divided by dwell time) as a function of the behavioral variable is then produced, and often smoothed for interpretability, using a fixed-sized (O'Keefe and Burgess, 1996) or variable-sized (Markus et al., 1995) kernel.

However, inhomogeneous sampling of behavioral variables by the animal's motion creates dependencies among them that can produce artifactual results. For example, inhomogeneity in the sampling of orientations will cause place cell firing to show an apparent preferential response to those head directions sampled most frequently when the animal is in the place field. Likewise, inhomogeneity in the sampling of places will cause head-direction cells to show an apparent preferential response to those places sampled most frequently with the preferred head direction. Inhomogeneity of sampling is unavoidable in freely moving

animals, and is often particularly acute at the boundaries of an environment, where locations can only be approached in particular directions. In this article, we present a method for estimating the effect of one variable (e.g., location) on firing rate while taking into account the effect of a second variable (e.g., orientation).

Suppose we are interested in the independent modulatory influences on firing rate as a function of location \underline{x} divided into N_l bins centered on the positions \underline{x}_i and head direction θ divided into N_d bins centered on directions θ_j . Our data consist of the number of spikes n_{ij} and the "dwell time" t_{ij} corresponding to locations \underline{x}_i and directions θ_j . The traditional spatial firing rate map would be defined as $f(\underline{x}_i) = n_i/t_i$, where $n_i = \sum_j n_{ij}$ and $t_i = \sum_j t_{ij}$ are the number of spikes and dwell time in the location bin at \underline{x}_i . Similarly, the traditional directional firing rate polar plot would be defined as $f(\theta_j) = n_j/t_j$, where $n_j = \sum_i n_{ij}$ and $t_j = \sum_i t_{ij}$ are the number of spikes and dwell time in the direction bin at θ_j .

The presence of a directional effect on place cell firing beyond the artifactual effect of inhomogeneous behavior interacting with a "true" locational effect can be detected using the "distributive hypothesis" (Muller et al., 1994). The directional firing predicted by the "Null" hypothesis (no influence of direction other than that caused by a locational effect) is calculated. Thus, the assumed "true" locational firing rate map $f(\underline{x}_i) = n_i/t_i$ is attributed to directions according to the time spent facing in each direction at each location to produce the predicted artifactual dependence on direction:

$$f^o(\theta_j) = \sum_i t_{ij}(n_i/t_i) / \sum_i t_{ij} \quad (1)$$

The observed firing rate polar plot $f(\theta_j)$ can then be tested to see whether it differs significantly from $f^o(\theta_j)$. This was used successfully to show that place cell firing in an open cylinder was not directionally modulated (i.e., $f(\theta_j)$ did not differ significantly from $f^o(\theta_j)$) (Muller et al., 1994). A similar procedure could be used to detect the presence of a locational effect beyond that due to inhomogeneous behavior interacting with a "true" directional effect. In addition, semi-partial correlation coefficients can be used to describe the amount of overall variance accounted for by one variable after taking into account the effects of other variables (Sharp, 1996). However, these procedures do not allow estimation and visualiza-

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Grant sponsor: Medical Research Council; Grant sponsor: Wellcome Trust, U.K.

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Accepted for publication 24 September 2004

DOI 10.1002/hipo.20058

Published online 19 November 2004 in Wiley InterScience (www.interscience.wiley.com).

tion of the relative effects of location and direction in cells for which there might be “true” effects of both.

We now outline how the parameters of a model of independent modulatory influences on firing rate can be estimated. Under such a “factorial” model, the expected number of spikes per location and direction bin is parameterized as:

$$E(n_{ij}) = p_i d_j t_{ij}, \quad (2)$$

where p_i represents the contribution of position x_i as a cause of firing and d_j represents the contribution of direction θ_j as a cause of firing. The maximum-likelihood approach (see, e.g., Duda et al., 2001) is to choose p_i and d_j to maximize the probability of the observed data n_{ij} under the model. For this we must define a probability distribution for the observed data, not just the expected values. The most obvious choice for the number of counts per bin of a random variable is the Poisson distribution. While this seems a reasonable model for firing as a function of position or direction, it would not be such a good model for firing as a function of time. Fenton and Muller (1998) note that, in contrast to the reliability of place cell firing as a function of location, averaged over several runs (i.e., n_i/t_i above), the firing rate on individual runs through a place field shows greater variability than consistent with a Poisson distribution. One reason for this might be that rate covaries with factors other than location, e.g., speed, which average out over many runs. The likelihood of the data in a single bin under a Poisson model is:

$$p(n_{ij}|p_i, d_j, t_{ij}) = (\lambda_{ij})^{n_{ij}} \exp(-\lambda_{ij}) / n_{ij}! \quad (3)$$

where $\lambda_{ij} = p_i d_j t_{ij}$ under the factorial model. Assuming independence over bins, the likelihood of the data L and the log likelihood l are, respectively:

$$L = \prod_{ij} p(n_{ij}|p_i, d_j, t_{ij}), \quad \text{and} \quad l = \sum_{ij} \log[p(n_{ij}|p_i, d_j, t_{ij})] \quad (4)$$

Under the factorial model with Poisson noise:

$$l = \sum_{ij} n_{ij} \log(\lambda_{ij}) - \lambda_{ij} - \log(n_{ij}!) = \sum_{ij} n_{ij} \log(p_i d_j t_{ij}) - p_i d_j t_{ij} - \log(n_{ij}!). \quad (5)$$

By setting the partial derivatives $\partial l / \partial p_i$ and $\partial l / \partial d_j$ equal to zero, we see that the values of p_i and d_j that maximize l (and thus maximize L) obey:

$$p_i = \sum_j n_{ij} / \sum_j d_j t_{ij}; \quad d_j = \sum_i n_{ij} / \sum_i p_i t_{ij}. \quad (6)$$

These $N_l + N_d$ equations can be iterated to find p_i and d_j , given a sufficiently large number of data points n_{ij} . (In principle, there can be $N_l \times N_d$ observations of n_{ij} but in practice far fewer are

sampled, and many of these will be zero).¹ Intuitively, the equations can be thought of as setting d_j to be the multiplicative factor by which the observed number of spikes for direction θ_j differs from that predicted by the locational firing pattern (using a distributive hypothesis) and vice versa for p_i . To display p_i or d_j independently of the other, as estimated firing rate plots, the values in each plot are scaled so as to match the total numbers of spikes recorded.

We next illustrate the use of our approach in assessing the effects of location and direction on cell firing, as compared with simply composing histograms relating firing rate to location or direction without consideration of the effects of one variable on the other. The cells shown were recorded using tetrodes (Recce and O’Keefe, 1989) from freely moving rats exploring for scattered food rewards in walled environments (for methods, see, e.g., Lever et al., 2002). The animal’s location and head direction are tracked by an overhead camera monitoring two LEDs (one bright, one dim) on the animal’s head (Axona, Ltd.).

Figure 1 shows examples of the firing of head-direction cells in the presubiculum displayed as uncorrected histograms of the directional and locational effects (*uncorr*) or when the combined maximum likelihood model is used to correct for the effects of direction and location on each other [*corr*, Eq. (6)]. Note the spurious positional correlate of firing in the uncorrected histogram by position, due to the preferred head direction occurring preferentially in locations near to the edge of the environment (e.g., HD1 where the rat tends to approach the north edge facing northward, and HD2–3, where it shows some unidirectional wall-following). These do not occur when using the combined maximum likelihood model. Applying the combined model tends to reduce the locational information content of the firing of these cells noticeably, but not so the directional information content (as estimated by the method of Skaggs et al., 1993) (see legend to Fig. 1). In a larger sample of 43 head-direction cells, locational information (in bits per spike) was reduced by 28% on average while directional information was reduced by 4%. However, the specific effect of the correction on a given cell was by no means uniform (standard deviation [SD] of reduction of information: 25% for location; 7% for direction).

Figure 2 presents examples of the firing of hippocampal place cells displayed as uncorrected histograms of the directional and positional effects (*uncorr*) or when the combined model is used (*corr*). Note the spurious directional correlate of firing observed in the uncorrected histogram by direction. Spurious peaks in the directional histogram occur at the directions along which the rat most often ran through the place field. This spurious directionality is reduced in the combined model. As noted in the legend, applying the combined model noticeably reduced the directional information content of the firing of these cells, but not so the locational

¹Starting with a uniform estimate for p_i , use Eq. (6) to calculate d_j ; then use the new values of d_j to recalculate p_i , and then use the new values of p_i to recalculate d_j , and so on, until the log likelihood of the data, i.e., l from Eq. (5), stops increasing. Equally, one can start from a uniform estimate of d_j .

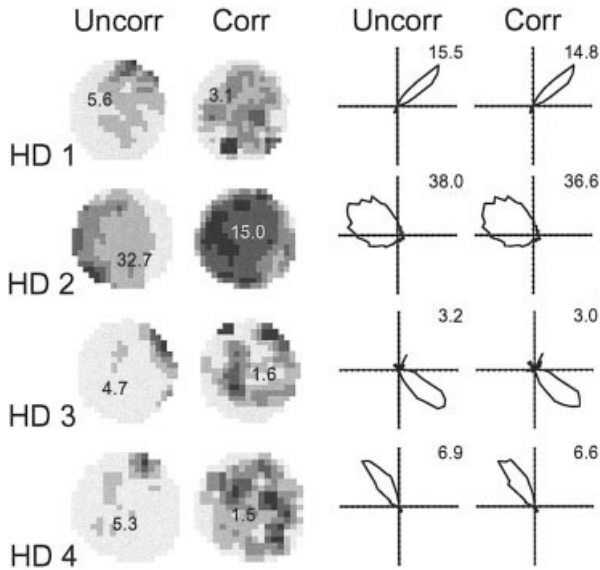


FIGURE 1. Firing rate histograms of four head-direction cells (HDs) recorded over 10 min. Left columns show locational rate histograms. Right columns show directional rate histograms. The peak firing rate (Hz) is shown on each plot. *Uncorr* shows the uncorrected histograms which simply plot the number of spikes divided by dwell time in each interval of the corresponding behavioral variable. *Corr* shows the histograms calculated under the maximum likelihood factorial model [MLM, Eq. (6)] to correct for the effects of direction and location on each other. Note the spurious positional effects shown using the uncorrected histograms. Applying MLM decreases locational information content (from *uncorr* to *corr*: 0.32–0.22, 0.18–0.04, 0.75–0.40, 0.35–0.21 bits per spike in cells 1–4, respectively, see also main text) and decreases locational peak rates, but produces similar directional information content (2.33–2.28, 0.63–0.60, 1.61–1.50, 1.93–1.97 in cells 1–4, respectively) and directional peak rates. To give reasonable spatial resolution and interpretability, binning of locations uses a square grid such that on average 245 locational bins were smoothed using a 3 × 3 kernel. Directions were divided into 60 bins (nearly always fully occupied, i.e., $N_d = 60$) and directional plots were not smoothed.

information content. In a larger sample of 44 place cells, directional information (in bits per spike) was reduced by 27% on average (SD 21%), while locational information was increased by 1% (SD 10%).

Figure 3 provides examples of a third cell type recorded in the pre- and parasubiculum (Cacucci et al., 2004) that has a genuine response to both place and direction, as shown by the combined model. Note the spurious “edge field” in the uncorrected histogram by position for TPD1. Figure 4 demonstrates the anecdotally well-known fact that the directional preference of head-direction cells is independent of position. Using uncorrected histograms over direction to make this point would be open to doubt due to differences in the sampling of head directions in the different parts of the environment.

It is possible that firing rate is affected by location and direction in ways not consistent with our factorial model (in which the influences multiply). It might be better to consider the influences of location and direction to combine additively. Unfortunately,

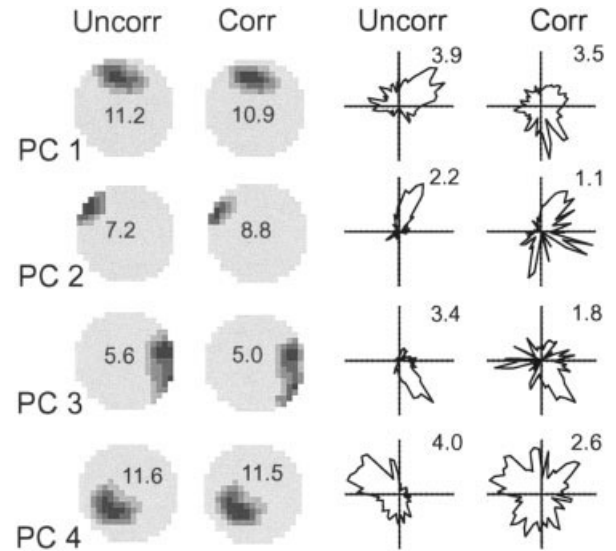


FIGURE 2. Firing rate histograms of four “place cells” (PCs) simultaneously recorded over 20 min. Left columns show locational rate histograms. Right columns show directional rate histograms. *Uncorr* columns show the uncorrected separate histograms, while *Corr* columns show the histograms calculated under the maximum likelihood factorial model (MLM), as in Fig. 1. Note the spurious directional effects shown using the uncorrected separate histogram. Applying the MLM decreases directional information content (from *uncorr* to *corr*: 0.27–0.08, 0.98–0.51, 0.73–0.28, 0.29–0.07 in cells 1–4, respectively) and directional peak rates, but produces similar locational information content (1.66–1.71, 3.12–3.26, 1.77–1.70, 1.57–1.58 in cells 1–4, respectively) and locational peak rates.

maximizing the log likelihood of the data [Eq. (5)] under an additive model, i.e., $\lambda_{ij} = (p_i + d_j)t_{ij}$ does not lead to easily solvable equations like Eq. (6). However, an additive model can be found, using the reasoning of the distributive hypothesis, by estimating the additive effect of location (p_i) above that predicted by an effect of direction (d_j) and vice versa, giving:

$$p_i = \frac{\sum_j n_{ij} \sum_j t_{ij} - \sum_j t_{ij} d_j}{\sum_j t_{ij}}; \quad d_j = \frac{\sum_i n_{ij} \sum_i t_{ij} - \sum_i t_{ij} p_i}{\sum_i t_{ij}}. \quad (7)$$

These equations can be solved iteratively, like Eq. (6), or by inversion of their matrix form.² The resulting model is a reasonable attempt to match the observed data with an additive model, but does not necessarily maximize the likelihood of the data under any given (e.g., Poisson) noise model. For comparison, we also evaluate the “naive” method based on the standard histograms (e.g., assuming that the firing rate at location i and direction j is simply the

²By substitution, Eq. (7) gives: $\underline{p} = (\mathbf{I} - \mathbf{T})^{-1} \underline{m}$, where \underline{p} is the vector of p_i , the vector \underline{m} has elements $m_i = n_i/t_i - \sum_j t_{ij} n_j/t_j$, \mathbf{I} is the identity matrix, and the matrix \mathbf{T} has elements $T_{ik} = \sum_j t_{ij} t_{kj}/t_j$. Variable \underline{d} can then be found from \underline{p} and Eq. (7). However, we found no better convergence for this solution over the iterative solution of Eq. (7); in both cases, convergence was worse than for Eq. (6), see Fig. 5 legend.

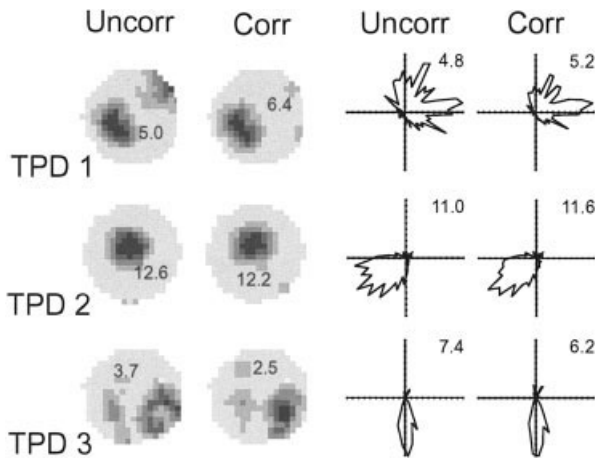


FIGURE 3. Firing rate histograms of 3 “theta-modulated place-by-direction cells” (TPD; Cacucci et al., 2004) recorded over 10 min. Left columns show locational rate histograms. Right columns show directional rate histograms. *Uncorr* columns show the uncorrected separate histograms, while *Corr* columns show the histograms calculated under the maximum likelihood factorial model (MLM), as in Fig. 1. MLM allows an unbiased picture of the dependencies on place and direction to be shown. Note the spurious positional effect at the edge of the environment using the uncorrected histogram in cell 1. The precise effect of applying the MLM on peak rates and information content cannot be simply predicted (locational information, from *uncorr* to *corr*: 0.47–0.67, 0.93–0.87, 0.74–0.60 in cells 1–3, respectively; directional: 0.42–0.63, 0.85–0.78, 1.86–1.54 in cells 1–3, respectively).

average of p_i and d_j , where each is calculated in isolation in the standard way).

Finally, a “simple normalization” approach would be to take the locational firing rate as the mean firing rate over all directions sampled at that location (to remove dependence on the time spent

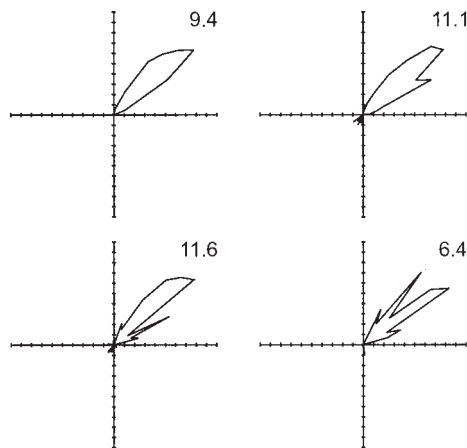


FIGURE 4. Effect of head direction on the firing rate of a “head-direction cell” calculated in each quadrant of the environment using the combined maximum likelihood model. Data from the Northeast quadrant is shown in the Top Left panel, etc. This allows an unbiased picture of the dependency on direction to be shown, despite inhomogeneous sampling of locations and directions. Note the parallel directional preferences in the different parts of the environment.

in each direction), and similarly the directional firing rate as the mean firing rate over all locations sampled at that direction i.e.: $p_i = \sum_j (n_{ij}/t_{ij})/N_d$ and $d_j = \sum_i (n_{ij}/t_{ij})/N_l$. However, the sampling of positions and directions in typical data is so sparse that the means are dominated by extreme rates from very short duration samples (while the medians are zero), so that this approach fares worse than the naive model, see Figure 5 legend.

The goodness of fit of different models can be assessed by comparing the log likelihood (l) of the data under each model. We do this using Eq. (5), under the assumption of Poisson noise, calculating: l_x with $\lambda_{ij} = p_i d_j t_{ij}$ from Eq. (6) for the factorial model; l_+ with $\lambda_{ij} = (p_i + d_j) t_{ij}$ from Eq. (7) for the additive model; l_n with $\lambda_{ij} = \frac{1}{2}(n_i/t_i + n_j/t_j) t_{ij}$ for the naive model; and l_u with $\lambda_{ij} = (\sum_{ij} n_{ij} / \sum_{ij} t_{ij}) t_{ij}$ for the prediction of a uniform firing rate model. The goodness of fit of the naive, additive, and factorial models to place, head direction, and TPD cell firing is shown in Figure 5 (as log scores of the factor by which the data are more likely under a given model than under the uniform model, e.g., $l_x - l_u$).

There is a clear advantage for the factorial model over the available alternatives. Although this does not conclusively rule out an additive model, since our estimate is not necessarily the additive model that maximizes the likelihood of the data, factorial rather

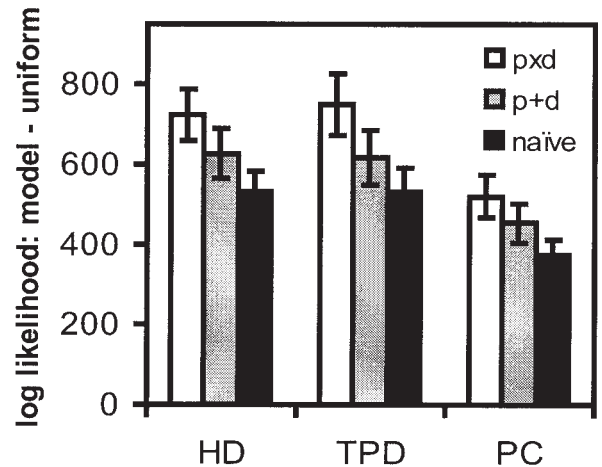


FIGURE 5. Relative goodness of fit of naive, additive (p+d), and factorial (p×d) models of the locational and directional influences on the firing rate of place cells (PC) head-direction cells (HD) and theta-modulated place-by-direction cells (TPD). The difference in the log likelihood of the data under the given model and under a uniform firing rate model is shown. For example, for the factorial model $l_x - l_u$ is shown, see text. The naive model is based on the traditional separate histograms of firing rate by location and direction. A square grid of location bins was used such that on average 68 bins were occupied (i.e., mean $N_l = 68$, this smaller number cf. Figs. 1–3 was used to aid convergence of Eq. (7) for the additive model) and 64 direction bins were used (i.e., $N_d = 64$). The mean and SEM are shown for the 29/46 PC, 30/46 HD, and 33/46 TPD cells for which Eq. (7) converged. The differences between the bar heights for each model are significant for each cell type ($P < 10^{-6}$, paired sample t -test). The “simple normalization” approach to estimating p_i and d_j (see text) showed a worse overall fit than any of the above models, with $l_s - l_u = 346$ for PCs, 485 for HDs, and 465 for TPDs when l_s is found using $\lambda_{ij} = \frac{1}{2}(p_i + d_j) t_{ij}$ in Eq. (5), and 414 for PCs, 450 for HDs, and 55.4 for TPDs when l_s is found using $\lambda_{ij} = p_i d_j t_{ij}$.

than additive models are consistent with the very low firing rates of TPD cells outside of their preferred location and direction (and with the low firing rate of place cells outside the place field, despite its modulation by running speed, McNaughton et al., 1983; Wiener et al., 1989; Huxter et al., 2003). Data may also contain more complex dependencies that are not well characterized by any of these models (e.g., two locational subfields with different preferred directions). Such data can sometimes be divided so that each subset of observations is well fitted. In this case, the (geometric) mean likelihood per observation in a subset of N observations [$\exp(l/N)$] can be used to indicate whether the division was justified.

In conclusion, we believe that our approach for separating the influences of multiple independent causes made dependent by inhomogeneous sampling, or something equivalent, is required before correct assessment can be made of the contribution of any one variable where there is a contribution from a second variable. This approach is related to the converse problem of how a factorial code can enable multiple cells to encode a single variable (see, e.g., Schneidman et al., 2003; Schmidhuber, 1992). We have illustrated the application of our approach to place and direction correlates of cell firing in single units recorded in freely moving rats. A spurious impression of positional or directional correlates created by inhomogeneous sampling of places and directions is commonplace when using the traditional method of separately forming histograms of numbers of spikes divided by dwell-times. The use of an explicit maximum-likelihood factorial model of the independent influences of position and orientation succeeds in reducing the problems posed by inhomogeneous sampling, and provides an improvement on the available alternatives (the standard separate histograms or an additive model). This opens the way to assess the firing patterns of cells for which both variables have an effect (e.g., Fig. 3), and to assess the contribution of one variable under manipulations of the other (e.g., Fig. 4). In principle, our method could be used to disentangle the combined effects of behavioral variables other than location and orientation (e.g., running speed) as long as there is a well-defined metric for the values observed.

Acknowledgments

The authors thank Peter Dayan and Suzanna Becker for useful discussions. The MatLab code for the factorial maximum-likelihood model can be found at www.icn.ucl.ac.uk/space_memory/resources/pxd.

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