APPENDIX

Estimation of the Demixing Matrix Based on the Templates

The linearity assumption implies that the template waveforms can be viewed as scaled versions of an intrinsic neuronal signal $\tilde{\boldsymbol{s}}^i$ with coefficients:

$$\boldsymbol{F}^i = \left[\boldsymbol{f}^i_1, \ \ldots, \boldsymbol{f}^i_M\right]^T = \left[a_{1i} \cdot \tilde{\boldsymbol{s}}^i, \ \ldots, a_{Mi} \cdot \tilde{\boldsymbol{s}}^i\right]^T. \qquad (10)$$

The mixing coefficients $a_{1i}, \ldots, a_{Mi}$ describe how $\tilde{\boldsymbol{s}}^i$ is mixed across the electrodes. If the neuron templates are known, the demixing coefficients in $\boldsymbol{A}$ can be directly derived by:

$$a_{ji} = \text{argmin}_a \|\boldsymbol{f}^i_j - a_{ji} \cdot \tilde{\boldsymbol{s}}^i\|; \quad \begin{matrix} i = 1, \ \ldots, N \\ j = 1, \ \ldots, M. \end{matrix} \qquad (11)$$

The average of the template waveforms is used for the intrinsic signal:

$$\tilde{\boldsymbol{s}}^i = \frac{1}{M} \sum_{j=1}^{M} \boldsymbol{f}^i_j. \qquad (12)$$

ICA-Based Spike-Sorting Algorithm

This section describes the individual steps of the spike-sorting algorithm and the parameters that were used for spike sorting of the simulated data sets. *1*) All data are band-pass filtered between 500 and 3,000 Hz. *2*) FastICA is applied to the full-length data, and the number of estimated ICs equals the electrode number. The following *steps 3–5* are successively performed for every IC signal. *3*) Spikes are detected on the IC by threshold detection. The noise level is estimated based on the median (Donoho and Johnstone 1994)

$$\sigma_n = \text{median}\left\{\frac{|x|}{0.6745}\right\} \qquad (13)$$

which has been shown to be robust for variable firing rates (Quiroga et al. 2004). A threshold level of $5 \cdot \sigma_n$ is used. *4*) PCA is applied to the aligned IC spike waveforms. The scores from the first three resulting principal components are clustered using KlustaKwik, which automatically estimates the number of clusters. The cluster with the largest average IC spike signal is selected for further processing, and other clusters are discarded. *5*) As spike traces from a well-isolated cluster are expected to have low variation, the standard deviation of the traces is used as a measure of cluster quality. The traces on the three electrodes with highest spike signals are normalized by the peak-to-peak amplitude of the cluster template, and the standard deviation of the normalized traces is computed (relative standard deviation, RSTD). The RSTD has been experienced to be a robust quality measure, as it compensates for the effect that the degree of spike trace variation also depends on spike amplitude. The resulting cluster from *step 4* is discarded if the RSTD exceeds a threshold of 0.12. *6*) After repeatedly conducting *steps 3–5* for all ICs, pairwise comparisons between the obtained clusters are performed, and two clusters, *A* and *B*, are merged if they appear to belong to the same neuronal unit according to the following criteria: *i*) if the number of spikes shared by both clusters exceeds 30% of the number of spikes in *cluster A* or *B*; and *ii*) the similarity between the aligned average waveforms of *clusters A* and *B* is measured by means of their normalized Euclidian distance

$$ED = \sqrt{\frac{1}{M} \cdot \frac{1}{L} \cdot \sum_j \sum_\tau \left(f^A_{j\tau} \cdot f^B_{j\tau}\right)^2} \qquad (14)$$

where $f^A_{j\tau}$ is the τth sample of the cluster template for *cluster A* at the *j*th electrode, *M* is the number of electrodes considered, and *L* is the waveform length. For this measure, only electrodes on which the clusters had significant energy were considered. The threshold value for merging was set empirically (merge if $ED < 4.3$). The merging is organized in the following way. In a first step, the clusters are compared for the criterion of common spike times and accordingly merged. In a second step, the distances for all cluster pairs are calculated, and the cluster pair with the smallest distance is merged if the condition is fulfilled. After merging, the cluster-pair distances are recalculated, and the merging condition is checked again for the pair with smallest distance. *7*) The STA waveforms of the identified neuronal clusters are subtracted from the raw data, and *steps 2–6* are subsequently applied to the residual data. This iterative scheme is repeated for a total of five iterations. After each iteration, newly identified clusters are aggregated with previously obtained clusters using the merging method described in *step 6*.

Computing the Overlap-Specific Error Probability

From the spike-sorting results, the observed probabilities of an FN error for nonoverlapping (p_E) and overlapping (p_{OE}) spikes can be extracted. The observed probability of missing an overlapping spike can also be formulated as:

$$p_{OE} = p_E + (1 - p_E) \cdot p_O. \qquad (15)$$

p_o is the specific error probability for overlapping spikes, i.e., the probability to miss a spike participating in an overlap, although it would have been detected if the other spike was not there:

$$p_O = \frac{p_{OE} - p_E}{(1 - p_E)}. \qquad (16)$$