Supplementary Material for Manns, J.R., Howard, M. W., and Eichenbaum, H.

Ensemble analysis of odor representations

To assess the specificity of the hippocampal population for odor identity, we conducted an ensemble analysis on population vectors constructed for each odor sampling event during the study phase of the experiment. We calculated the average firing rate for each cell (n = 4 to 29 cells per session; total number of cells analyzed = 302) for the one-second interval starting from each odor sampling event. We used the firing rate of each neuron to generate a point in an *N*-dimensional space (where *N* was the number of neurons in that session) for each odor sampling event. To assess the degree to which these study event vectors carried information about odor identity, we conducted several analyses to attempt to reconstruct odor identity from the ensemble response, ignoring the location at which the odor was sampled. A linear discriminant analysis (Bower, et al., 2005) with leave-one-out cross-validation showed significantly better-than-chance prediction (multinomial distribution $p < .05$) for 8/19 sessions. Summing across sessions, the linear discriminant analysis correctly predicted the odor presented on 258/1895 study events ($p < .001$). To quantify the degree of fidelity of the representation of odor in the hippocampal ensembles we conducted an analysis in which we sampled cells randomly across sessions and attempted to reconstruct the identity of the odor presented on ninety randomly-chosen study events (this was necessary because not all sessions contained the same number of presentations of each odor). This reconstruction used a support vector machine with linear kernel using leave-one-out cross-validation (Hung, et al., 2005). We measured the mutual information between the actual odor sequence and the predicted odor sequence and found an approximately linear relationship between the information transmitted about odor and the number of cells (see Supplementary Figure 1). Although there are a number of factors that prevent a precise estimate from this analysis, an extrapolation of this calculation suggests that odor could have been

represented at the same level of accuracy as the average of rats' performance (78.6% correct) by an ensemble of approximately one thousand cells.

Single-unit and ensemble analyses of recency

To address the possibility that activity in the hippocampus might have signaled the recency of test odors, we compared the firing rates of individual neurons during the test period between instances in which the rat sniffed the odor that had appeared earlier in the sample period (the correct "earlier" odor) to instances in which the rat sniffed (but did not select) the odor that had appeared later in the sample period (the incorrect "later" odor). The sniffing event was defined as onset of sniffing to 1 sec later. Out of 123 different CA1 pyramidal neurons considered in the single-unit analyses, the firing rates for only 3 neurons reliably differed between the earlier and later odors (t-tests, alpha = 0.05, uncorrected for multiple comparisons). The number of significant results expected from this test by chance alone would have been 6 out of 123. Thus, this analysis suggested that the firing rates of individual CA1 pyramidal neurons did not provide above-chance information regarding the relative recency of the two test odors.

We also asked whether the overall activity from a population of simultaneously recorded neurons reflected the recency of test odors. For each odor sniffing event during the test phase, we calculated the firing rate for each cell (n = 4 to 29 cells per session; sniffing event = onset of sniffing to 1 second later). The firing rate from each neuron was then represented as a point in an *N*-dimensional space, as in the analysis of odor coding above. The length of a vector drawn between the origin and this point in *N*-dimensional space represented the overall activity from all neurons included in the analysis. If overall hippocampal activity recorded during sampling of test odors had differed according to whether the odor had appeared earlier or later in the sample phase, one would have expected the length of this population vector to differ between earlier and later odors. However, across all sessions, the average lengths of these two vectors were similar

(mean length in hertz $\pm$ SEM = 5.99 $\pm$ 0.59 and 6.37 $\pm$ 0.87, for earlier and later odors, respectively; paired-samples t-test: $t(18) = 0.78$, $p > 0.1$). The results were similar when the vector lengths were standardized by dividing by the number of recorded neurons to allow for fair comparisons across recording sessions that differed in the number of recorded neurons (mean length in hertz per neuron $\pm$ SEM = 0.41 $\pm$ 0.05 and 0.43 $\pm$ 0.07, for earlier and later odors, respectively; paired-samples t-test: $t(18) = 0.44$, $p > 0.1$).

Regarding the possibility that hippocampal activity supported recency judgments, we also considered the possibility that some neurons might reflect recency by an increase in firing rate, whereas others might reflect recency by a decrease in firing rate. If so, one would expect a different pattern of firing between earlier and later test odors rather than a simple difference in overall activity. Therefore, measured the distance between endpoints of pairs of event vectors using Mahalanobis distance. For each session, a Distance Index was obtained was obtained by dividing the Mahalanobis distance by two times the number of units. If the pattern of hippocampal activity differed between earlier and later test odors, one would have expected large distances between these events (a higher Distance Index corresponded to dissimilar events). However, the average Distance Score between earlier and later test items for each session (mean $\pm$ 95% confidence intervals = 0.86 + 0.19) was actually slightly less than the average score obtained after randomly reshuffling earlier and later items 100 times for each session (mean = 0.94). Thus, the dissimilarity of ensemble activity between test odors that had been encountered earlier in the sample phase and test odors that had been encountered later in the sample phase was no greater than one would expect by chance.

We conducted the above analysis again but this time took into account the fact that odors could have been presented either on the left or the right side of the testing enclosure (see Figure 1). Previous results have indicated that odor-related activity in the hippocampus can be

modulated by the location of the odor (Wood et al., 1999). Therefore, we separately obtained all pairwise Distance Indices between earlier and later odors encountered on the left side of the enclosure and then averaged those scores with all pairwise Distance Indices on the right side of the enclosure. The purpose of accounting for spatial location was to reduce the variability in firing rates associated with location and increase the likelihood of observing differences between earlier and later odors. However, in this analysis the average Distance Index between earlier and later odors was not significantly different from what one would expect by chance (Figure 2A).

Single-unit and ensemble analyses of ordinal position

We next explored the possibility that the hippocampal neural representations enabled rats to associate an ordinal tag (e.g., "third" or "fifth", by analogy) with each odor it encountered during the study period and that the activity of individual CA1 neurons might have reflected this association. If this were the case, one would expect neural activity to differentiate ordinal positions on each trial but to be similar across trials for a given ordinal position. However, we found that out of 123 different neurons considered in the single-unit analyses, only 3 neurons showed differential firing across the five ordinal positions (one-way repeated measures ANOVA, alpha = 0.05, uncorrected for multiple comparisons). We again considered that the location of odors might modulate odor-related activity and specifically explored the possibility that individual neurons might show conjunctive encoding of ordinal position and spatial location. All 123 pyramidal neurons considered in the single unit analysis were entered into a 2 X 5 (spatial location X ordinal position) repeated measures ANOVA. Only 8 neurons showed a significant spatial location by ordinal position interaction with an alpha level of 0.05 (uncorrected for multiple comparisons), which represents a percentage (6.5 %) that did not significantly differ from the 5 percent one would expect by chance. Additionally, only 2 neurons showed a main effect of ordinal position in this analysis. In comparison, 34 (27.6 %) of the neurons showed a

main effect of spatial location during odor sampling (probability of obtaining 34/123 by chance was less than 0.001). Thus, the firing rates of individual neurons had a tendency to correlate with the spatial location of odors but did not provide above-chance information about the ordinal position of odors encountered during the sample phase of each trial.

We also asked whether hippocampal population activity during the sample phase consistently differentiated the ordinal position in which odors were encountered. If ordinal position was encoded by hippocampal ensemble activity, an odor sniffing event that occurred at the second ordinal position, for example, would have been represented more similarly to odors on other trials occurring in the second ordinal position as compared to events from the first, third, fourth, or fifth ordinal positions. Based on the prominent spatial correlates observed in the previous analysis, we accounted for spatial location in the present analysis by restricting event-event distances not only to the same ordinal position but also to the same spatial location. For example, for each session we obtained all pairwise Distance Indices between odors encountered in the second ordinal position on the left side of the enclosure. We then averaged those scores with all pairwise Distance Indices between odors encountered in the second ordinal position on the right side of the enclosure. The purpose of accounting for spatial position in this way was to reduce the variability in firing rates associated with differing locations and to increase the likelihood of observing activity related to ordinal position. However, as in the single-unit analyses, the ensemble analyses failed to find evidence of direct encoding of ordinal position.

Supplementary Figure 2B shows the average Distance Indices between pairs of items encountered at the same ordinal position during the sample phase and plots the results separately for the first, second, third, fourth, and fifth position. These Distance Indices for each ordinal position are similar to the average of all event-event Distance Indices between different ordinal positions, which represents the chance level of similarity between pairs of odors. **References**

Bower, M.R., Euston, D.R., and McNaughton, B.L. (2005). Sequential-context-dependent hippocampal activity is not necessary to learn sequences with repeated elements. J. Neurosci. 25, 1313-23.

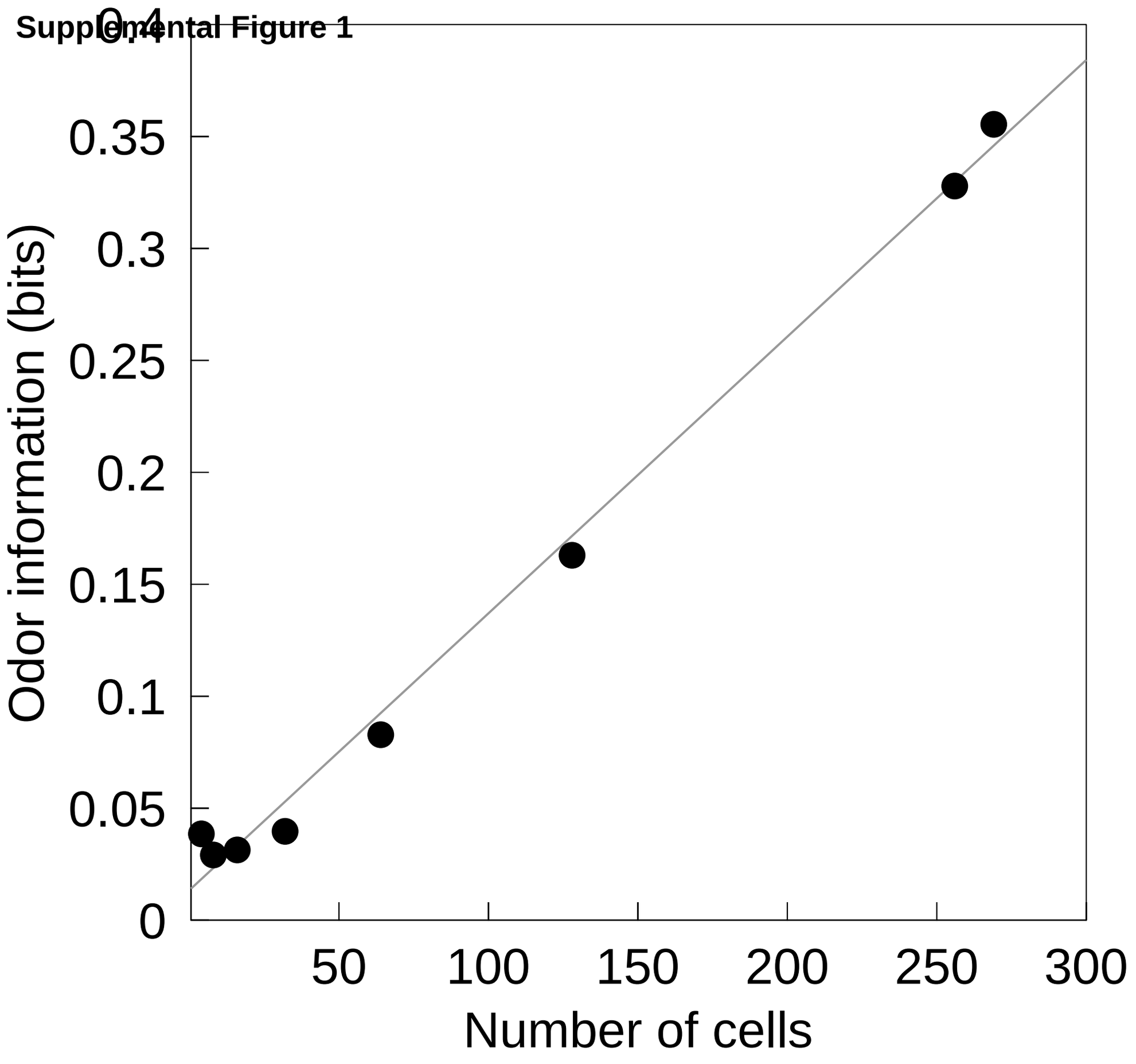
Hung, C.P., Kreiman, G., Poggio, T., and DiCarlo, J.J. (2005). Fast readout of object identity from macaque inferior temporal cortex. Science 310, 863-6.

Wood, E.R., Dudchenko, P.A., and Eichenbaum, H. (1999). The global record of memory in hippocampal neuronal activity. Nature 397, 613-6.**Figure Captions**

Supplementary Figure 1. Information transmitted about odor identity as a function of the number of neurons in the hippocampal ensemble. An approximately linear relationship exists over the range that could be considered (R^2 = .98, see Supplementary Information for more details). The minimum level of information about odor identity needed to produce performance on the judgment of recency at the level observed by the rats (78.6 percent correct) would correspond to approximately 1.85 bits. An extrapolation of the data would suggest that about 1000 neurons would be required to produce this level of performance.

Supplementary Figure 2. A. No evidence for a recency effect. The hippocampal ensemble representations of probe odors did not show above-chance differences between odors that had appeared earlier versus later in the sample sequence. **B.** No evidence for direct encoding of ordinal position in hippocampal activity. The hippocampal ensemble representations of odors encountered at the five ordinal positions were no more likely to resemble one another than they are to resemble any other ordinal position ("chance"). A lower Distance Index corresponds to greater similarity. Error bars show 95% confidence intervals.

Supplemental Figure 1



Supplemental Figure 2