William E. Skaggs and Bruce L. McNaughton state, in the title of their report, that they found a "Replay of neuronal firing sequences in rat hippocampus during sleep following spatial experience" (1). On the basis of the evidence they present, we question that finding.

Skaggs and McNaughton analyzed recordings from pairs of rat hippocampal neurons during a period of running on a closed track and a period of sleep immediately after. Each cell typically fires at a high rate when the animal enters a zone on the track called its "place field." When the fields of the two cells do not overlap, the cells fire in the order that the animal encounters their respective fields. During subsequent sleep, the same cells tend to fire nearly synchronously.

Skaggs and McNaughton state that the temporal order of activation of two cells—not their "firing sequences" but their sequence in firing—is the same during track running and in the sleep period that follows. Skaggs and McNaughton do not base this statement on the observed firing of the cells, but rather on a novel "measure of temporal ordering" they call "temporal bias." This bias is computed from a crosscorrelogram of the spike trains of a pair of cells, and is therefore dependent on the detailed timings of both spike trains. During running, those spike trains are influenced in a complex way by the shape and arrangement of the two place fields, the running speed of the animal (which may be age-dependent), track geometry and its familiarity, ongoing theta activity, and the propensity of the animal to stop and receive a food reward [see the legend of figure 1 and note 11 in the report (1)]. Thus, although the temporal order in which the animal encounters place fields may affect bias, these other factors render it an unsuitable and unreliable indicator of temporal order of firing under most conditions.

Furthermore, the algebraic sign of bias is neither a necessary nor sufficient condition for fixing the temporal order of firing of two cells. To give a simple example, consider the following three spike sequences

Cell A: 

Cell B: 

Cell C: 

Although cell A fires before cells B and C, the sign of the bias of cells A and B is opposite to that of cells A and C. Skaggs and McNaughton provide no evidence that a positive bias of their cell pairs consistently implies one temporal order of firing while a negative bias implies the reverse order. To establish this relationship they would first need an independent, unambiguous definition of temporal order, a subtle issue not addressed in their report.

Thus, even if Skaggs and McNaughton could demonstrate a statistically significant "replay" of bias in sleep, that would not logically imply that firing sequences or sequences of firing were "replayed."

If Skaggs and McNaughton used data from one session to select the most favorable "window" duration over which to compute bias from the correlogram [see note 11 in (1)] and then used this window duration to calculate bias for the other six sessions, they would then be left with six, not seven, sessions on which to use the Sign Test to determine the statistical significance of their observations. But if [see note 14 in (1)] they "experimented" with data from several (or all) of the sessions in choosing the window duration (achieving "consistent" results only with a value of 200 ms), they would be left with even fewer sessions in which to test for significance.

Depending on how many cell pairs were used in determining an optimal window, to that extent would the number of pairs be diminished on which to test the statement that "a significant majority of cell pairs showed the same direction of bias during the maze-running session as they did during sleep afterward. . . ." That number would certainly be far less than the number of pairs shown in figure 2B of the report (1), all of which were used in obtaining the "highly significant" result on which that statement is based.

The results [figure 2B of (1)] do not support the bias "replay" hypothesis in the majority of the remaining sessions even when the most favorable window duration is used. Even then, the null hypothesis—that bias is not replayed—could not be rejected in at least half the remaining sessions. Using other window durations, they were apparently unable to reject the null hypothesis in more than half the sessions. On the basis of this report, it seems just as logical to attribute the sessions with significant results to the age of the animal (there were three young and three old animals), or the shape of the track (triangular or square), or the experience of the animals with that track (familiar or unfamiliar), because each of these variables could affect the value of bias.

George P. Moore
Department of Biomedical Engineering,
University of Northern California,
Petaluma, CA 94952, USA

Jay R. Rosenberg
Division of Neuroscience,
Editor's Summary

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