"Replay" of Hippocampal "Memories"

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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 synchronous bursts.

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.

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1. W. E. Skaggs and B. L. McNaughton, *Science* **271**, 1870 (1996).

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Response: Moore *et al.* state that our title (*1*) was misleading in that the report is really about temporal bias rather than firing sequences per se. This criticism has some validity, and it might have been better to title the report "Replay of temporal bias . . . ," or "Replay of temporal order . . . ," rather than "Replay of neuronal firing sequences . . . ." We used the latter phrase because we thought it would be easier for the general reader to understand, but we agree that it might have misleading connotations for some. In any case, the text of our report made clear what we are describing.

The problem with the argument that temporal bias is not the same thing as temporal order is that "temporal order" is not a well-defined entity. We chose our temporal bias measure because it is a simple and robust indicator of temporal structure. Other measures of temporal order are possible, but we doubt that there are any whose virtues are manifestly superior to those of the temporal bias measure we have used. In the example of cells A, B, and C, Moore *et al.* state that cell A fires before cells B and C, apparently because, in the burst of spikes they illustrate, the first spike from cell A comes before the first spikes from cells B and C. This "first spike in a burst" principle would be difficult to turn into an unambiguous measure without making strong assumptions about the temporal structure of the spike trains, and even so it would probably not be very robust. In any case, we agree wholeheartedly that it would be interesting to examine the consequences of using different definitions of temporal order or temporal sequence, and we invite suggestions in this regard.

With regard to the criticism that the Sign Test analyses should have been based on six rather than seven sessions: Strictly speaking, this is correct. This makes the test significant at $P < 0.05$ rather than $P < 0.01$.

Moore *et al.* state that there should have

been a correction for the number of different window durations examined. This too, strictly speaking, is correct. Because five different durations were examined, we should have used a Bonferroni correction, multiplying the final significance level by 5. In fact, with the grouped data showing a significant effect at the level $P < 0.00001$ without any correction, such a correction makes little practical difference. Moreover, it is not accurate to state that the only significant effects occurred for a time window of 200 ms. There were also significant effects for time windows of 50, 100, and 500 ms, but, as we stated in our report, the effect for 200 ms was most consistent. Space did not permit us to describe these relationships, or their statistical ramifications, in detail. The bottom line is that we are completely comfortable that our results cannot be accounted for by statistical malfeasance. If we were dealing with a marginal phenomenon, the objections raised in this comment would be telling, but the phenomenon we described is actually quite robust, and has, in fact, been replicated several times in our laboratory, with different data.

Moore *et al.* appear to argue that the pattern of bias replay among sessions was incorrectly described. This criticism seems to be based on a misreading of our report. We did not state that we *proved* that temporal bias is replayed in every session. Our data suggest that it is, but do not prove it. Moreover, we did not try to determine which factors (for example, age or apparatus) were associated with sessions yielding individually significant results. Given the small number of sessions in the study, this would not be possible. Perhaps the most likely explanation is that the individually significant sessions tended to be those in which the largest numbers of cells were recorded.

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