Figure S1

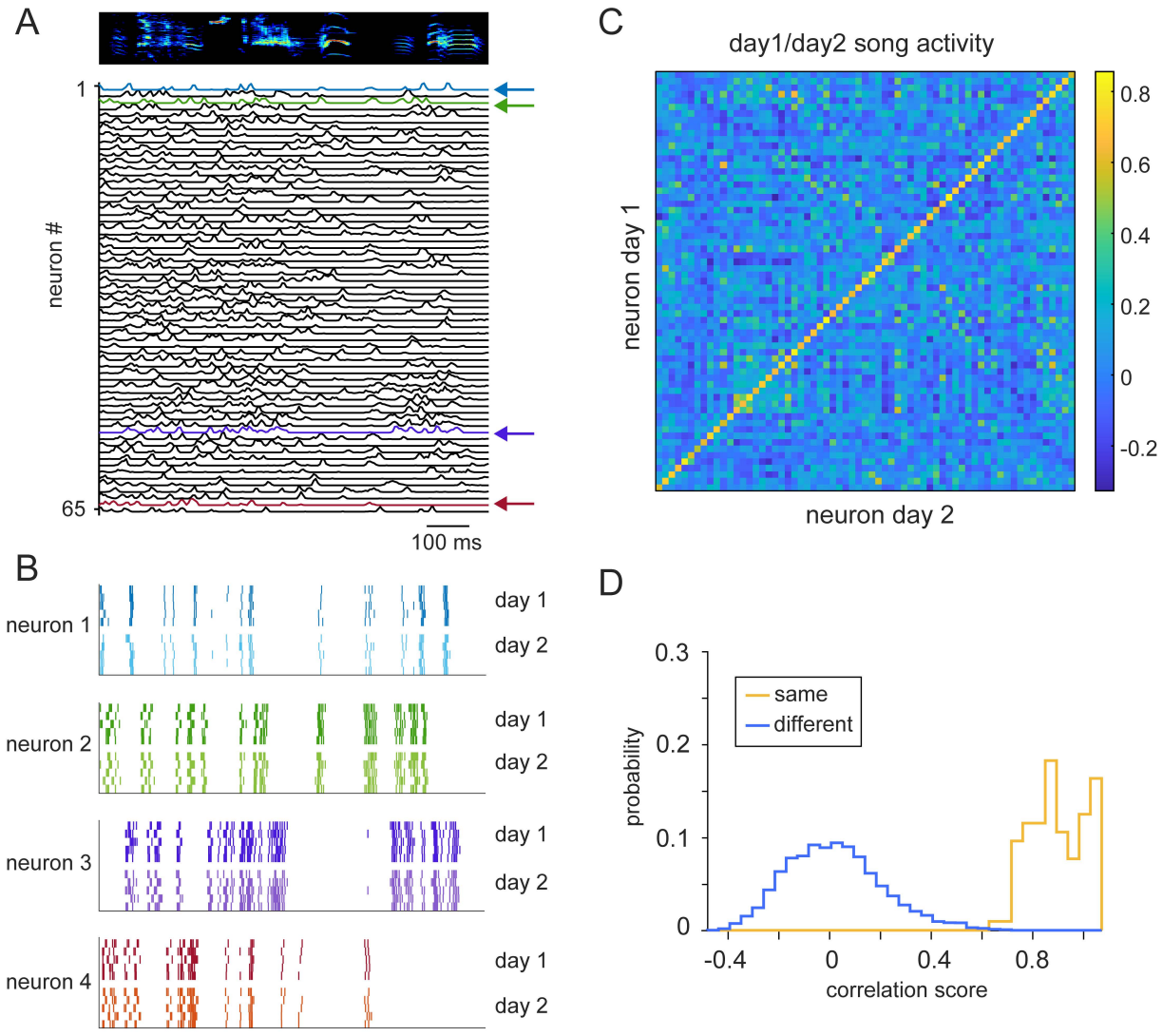

Figure S1. Stability of RA recordings across a night of sleep. Related to Figure 3.
(A) Normalized average instantaneous firing rate of 65 neurons from Bird C34 simultaneously recorded during song.
(B) Aligned spiking activity of four example cells, indicated by arrows in (A), during four trials on day 1 and four trials on day 2.
(C) Cross-correlation matrix of song activity recorded on day 1 vs day 2.
(D) Distribution of correlation scores within and across neurons (n = 104 neurons from 3 birds).

Figure S2

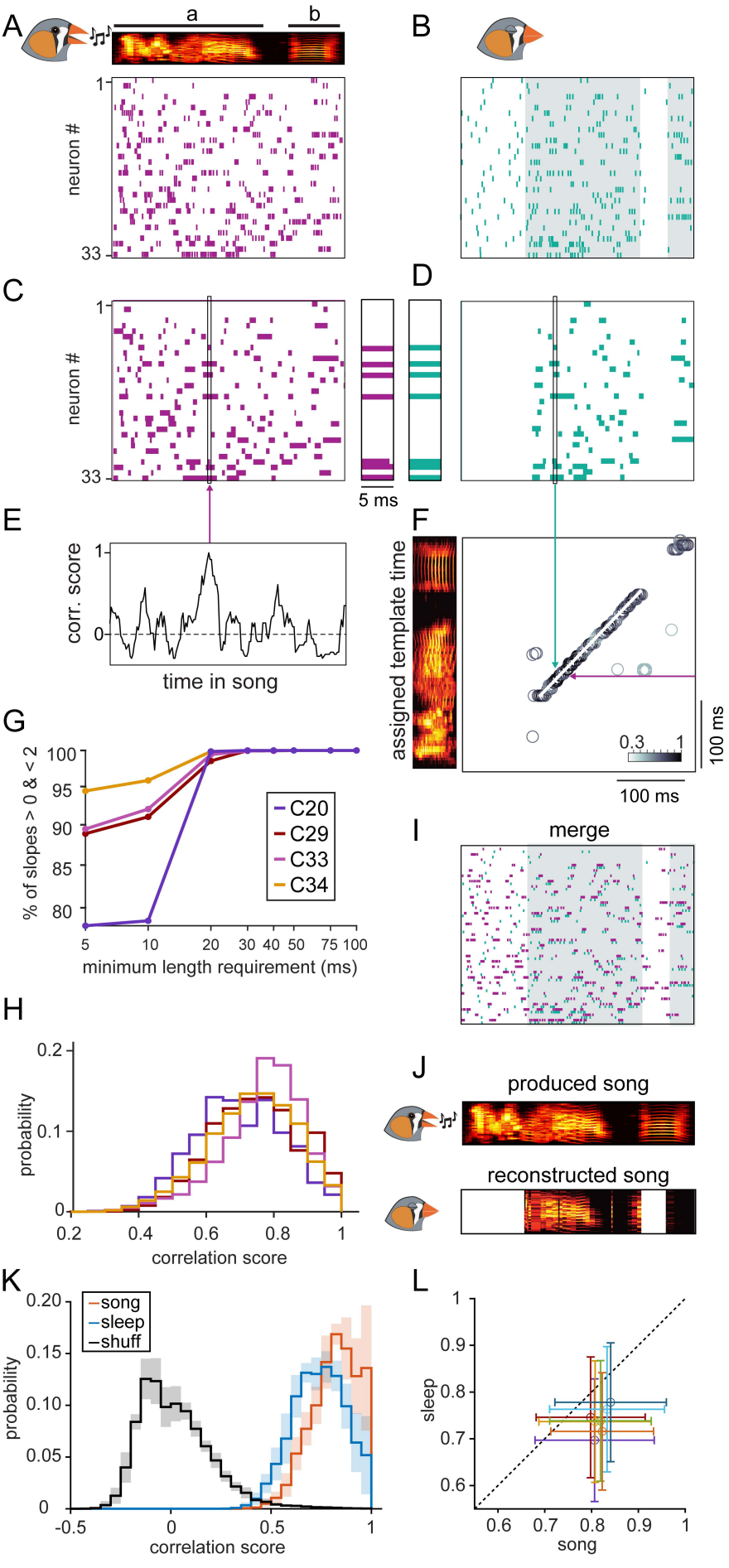
A
a
b
neuron #
1
33
B
C
D
5 ms
E
corr. score
time in song
F
assigned template time
0.3
1
100 ms
100 ms
G
% of slopes > 0 & < 2
minimum length requirement (ms)
C20
C29
C33
C34
I
merge
H
probability
correlation score
J
produced song
reconstructed song
K
song
sleep
shuff
probability
correlation score
L
sleep
song

Figure S2. Template matching strategy used for decoding the song content of sleep activity. Related to Figure 3.

(A and B) Simultaneous spiking activity of 33 RA neurons from Bird A413 during singing (A) and sleep (B).

(C and D) Binarized activity (threshold: > 100 Hz) for data shown in (A) and (B). Narrow black boxes indicate the 6 ms query bins in sleep (D) and the winner-take-all bin assignment from the song template (C).

(E) Correlation score between the query bin in D and all bins of the template shown in (C). Magenta arrow: maximal correlation score.

(F) Each data point is aligned to assigned bin of song template; color indicates Pearson correlation score. White line: detected replay event.

(G) Parameter search for the minimum length requirement to meet detection threshold for a replay event. Slopes greater than 2 or less than 0 were not observed during sleep and therefore were used to identify errors in event detection. Each color represents data from a single control bird (n = 4 birds).

(H) Distribution of maximum correlation scores (E) of all template matching assignments occurring within detected events for 4 birds.

(I) Spiking activity during song (magenta) and sleep (green) from (A) and (B), aligned according to template matching results.

(J) Spectrograms of the produced song (top) and reconstructed song (bottom) using the template matching assignments from (F).

(K) Distributions of correlation scores for all analyzed bins consisting of either replay events (blue) or non-template song renditions (red). The shuffled distribution (black) includes the correlation scores for all analyzed sleep and song bins assigned to random bins of the template.

(L) Direct comparison of correlation scores (mean ± SD) for each template bin in each bird (n = 7) for song renditions and replay events.

Figure S3

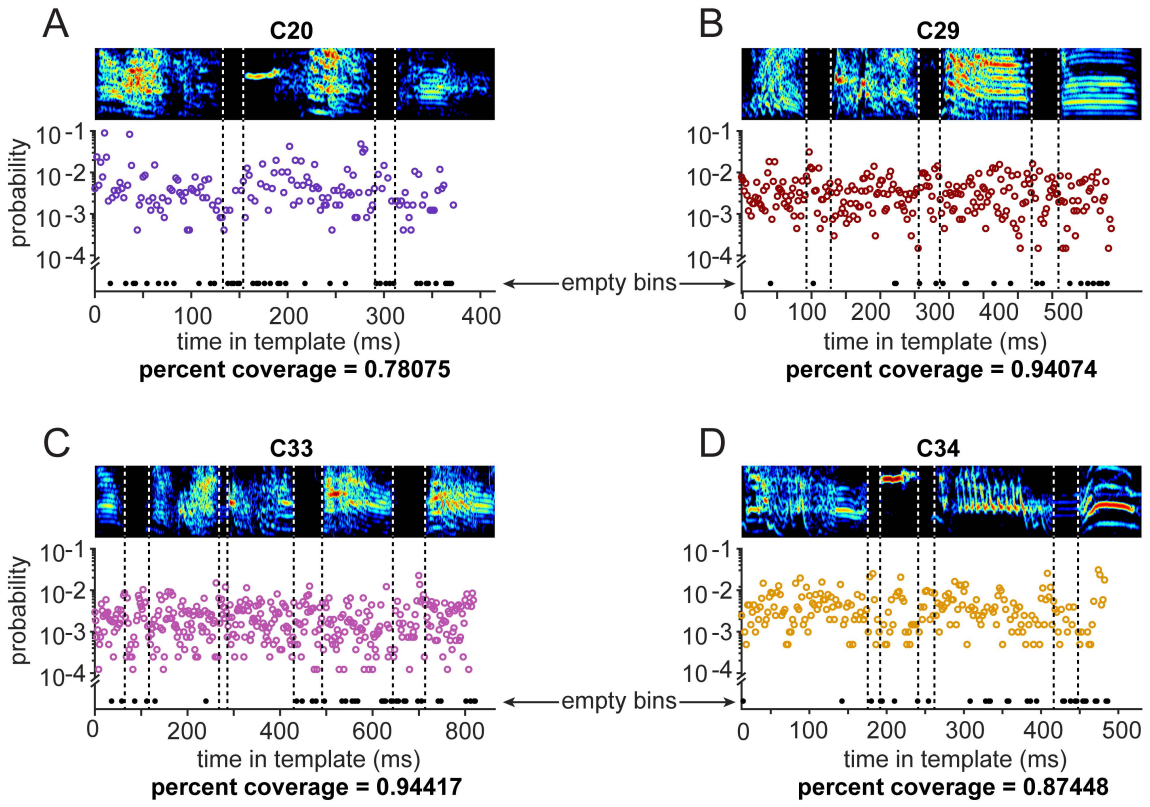

Figure S3. Replay events initiate throughout the song sequence. Related to Figure 3.
The probability of sleep replay events beginning at any timepoint throughout the song (bin size: 6 ms) for 4 birds.

Figure S4

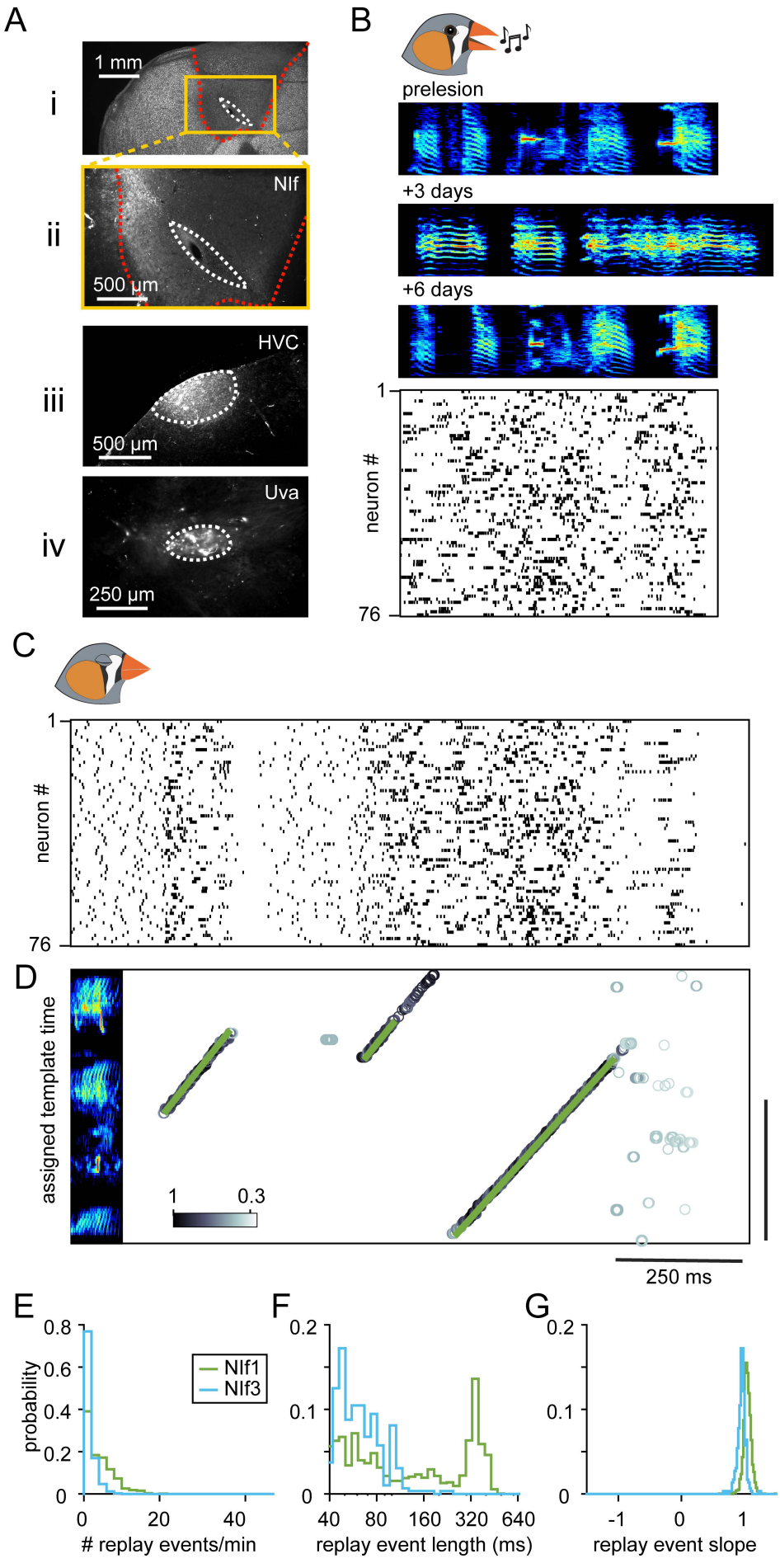
A
i
1 mm
ii
NIf
500 µm
iii
HVC
500 µm
iv
Uva
250 µm
B
prelesion
+3 days
+6 days
1
neuron #
76
C
1
neuron #
76
D
assigned template time
1
0.3
250 ms
E
probability
0.8
0.6
0.4
0.2
0
NIf1
NIf3
0
20
40
replay events/min
F
0.2
0.1
0
40
80
160
320
640
replay event length (ms)
G
0.2
0.1
0
-1
0
1
replay event slope

Figure S4. Replay persists following NIf lesion. Related to Figure 4.
(A) Histology showing NIf lesion, using NeuN (i) and dextran labeling (ii). Red dashed line shows border of NIf lesion. White dashed line represents estimated location of NIf before lesion. Dextran injection into HVC (iii) retrogradely labeled cell bodies in Uva (iv).
(B) Spectrograms of adult zebra finch songs: prior to NIf lesion (Top), 3 days postlesion (Middle) and 6 days postlesion (Bottom). Simultaneously recorded spiking activity of 76 RA neurons during song corresponding to above spectrogram (i.e., 6 days postlesion).
(C) Observed sleep activity of RA neurons shown in (B).
(D) Template matching applied to RA activity in (C). Each data point is aligned to the assigned bin of song template (y-axis) and color indicates Pearson correlation score. Detected events fitted by green line.
(E-G) Characterization of replay event frequency during each 1-minute period of the recording (E) as well as the replay length (F) and slope (G) for both NIf lesioned birds.

Figure S5

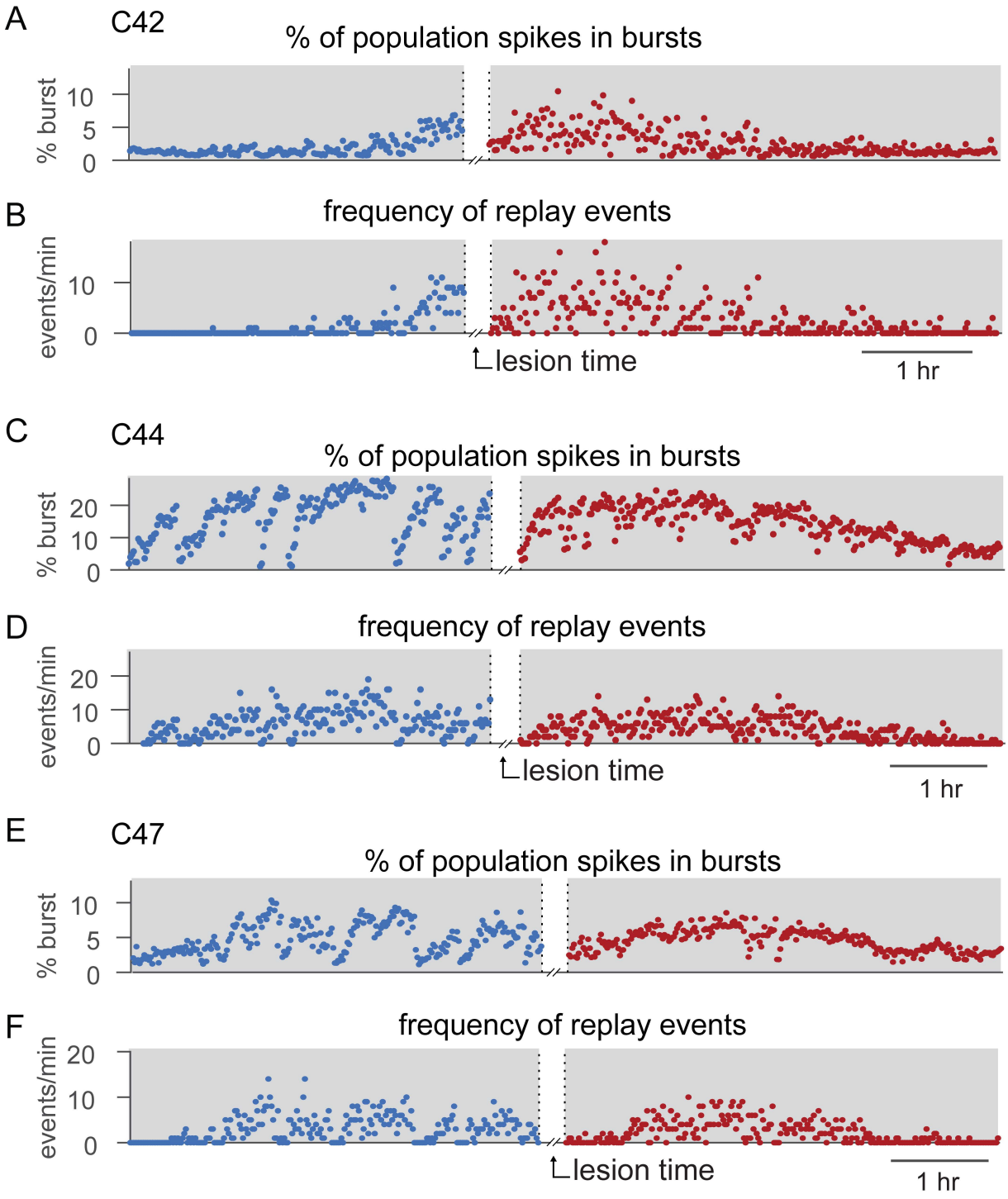

Figure S5. Population bursting and replay events before and after Uva lesion. Related to Figure 5.
Proportion of spikes from the population meeting burst criteria and detected network events for 3 birds. Lights off indicated by the shaded region.

Figure S6

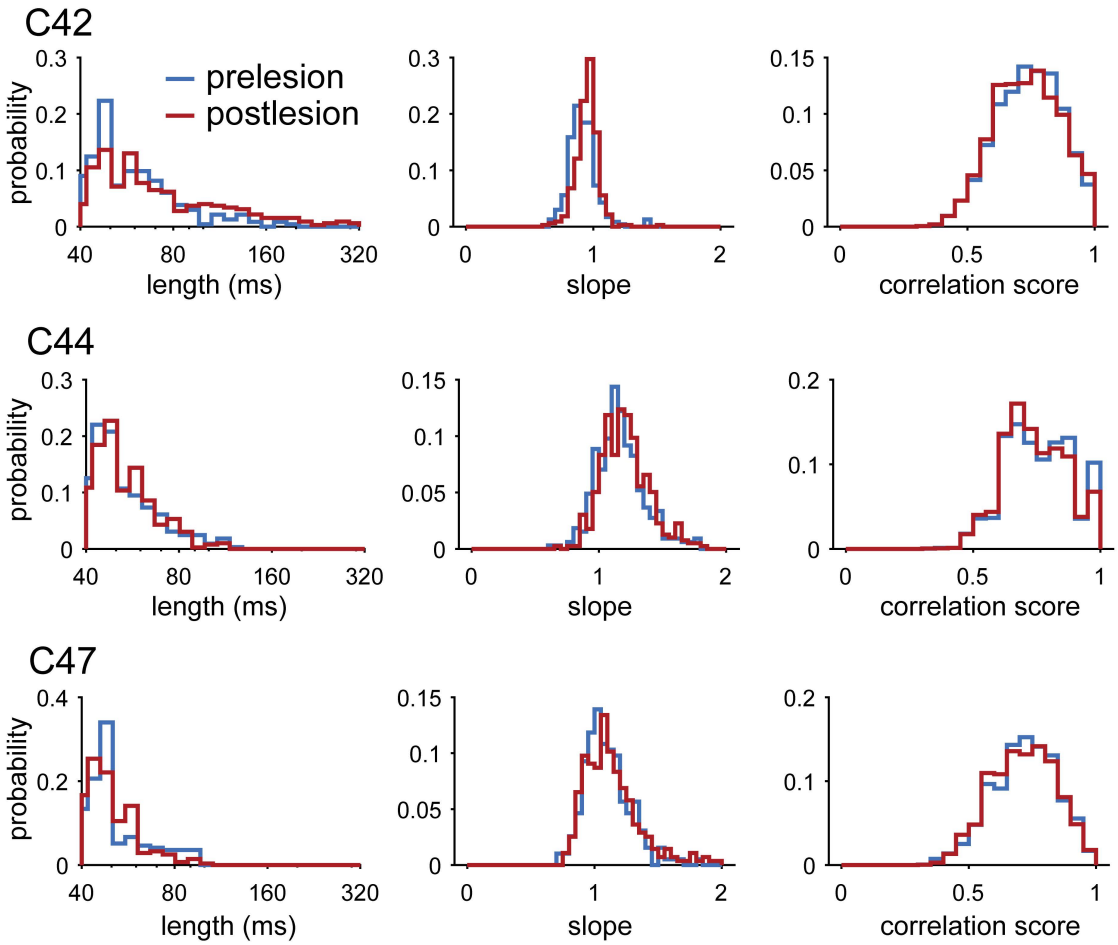

Figure S6. Characterization of detected replay events prelesion and postlesion. Related to Figure 6.
Replay event length (left), slope (middle), and Pearson correlation coefficient (right) of template matching assignments for 3 birds (C42, C44 and C47).