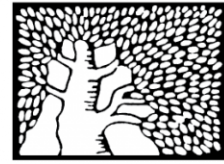


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## Affective memory rehearsal with temporal sequences in amygdala neurons

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1 **Affective Memory Rehearsal with Temporal Sequences in Amygdala Neurons**

2

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6

7

8

9 **Abstract**

10 **Affective learning and memory are essential for daily behavior, with both adaptive and**  
11 **maladaptive learning depending on stimulus-evoked activity in amygdala circuitry.**  
12 **Behavioral studies further suggest that post-association offline processing also contributes**  
13 **to memory formation. Here, we investigated spike-sequences across simultaneously**  
14 **recorded neurons while monkeys learned to discriminate between aversive and pleasant**  
15 **tone-odor associations. We show that triplets of neurons exhibit consistent temporal**  
16 **sequences of spiking activity that differed from firing patterns of individual neurons and**  
17 **pairwise correlations. These sequences occurred throughout the long post-trial period,**  
18 **contained valence-related information, declined as learning progressed, and were**  
19 **selectively present during the recent CS-US evoked activity. Our findings reveal that**  
20 **temporal sequences across neurons in the primate amygdala serve as a coding**  
21 **mechanism, and might aid memory formation by rehearsal of the recently experienced**  
22 **association.**

23

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26

27

## 28 Introduction

29 The role of the amygdala in learning with aversive and pleasant outcomes is well  
30 established<sup>1-11</sup>, and impaired processing can result in maladaptive expression and  
31 discrimination of fear from safety<sup>12-15</sup>. During learning, an initially neutral conditioned-  
32 stimulus (CS) is paired with an unconditioned-stimulus (US) to produce plasticity that  
33 enables memory formation<sup>6, 16-21</sup>, leading most studies to focus on stimuli-evoked responses.  
34 Nevertheless, behavioral studies have shown that the length of the post-trial interval  
35 contributes to the acquisition rate<sup>22, 23</sup> and that learning is diminished when introducing a  
36 novel association during that time<sup>24</sup>. Together, these results suggest a memory rehearsal  
37 mechanism<sup>24, 25</sup> that is also in-line with an amygdala-dependent fast consolidation process<sup>26-</sup>  
38 <sup>28</sup>. Despite evidence for affective-state-specific tonic responses that continue in the absence  
39 of external stimuli<sup>29, 30</sup>, it remains unclear how amygdala circuitry process specific  
40 associations after the stimuli terminated to aid memory formation. Here, we demonstrate  
41 that amygdala ensembles carry such information in timing and order of spiking activity  
42 during the post-trial offline period. The focus on the order of spikes across several neurons  
43 allows examination of a lower dimension compared to that of all possible spatiotemporal  
44 patterns, and is therefore computationally tractable.

45 We recorded neurons in the amygdala of two monkeys acquiring pleasant and aversive tone-  
46 odor conditioning on a daily basis (Fig.1A, Supplementary Fig.1A, n=119 neurons). A  
47 discriminatory conditioned-response (CR, higher inhale volume in response to the pleasant-  
48 or aversive- associated tone) occurred in 74% of the days (n=31/42, 2-way ANOVA, p<0.05  
49 for main effect of valence). The discriminatory CR developed after the first trial and  
50 progressed along the acquisition session (Fig.1B).

51 To establish the role of temporal-sequences as a coding and rehearsal mechanism, we  
52 examined all groups of three simultaneously recorded units (n=355) and tested for the  
53 following criteria: First, that the structure of the sequence occurs beyond what is expected  
54 from single-neuron activity; Second, that sequences are consistent across time; Third, that  
55 sequences are valence-specific, namely they are consistent within valence, hold information  
56 about the trial-type, and allow decoding of the trial valence; Fourth, that sequences contain  
57 more information early in learning than when memory formation is complete; Fifth, that the  
58 sequences that occur during the post-trial period also occur during the stimuli-pairing  
59 evoked activity. Finally, we used several shuffling approaches to validate that sequences  
60 occur beyond independent changes in firing rates, beyond single pairwise correlations, and  
61 used maximum-entropy models to demonstrate 3<sup>rd</sup> order correlations. Together, fulfilling  
62 these criteria would constitute evidence for the use of spatiotemporal spiking sequences for  
63 memory rehearsal in the post-trial offline epoch during learning.

64

## 65 Results

### 66 *Structure in amygdala spike sequences*

67 For an unbiased selection of time window, we quantified the number of spike-*triplets*  
68 (Fig.1C; 3-spikes across 3-neurons) in sliding windows of different sizes, and a-priori chose  
69 150ms because it captured the majority of sequences (Fig.1D). To identify triplets with

70 spatiotemporal patterns that do not result from single-unit firing-rate (FR) modulations, we  
71 compared sequences to circular shuffling of the entire spiking pattern of the neurons in a  
72 random bounded duration (between  $\pm 150$ -300ms, Extended Data Fig.1A,B). This shuffle  
73 preserves single neuron activity and destroys inter-neuron correlations, and we therefore  
74 term triplets with distinctive activity '*Structured triplets*'. Other shuffling approaches  
75 (methods, e.g. trial-shuffle) produced stronger-better results, indicating that the circular  
76 shuffle is indeed the most stringent approach.

77 We first examined whether sequences existed in amygdala triplets by comparing the actual  
78 distribution to that expected from single-neuron firing rates (Fig.1E, left and middle triplets  
79 vs. rightmost, Extended Data Fig.1B). During pre-task activity, a notable proportion of  
80 amygdala triplets exhibited significant structure (Fig.1F, 49% of  $n=355$ , Monte Carlo [MC] p-  
81 values with Benjamini-Hochberg [BH] correction for multiple-comparisons). In comparison,  
82 independent neurons recorded on different days (across-days-triplets) and independent  
83 activity of the neurons from within-day did not show structured sequences (trial shuffle,  
84 Fig.1F). A similar somewhat weaker effect (20% of  $n=104$ ,  $\chi^2_{df=1} = 27.7, p < 10^{-6}$ ) was  
85 found using only neurons recorded on different electrodes (Fig.1F, inset). This difference  
86 could partly stem from the proximity between the neurons, as physical distance between  
87 electrodes was smaller for triplets exhibiting structure compared to triplets with no  
88 structure (Extended Data Fig.2A). Moreover, the magnitude of sequence structure (structure  
89 score) was higher than that of across-days-triplets (Fig.1G; unpaired t test; all triplets:  
90  $t_{699} = 12.85, p < 10^{-35}, d = 0.97$ ; different electrode triplets:  $t_{201} = 2.16, p = 0.016, d =$   
91 0.3).

92 Importantly, we found that a large proportion of the structured triplets exhibited sequences  
93 that could not be explained even when taking into account the pairwise correlated activity  
94 of either pair (Fig.1H, Supplementary Fig.2). In addition, although we initially selected an  
95 unbiased temporal window of 150ms, we further quantified the proportion of structured  
96 triplets for different durations and found that sequences occurred even in shorter time  
97 scales (Fig.1I, 25-50ms).

98 Taken together, these results demonstrate that triplets of neurons in the amygdala exhibit  
99 sequence structures that are different than expected from single neuron as well as pairwise  
100 activity.

101

### 102 *Amygdala sequences are consistent across time*

103 If sequences are indeed used as a coding mechanism in the amygdala, they should  
104 consistently and repeatedly occur across time. To evaluate this, we compared the  
105 dissimilarity between two different time segments (Extended Data Fig.3), and identified  
106 *consistent triplets* that are more similar across time (Fig.2A, left and middle triplets vs.  
107 rightmost). A large proportion of triplets exhibited consistency (Fig.2B, all triplets: 33%;  
108 Different electrodes: 11%, MC p-value BH corrected), whereas independent across-days-  
109 triplets and within-day trial-shuffle did not show consistent sequences (Fig.2B). In  
110 accordance, the distribution of consistency scores was positively skewed (Fig.2C) and higher  
111 than that of independent across-days-triplets (unpaired t test; all triplets:  $t_{694} = 10.2, p <$

112  $10^{-22}$ ,  $d = 0.77$ ; Different electrode triplets:  $t_{198} = 2.66$ ,  $p = 0.004$ ,  $d = 0.38$ ). Here again,  
113 many triplets exhibited consistency exceeding that expected from the pairwise correlations  
114 of either pair (Fig.2D). Similarly, sequences were consistent also in shorter time scales  
115 (Fig.2E) and the physical distance was smaller for triplets exhibiting consistent activity  
116 (Extended Data Fig.2B). Finally, there was a large overlap between structured and consistent  
117 triplets (Fig.2F). These results show that spike sequences in the amygdala are consistent  
118 throughout time.

119

#### 120 *Sequences are more abundant in the Amygdala than in the dACC*

121 Next, we examined if spike-sequences during valence discriminatory learning occur more in  
122 the amygdala than in another region. We obtained recordings in the dorsal-anterior-  
123 cingulate-cortex (dACC,  $n=228$ ; simultaneously recorded triplets:  $n=564$ ; Supplementary  
124 Fig.1A), and repeated the same analyses. We found that a larger proportion of amygdala  
125 triplets were significantly structured compared to dACC triplets (Extended Data Fig.4A), with  
126 higher mean structure score (Extended Data Fig.4B). Similarly, the proportion of consistent  
127 triplets in the amygdala was larger than in the dACC (Extended Data Fig.4C), with higher  
128 mean consistency score (Extended Data Fig.4D). Because the BLA is smaller in size, we  
129 validated the analysis on triplets with similar anatomical distance in the dACC as in the  
130 amygdala and found similar results. We note that a similar proportion of dACC and  
131 Amygdala neurons exhibit FR response to the aversive and pleasant CS or US (aversive:  
132 amygdala: 11%, dACC: 8%,  $\chi^2_1 = 1$ ,  $p = 0.32$ ; pleasant: amygdala: 30%, dACC: 29%,  
133  $\chi^2_1 = 0.0008$ ,  $p = 0.97$ ) and differentiate between aversive and pleasant CS or US  
134 (amygdala: 22%, dACC: 25%,  $\chi^2_1 = 0.44$ ,  $p = 0.5$ ).

135 This strengthens the finding that Amygdala triplets exhibit spike-sequences in this context of  
136 discriminatory affective learning compared to another region, the dACC, that is also involved  
137 in affective learning and shows similar stimulus-evoked responses. The spike-sequences  
138 might also underlie previous findings of more synchronized activity in the amygdala<sup>31</sup>.

139

#### 140 *Amygdala sequences are consistent within valence*

141 After having established the existence of temporal spike sequences across neurons, we  
142 sought to examine if they code for valence during the learning of affective-associations. To  
143 examine this, we sampled the distribution of sequences during the offline post-trial epoch  
144 and identified triplets that exhibited different distributions of spike-sequence for the  
145 pleasant versus the aversive trials (Fig.2G, trials sorted by type for presentation only). To  
146 confirm such valence-specific sequences, we examined whether the similarity between  
147 sequences following trials with similar valence, is higher than that following trials of  
148 different valence.

149 For all available triplets, the mean dissimilarity between aversive-related sequences was  
150 significantly lower than the mean dissimilarity between sequences of different valence  
151 (Fig.2H), and this difference was larger than the difference in independent across-days-  
152 triplets (Fig.2H middle-left). To further control for single-neuron activity and correlations, we

153 compared consistency scores and found that their mean within valence was significantly  
154 higher than the mean score between valence, and larger than the difference in across-days-  
155 triplets (Fig.2H middle-right). Similar results were found when comparing to independent  
156 trial-shuffle triplets (Supplementary Fig.3A). As the lower dissimilarity and higher scores  
157 imply higher similarity within the aversive post-trial comparisons, these results suggest that  
158 a significant subset of the triplets exhibit aversive specific sequences.

159 Similarly, the mean dissimilarity between distributions of pleasant-related sequences was  
160 lower than the mean dissimilarity between sequences of different valence (Fig.2I), also  
161 compared to independent across-days-triplets (Fig.2I middle-left), and the mean consistency  
162 score was higher within the pleasant post-trial epochs than between sequences of different  
163 valence, also compared with across-days-triplets (Fig.2I middle-right). Here again, similar  
164 results were found for trial-shuffle control (Supplementary Fig.3B). As for aversive, these  
165 results suggest that a significant subset of the triplets exhibit pleasant specific sequences.

166 Therefore, a significant proportion of triplets of amygdala neurons produce sequences that  
167 are specific to the valence of the recently presented (learned) association.

168

#### 169 *Sequences hold information about recent valence associations*

170 To test if sequences hold information about aversive vs. pleasant associations of the recent  
171 trial, we examined the difference between decoding of valence using the sequences and  
172 decoding based on independent neurons (Supplementary Fig.4). We found that 20% of  
173 amygdala triplets were able to decode the valence of the previous trial above chance level  
174 (Extended Data Fig.5D, binomial test for each triplet, BH corrected). This proportion of  
175 correctly classifying triplets (20%) was higher than the proportion in independent across-  
176 days-triplets (Fig.3A, 6.7%), higher than in trial-shuffle data (Extended Data Fig.5A, MC p-  
177 value, all triplets:  $p = 0.024$ ) and higher compared to the dACC (0.5%,  $\chi^2_{df=1} = 111, p <$   
178  $10^{-10}$ ). Similarly, the mean decoding hit rate was higher than in trial-shuffle triplets  
179 (Extended Data Fig.5B). We also found that these sequences differ from pre-task sequences,  
180 namely before associative-learning started (Extended Data fig.5C), because decoding based  
181 on valence-related triplets allowed correct discrimination between post-trial activity and  
182 pre-task activity ( $n = 71$ , BH corrected [FDR<0.05], aversive: 90%, pleasant: 45%). Note that  
183 there was no stereotypic or preparatory inhale behavior during this post-trial period  
184 (Supplementary Fig.5).

185 Notably, the discrimination was achieved using sequences that occur long after the stimuli  
186 terminated (2-12sec after the CS and the US). Moreover, a high proportion of decoding  
187 triplets exhibited stable decoding for more than 25 seconds after US offset (Fig.3B, ranging  
188 from 15-35%,  $p < 0.05$ , one-tailed  $\chi^2$  test) and this decoding was enabled by similar  
189 sequences across different times (Supplementary Fig.6).

190 Interestingly, highly discriminating triplets achieved better decoding than inter-spike-  
191 intervals (ISI) or firing-rates, as the mean hit rate was higher based on sequences than on ISI  
192 (Extended Data Fig.5D,  $n=76, 130$  respectively, one tailed independent t-test:  $t_{204} = 1.69,$   
193  $p = 0.046, d = 0.24$ ). Similarly, sequence-based decoding achieved higher hit rates than ISI  
194 or FR based decoding in up to 10% of the significant triplets (Fig.3C, 28% of sequence-

195 significant triplets, one-tailed Wilcoxon rank-sum test,  $p < 0.05$ ). This benefit was not  
196 observed in independent across-days-triplets or in trial-shuffle triplets (Fig.3C, insets).

197 These results show that a significant proportion of the triplets hold more information than  
198 independent firing patterns and that this information is available long after the stimulus has  
199 terminated.

200

#### 201 *Sequences hold more information in early than in late learning*

202 If temporal sequences are used to strengthen the learning of a recent association, their  
203 information should fade as learning progresses and the memory strengthens, in a teaching-  
204 signal like manner. Indeed, repeating the decoding with ten trials extracted from different  
205 phases of the learning (Fig.3D,E), we found a higher hit rate in the initial phase of acquisition  
206 (trials 1-10) compared to the intermediate phase (trials 11-20, Bonferroni corrected sign-  
207 rank test,  $Z = 5.85, p < 10^{-8}$ ), and compared to the final phase (Fig. 3D,E, trials 21-30,  
208  $Z = 6.47, p < 10^{-9}$ ). This decline in decoding performance was not due to changes in FR or  
209 ISI distributions (Supplementary Fig.7), or a result of changes in local-field-potential (LFP)  
210 that could point to a different overall brain-state (Supplementary Fig.8). Furthermore, we  
211 quantified trial-by-trial decoding performance (proportion of correct classification across  
212 triplets) and found a negative correlation with the mean conditioned response (CR, Fig.3F,  
213 rank-order correlation  $r = -0.41$ , resampling p-value:  $p = 0.016$ ,  $n=29$ ; when removing the  
214 first trial- bottom dot- as outlier:  $r = -0.44$ ,  $p = 0.008$ ).

215 To further demonstrate that this reduction occurs also in information in addition to the  
216 decoding approach, we calculated the mutual information (MI) between sequence activity  
217 and recent trial valence (29% of  $n=328$  triplets contain significant information about valence,  
218 BH corrected permutation test). Here also, we found that the proportion of triplets with  
219 significant MI during the initial phase of learning (46% of  $n=258$ ) was larger compared to the  
220 final stage (Fig.3G-I, 26% of  $n=269$ ,  $\chi^2$  test for independence,  $\chi^2_{df=1} = 22.3, p < 10^{-5}$ ), as  
221 well as a significant reduction in MI between the initial and later phases (Fig.3G-I, initial vs.  
222 intermediate:  $n=249$ , Bonferroni corrected sign-rank test,  $Z = 6.53, p < 10^{-9}$ ; initial vs.  
223 final:  $n=243$ ,  $Z = 3.74, p < 0.001$ ; intermediate vs. final:  $n=254$ ,  $Z = -2.03, p = 0.064$ ).  
224 These results were specific to the task-related information, as the overall number of  
225 sequences did not decrease along the learning (Fig.3D-top inset).

226 We conclude that sequences hold information in the post-trial epoch when the association is  
227 still being acquired and this information decreases as learning progresses, a characteristic of  
228 a memory-rehearsal process.

229

#### 230 *Trial-specific sequences are repeated in the post-trial epoch*

231 Finally, if the sequences indeed serve as a post-trial rehearsal mechanism, then we can  
232 hypothesize that the same sequences should be present also in evoked responses during the  
233 CS-US presentation (Fig.4A). We therefore examined whether post-trial valence-specific  
234 sequences occurred also during the preceding CS-US presentation. We repeated the  
235 decoding approach that was trained on post-trial sequences only, but this time tested the

236 performance on activity during the CS-US presentation. Post-trial triplets that significantly  
237 decoded preceding trial valence ( $p < 0.05$ ,  $n=101$ ), displayed an average hit rate  
238 significantly above chance also when tested on CS-US evoked activity (Fig.4B, inset). This hit  
239 rate was also higher than the hit rate in triplets with no post-trial decoding (Fig.4B, inset,  
240  $n=254$ ). Accordingly, there was a positive correlation between the post-trial decoding and  
241 the hit rate based on activity during the CS-US presentation (Fig.4B, Spearman rank-order  
242 correlation:  $r = 0.28$ ,  $p < 10^{-6}$ ). This suggests that some of the sequences that occur in the  
243 stimulus-evoked activity are later repeated during post-trial activity.

244 To demonstrate this more directly, we examined the occurrence of aversive or pleasant -  
245 specific post-trial sequences during CS-US related activity. For each triplet, we identified  
246 aversive-/pleasant- specific sequences in post-trial epochs (Fig.4C) and quantified their  
247 presence in evoked activity during the CS-US presentation. As expected, aversive-specific  
248 post-trial sequences were more abundant in aversive CS-US activity (Fig.4D, one tailed sign-  
249 rank test:  $Z = 2.7$ ,  $p = 0.004$ ) whereas pleasant-specific post-trial sequences were more  
250 frequent in pleasant CS-US activity (Fig.4D,  $Z = 3.89$ ,  $p < 10^{-4}$ ). This rehearsal activity did  
251 not exhibit itself in pre-task activity (Supplementary Fig.9).

252 Together, these results suggest that a portion of post-trial sequences are repetitions of the  
253 activity that occurs during the acquisition trial, implying a rehearsal mechanism for the  
254 recent association in post-trial activity.

255

#### 256 *Maximum entropy (ME) models validate the role of sequences*

257 To further demonstrate sequence activity in triplets, we fitted two types of ME models<sup>32-35</sup> to  
258 amygdala activity. We first implemented the standard spatial model fitted on simultaneously  
259 recorded quadruplets of neurons in order to quantify the gain obtained by using triplets  
260 compared to pairwise (Extended Data Fig.6A, 'spatial-ME',  $n=358$ ). In addition, because the  
261 spatial model does not consider the order of spikes in a triplet, we further developed a novel  
262 ME model to examine the sequential activity of triplets (Extended Data Fig.6B, 'Sequence-  
263 ME',  $n=291$ ). For both models, we re-tested structure, consistency, decoding, and CS-US  
264 rehearsal.

265 We computed the independent, pairwise and triple-wise models on the data of individual  
266 trials (Fig.5A,B). We then quantified the reduction in total entropy due to the pairwise and  
267 triple-wise correlations, reflecting the contribution of these interactions to the overall  
268 activity. There was a significant contribution of triple-wise interactions to the reduction in  
269 entropy, beyond that expected from pairwise activity (Fig.5C,D, comparing to surrogate data  
270 sampled from the pairwise ME model, and see Supplementary Fig.10A,B for pairwise vs.  
271 independent). These results strengthen the conclusions of sequence-structure in triplets,  
272 demonstrating a triple-wise interaction in the sequences.

273 To assess consistency, we compared the JSD dissimilarity between the model in one time-  
274 segment and the data in another time-segment. We first identified groups with pairwise  
275 consistent activity (see methods), and found that in 15.3% of these quadruplets (Spatial-ME,  
276  $n=13/85$ ) and in 26.5% of these triplets (Sequence-ME,  $n=81/307$ ), the dissimilarity in the



277 triple-wise model was smaller than in the pairwise model (BH corrected,  $FDR \leq 0.05$ ). These  
278 results further demonstrate consistency in triplets of neurons.

279 For valence decoding from post-trial activity, we found better performance of the triple-wise  
280 compared to the pairwise model in the spatial-ME (Fig.5E). Similarly, there was a trend in the  
281 sequence-ME for higher performance of the triple-wise compared to the pairwise model  
282 (Fig.5F, and see Supplementary Fig.10C,D for pairwise vs. independent).

283 Finally, we compared the decoding of CS-US activity from the model trained on post-trial  
284 activity (as in Fig.4B). In the spatial-ME, the hit rate for decoding CS-US activity from the  
285 triple-wise and pairwise models were higher than the independent model (Extended Data  
286 Fig.7A). In the sequence-ME, the hit rate of the triple-wise model was higher than that of the  
287 independent model (Extended Data Fig.7B), and higher than the pairwise model.

288 These results further support the findings of the shuffle approach, and hence the notion that  
289 sequence activity during CS-US presentation is repeated during the post-trial period.

290 We also validated that the main findings are not different between putative excitatory  
291 projection cells and interneurons (Supplementary Fig.11), and further cannot be explained  
292 by unit-isolation (Supplementary Fig.12), non-stationarity of firing-rates (Extended Data  
293 Fig.8), or short phasic FR modulations and correlations (Supplementary Fig.13).

294

## 295 **Discussion**

296 Overall, our findings show that temporal sequences across multiple amygdala neurons  
297 maintain information about discriminatory valence associations. We find that specific  
298 sequences exist at baseline, as structure and consistency of triplet sequences were identified  
299 during pre-task activity and beyond pairwise and independent (firing-rate) patterns. In  
300 addition, sequences further develop according to trial valence when conditioning begins,  
301 suggesting a coding mechanism. Because these sequences were identified during the long  
302 post-trial periods, diminished as learning progressed (similar to a teaching signal), and were  
303 repetitions of CS-US evoked sequences, they likely serve as a rehearsal mechanism of the  
304 recently acquired association. This is a first demonstration of post-trial rehearsal during  
305 learning in amygdala neurons, and of coding with temporal sequences across several  
306 neurons in this circuitry. It suggests that the affective association is repeated to enhance  
307 synaptic plasticity<sup>20, 21, 36, 37</sup>, and moreover, the short time-scales of sequences compared to  
308 the CS-US gap might reconcile previous debates about plasticity constraints during the  
309 pairing itself<sup>21</sup>.

310 Although it is reminiscent of offline replay in the hippocampus<sup>38, 39</sup>, there is a major  
311 difference between the findings. In the hippocampus, specific cells increase firing rates at  
312 specific spatial locations along the behavioral trajectory<sup>40</sup>, so that the ordering of single-cell  
313 activity is behaviorally imposed and a time compressed sequence is repeated offline<sup>38</sup>.  
314 Affective conditioning does not impose external ordering, implying a different rehearsal  
315 mechanism and further introducing a technical difficulty to detect these sequences.

316 Our findings in triplets that exceed pairwise-correlations therefore point to a spatiotemporal  
317 code<sup>41-45</sup> and a first demonstration for its role during affective learning in the primate.

318 Therefore, the results suggest that associations are not encoded solely by firing rate (FR)  
319 changes, but also by sequences of spikes that are rehearsed offline to enhance learning.  
320 Although circuit mechanisms that can generate such reliable sequences and their readout  
321 are yet to be demonstrated conclusively, such ordinal activity as we identify here can result  
322 from the sparse sampling of three neurons (as the case in extracellular recordings) from  
323 three different yet connected sub-populations. This is in line with the varying and relatively  
324 long temporal lags of dozens of ms we observed between the spikes. In such a case, our  
325 findings are consistent with many studies showing phasic changes in FR synchrony across  
326 subpopulations of neurons<sup>46</sup>. Together with our findings that the reported activity exceeds  
327 short time-scale FR modulations, we argue that spike-sequences are the best explanation for  
328 the results presented here.

329 The sequence code and rehearsal, as well as the large proportion of triplets, suggest that  
330 they are part of a larger memory-coding ensemble in the amygdala<sup>11, 47-50</sup>. It remains to be  
331 seen how such larger ensembles are activated during learning and how they are enhanced or  
332 constrained by temporal patterns as shown here. Overall, we conclude that temporal-  
333 sequences in primate amygdala neurons replay recent affective associations between trials  
334 to aid memory formation.

335

336

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343

### 344 **Author Contributions**

345 T.R.S and R.P conceived and designed the experiments; T.R.S. planned and performed the  
346 analyses; T.R.S and R.P. wrote the manuscript.

347

### 348 **Competing interests**

349 The authors declare no competing interests.

350

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**Figure 1. Experimental setup and Structure of spatiotemporal sequences in the amygdala**

460 (A) Each trial began with a pure tone, followed by an aversive (Propionic acid) or pleasant  
461 (banana and melon organic extract) odor. Analyses were performed prior to any stimuli  
462 ('baseline activity') and during post-trial epochs, starting 2 seconds after the termination of  
463 odor delivery. Shown also is an example raster plot of a single amygdala neuron during 5  
464 seconds of the post-trial epoch without any external stimuli.

465 (B) The mean conditioned response (CR, measured as difference in full width at half  
466 maximum [FWHM] of inhale duration, see methods) showed fast initial learning (bottom  
467 inset), and progression along the session (main panel, discriminatory days, n=31, trials 1-10  
468 vs. trials 11-20, one tailed paired t-test,  $t_{df=30} = -2.13$ ,  $p=0.021$ ,  $d=0.21$ ; trials 1-10 vs. 21-  
469 30,  $t_{df=30} = -2.2$ ,  $p=0.018$ ,  $d=0.25$ ; and trials 11-20 vs. 21-30,  $p=0.27$ ). Top inset: single  
470 inhalation example (CR) with shorter inhale duration upon presentation of the pleasant  
471 (purple) compared to the aversive (red) conditioned stimuli (CS). FWHM are marked by  
472 corresponding dashed lines.  $\Delta$ FWHM score takes the absolute value of the changes, so  
473 inhale volume can change in either direction (see methods).

474 (C) Estimating the probability distribution of three spike-sequences of three neurons. Left:  
475 Surrogate example of voltage traces from three neurons. The boxes symbolize a running  
476 window that starts with a spike in any of the neurons. A sequence is counted if three spikes  
477 occurred within the time window. Right: the estimated sequence probability distribution.

478 (D) Proportion of three spikes sequences within a time duration for amygdala triplets during  
479 the post-trial epoch. Dashed line: the unbiased a-priori chosen time duration used  
480 throughout the study unless specifically mentioned otherwise (150ms).

481 (E) Examples of structured (two left examples) and non-structured triplets (right). Top: mean  
482 data (blue) and shuffled data (green) sequence probability distributions, sorted by the  
483 shuffled distribution (log scale). The data and shuffled distributions are different in the  
484 structured triplets ( $p=0.002$ , right tailed Monte Carlo) and similar in the non-structured  
485 triplet ( $p=0.8$ ). The shaded areas represent standard error of the mean (SEM) over 10s time  
486 segments ( $n=30$ ), averaged over shuffled instances. Bottom: In the structured triplets, the  
487 mean Jensen-Shannon-divergence (JSD) dissimilarity between shuffled data sequences  
488 ( $\bar{D}_{1,2,\dots,500}$ , green histogram) is smaller than the mean dissimilarity between the data and the  
489 shuffled sequences ( $\bar{D}_{data}$ , blue line).

490 (F) Distribution of p-values (right tailed Monte Carlo, as in E) for all simultaneously recorded  
491 triplets (blue,  $n=355$ ), independent across-days-triplets (gray,  $n=355$ ) and independent trial-  
492 shuffle control (turquoise,  $n=355$ ). Many simultaneously triplets showed significant structure  
493 ( $p \leq 0.05$ ). Inset: triplets from different recording electrodes ( $n=104$ ).

494 (G) Frequency of scores for simultaneously recorded triplets (blue), independent across-  
495 days-triplets (gray) and independent trial-shuffle control (turquoise). The right tail of the  
496 simultaneously recorded distribution suggests that many triplets exhibit structure that is  
497 highly different from single neurons. Inset: triplets from different recording electrodes.

498 (H) Proportion of significantly structured triplets beyond either of the three pairwise  
499 activities (i.e. compared to all three single unit shuffles, right tailed Monte Carlo, as in E,  
500  $p < 0.05$  for all three,  $n=195$ ). The proportion was significantly higher than chance (dashed  
501 black) for all triplets (38%,  $75/195$ ,  $\chi^2$  test for goodness of fit for  $p=0.05$   $\chi^2_{df=1} = 459$ ,  $p <$   
502  $10^{-20}$ ) as well as for triplets recorded on different electrodes (14%,  $\chi^2_{df=1} = 4.72$ ,  $p = 0.03$ ).  
503 Note that these triplets are structured beyond pairwise activity of single pairs (third order  
504 structure is demonstrated in Fig.5D).

505 (I) Proportion of structured triplets as a function of maximal sequence durations ( $n=355$ ).

506 Error bars: standard error of the mean (SEM).

507 In all panels error bars mark the standard error of the mean (SEM);  
508 \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$

509 **Figure 2. Consistent amygdala sequences throughout time and within valence.**

510 (A) Consistent (two left examples) and non-consistent (right) triplets. Top: data (blue) and  
511 shuffled data (green) sequence probability distribution of the two subdivisions (as  
512 exemplified in the left blue bar,  $n=15$  for each). In the two left examples the similarity  
513 between the solid blue and dashed line shows that the data sequences are similar to each  
514 other. The difference between these blue lines and the green lines shows that the data  
515 sequences are different from the shuffled sequences ( $p=0.002$ , left tailed Monte Carlo). In  
516 the right example the data sequences are similar to the shuffled sequences ( $p = 0.83$ ).  
517 Bottom: histogram of mean JSD dissimilarity between the data and shuffled sequences  
518 ( $\bar{C}_{1,2...500}$ , green) and a line indicating the dissimilarity between the data sequences  
519 ( $\bar{C}_{data}$ , blue). The higher similarity between the data sequences suggest that they are  
520 consistent.

521 (B) Distribution of p-values for all possible simultaneously recorded triplets (blue,  $n=355$ ),  
522 independent across-days-triplets (gray,  $n=355$ ) and independent trial-shuffle control  
523 (turquoise,  $n=355$ ). Many simultaneously recorded triplets showed significant consistency ( $p$   
524  $\leq 0.05$ , right tailed Monte Carlo, as in A). Inset: triplets from different recording electrodes  
525 ( $n=104$ ).

526 (C) Frequency of consistency scores of simultaneously recorded triplets (blue), independent  
527 across-days-triplets (gray) and independent trial-shuffle control (turquoise). Inset: triplets  
528 from different recording electrodes. Scores are larger for simultaneously recorded triplets,  
529 indicating consistent sequences.

530 (D) Proportion of triplets significantly consistent beyond expected from either of the three  
531 pairwise activities (i.e. compared to all three single unit shuffles, right tailed Monte Carlo, as  
532 in A,  $p<0.05$ ). The proportion was significantly larger than chance level (dashed black) for all  
533 triplets (20%,  $28/139$ ,  $\chi^2$  test for goodness of fit for  $p=0.05$ ,  $\chi^2_{df=1} = 67$ ,  $p < 10^{-15}$ ) and for  
534 triplets from different recording electrodes (15%,  $\chi^2_{df=1} = 4.21$ ,  $p = 0.04$ ).

535 (E) Proportion of consistent triplets as a function of maximal sequence durations ( $n=355$ ).

536 (F) The number and overlap between structured (pink) and consistent (purple) - triplets.

537 (G) Two examples of amygdala triplets with different sequence distributions in aversive (red)  
538 and pleasant (purple) post-trial epochs. Upper sections: sequence probability distributions  
539 averaged over all trials (mean and SEM). Lower sections: color maps of sequence probability  
540 distributions of single trials in the pleasant (top half) and aversive (lower half). Note that  
541 pleasant-aversive separation is only for presentation purposes; trials were interleaved.

542 (H) Comparison of JSD dissimilarity and consistency scores between sequence probability  
543 distributions estimated in the post-trial of two halves of the aversive trials ('aversive',  $n=15$   
544 vs. 15) and between the sequence probability distributions estimated in post-trial epoch of  
545 half of the aversive trials and half of the pleasant trials ('between').

546 Top: Single triplets' JSD (mean and SEM over subdivisions) between aversive-related  
547 sequences (x axis) and between aversive and pleasant related sequences (y axis). The JSD of  
548 many triplets is above the black identity line, implying higher similarity between aversive-

549 related sequences compared to the similarity across valence. Right top corner: histogram of  
550 differences between the two JSD.

551 middle-left: The mean JSD over all triplets between aversive-related sequences ('within day',  
552 red) was smaller than the mean JSD between aversive and pleasant related sequences  
553 ('within day', pink, one tailed paired t-test:  $t_{df=354} = -3.53, p < 10^{-3}, d_{Cohen} = 0.12$ ),  
554 beyond the difference in the across-days-triplets control ('across days', red and pink, 2X2  
555 mixed model ANOVA within stimulus valence and between triplet type; interaction  
556  $F_{df=1} = 13.7, p < 10^{-3}$ ). Shaded area: triplets from different recording electrodes.

557 Bottom-left: Violin plot of the difference between the JSD of aversive related sequences  
558 (corresponding to the red bar in the middle-left plot) and the JSD between aversive and  
559 pleasant related sequences (pink bar) for individual triplets (black dots, n=355). The colored  
560 surface marks the kernel density estimate of the corresponding probability distribution, the  
561 thick gray line marks the interquartile range and the black dashed line marks mean  
562 difference.

563 middle-right: The mean consistency score over all triplets between aversive-related  
564 sequences ('within day', red) was larger than the mean consistency score between aversive  
565 and pleasant related sequences ('within day', pink, one tailed paired t-test:  $t_{df=354} =$   
566  $2.71, p < 0.01, d_{Cohen} = 0.1$ ), beyond the difference in the independent across-days-triplets  
567 ('across days', red and pink, 2X2 mixed model ANOVA within stimulus valence and between  
568 triplet type; interaction  $F_{df=1} = 4.26, p = 0.04$ ). Shaded area: triplets from different  
569 recording electrodes.

570 Bottom-right: Violin plot of the difference between the consistency score of aversive related  
571 sequences (corresponding to the red bar in the middle-right plot) and the consistency score  
572 of aversive and pleasant related sequences (pink bar) for individual triplets (black dots,  
573 n=355). Violin elements are as in the bottom left panel.

574 (I) Arranged as (H) for the pleasant trials.

575 middle-left: within day t-test:  $t_{df=354} = -4.37, p < 10^{-5}, d_{Cohen} = 0.18$ ;  
576 interaction:  $F_{df=1} = 5.7, p < 0.05$ .

577 middle-right: within day t-test:  $t_{df=354} = 4.32, p < 10^{-4}, d_{Cohen} = 0.17$ ; interaction  
578  $F_{df=1} = 10.1, p < 0.01$ ,

579 In all panels error bars and shaded area mark SEM, \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$



580 **Figure 3. Sequence based decoding and information in the post-trial epoch.**

581 (A) Proportion of triplets with higher-than-chance hit rate (binomial test for each triplet, BH  
582 corrected, false discovery rate [FDR]≤0.05) was larger for within day triplets than  
583 independent across-day-triplets ( $\chi^2$  test for independence:  $\chi^2_{df=1} = 26.8, p < 10^{-6}$ ). Inset:  
584 triplets from different recording electrodes ( $\chi^2_{df=1} = 5.15, p = 0.023$ ).

585 (B) Proportion of triplets with significant decoding performance ( $\chi^2$  test) as a function of  
586 time from stimulus offset, calculated on 5 seconds running window (with 4 seconds overlap,  
587  $n = 355$ ). At all times, the proportion was significantly higher than chance (dashed line).

588 (C) Mean decoding hit rate as a function of the proportion of triplets included. Triplets  
589 ( $n=193$ ) are sorted in a descending manner based on hit rates of: sequences distribution  
590 (blue), ISI distribution (green, solid) and FR distributions (green, dotted). The hit rate of high  
591 performance triplets was higher based on sequences compared to ISI and FR (significance  
592 marked by black dots). Top left inset: across-days control ( $n=201$ ). Bottom right inset: trial-  
593 shuffle control (mean over  $n=250$  repetitions).

594 (D) Mean decoding hit rate as a function of acquisition trials in a session ( $n=355$ ). The hit rate  
595 was significantly higher in the first 10 trials of learning. Left bottom inset: boxplot of the hit  
596 rates as a function of acquisition trials, normalized (Z-score) for each triplet along the  
597 acquisition trials. Right top inset: the overall sequence rate averaged over all days.

598 (E) Hit rate of individual triplets in the first vs. last 10 trials (x and y axes, respectively). Blue:  
599 all triplets; purple: triplets with significant decoding performance for the entire day. Most  
600 triplets are below the black dashed identity line, suggesting higher hit rate early in learning.

601 (F) Trial by trial CR is negatively correlated with the proportion of classifying triplets in each  
602 trial (2-trials smoothing,  $n=29$ ). Dashed line: linear regression ( $r = -0.44, p = 0.008$ ).

603 (G) Mutual information in triplets (MI, mean and SEM, left y axis, blue,  $n=328$ ) and  
604 proportion of triplets with significant MI (right y axis, pink) as a function of learning trials.  
605 Inset: boxplot of MI as a function of acquisition trials, normalized (Z-score) for each triplet  
606 along the acquisition trials.

607 (H) Same as (G) for MI rate (mean and SEM), i.e. bits per second.

608 (I) MI of individual triplets in the first vs. last 10 trials (x and y axes, respectively). Blue: all  
609 triplets ( $n=243$ ); pink, dark purple, light purple: triplets with significant information for trials  
610 1-10, 21-30 and both phases, respectively. Most triplets are below the black dashed identity  
611 line, suggesting higher information early in learning.

612 In all box plots, boxes include 25 to 75 percentile with the median marked by the middle  
613 line, whiskers mark the last data point within 1.5 interquartile range from the median.

614 In all panels error bars and shaded area mark SEM, \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$

615 **Figure 4. Valence-specific post-trial sequences are repetitions of sequences that occurred**  
616 **during CS-US presentations.**

617 (A) Example of CS-US evoked firing rate response and post-trial activity. Top panels: Raster  
618 plot and PSTH of a single amygdala neuron in response to pleasant (purple) and aversive  
619 (red) CS (top left panel), US (top right panel) and post-trial activity (bottom panel).

620 (B) Valence-decoding from trial (CS-US) sequences can be achieved based on valence-specific  
621 post-trial sequences. Main panel: decoding hit rate tested on CS-US sequences (but trained  
622 on post-trial-sequences; y-axis) is positively correlated with post-trial (train and test)  
623 decoding hit rate (x-axis, n=355). Purple and blue: triplets with significant/non-significant  
624 post-trial decoding, and a significant linear regression line (black dashed). Inset: mean hit  
625 rate for decoding CS-US valence (from post-trial training) for significant post-trial triplets  
626 (purple, n=101) is significantly higher than chance level (gray, one sample t-test,  $t_{df=100} =$   
627  $4.9, p < 10^{-5}, d = 0.43$ ) and higher than post-trial non-significant triplets (blue, n=254,  
628 independent samples t-test,  $t_{df=353} = 5.48, p < 10^{-7}, d = 0.45$ ). Notice that this analysis  
629 does not require cross-validation as the training sequences are taken from post-trial activity  
630 and the test sequences are taken from trial (CS-US) activity.

631 (C) Two single triplet examples of aversive and pleasant-specific post-trial sequences. Top  
632 part: sequence probability distribution for aversive (red) and pleasant (purple). Bottom part:  
633 sequence probability ratio ( $P(\text{seq}|\text{aversive})/P(\text{seq}|\text{pleasant})$ ). Differentiating sequences  
634 for aversive-specific (red rectangle) and pleasant specific (purple rectangle) were selected  
635 for each triplet. The sum of proportions of these example sequences in CS-US activity is  
636 marked by full (top example) and dashed (bottom example) gray squares in (D), where the  
637 proportions of pleasant specific sequences are marked by purple dots and aversive by red  
638 dots.

639 (D) For each post-trial decoding triplet (n=101), the sum of proportion of sequences that are  
640 associated with aversive (red) or pleasant (purple) post-trial activity out of all sequences  
641 present during aversive (x-axis) and pleasant (y-axis) CS-US pairings. For example, the  
642 sequences [231, 133, 112], were aversive-specific in post-trial activity (bottom example in  
643 [C]). The summed proportion of these sequences in aversive CS-US activity (0.34) was higher  
644 than the summed proportion in pleasant CS-US activity (0.27). Across all post-trial decoding  
645 triplet, aversive post-trial sequences were more frequent during aversive CS-US pairings  
646 (below the dashed black identity line) whereas pleasant post-trial sequences were more  
647 frequent in pleasant CS-US pairings (above the identity line). Main panel: Using three  
648 aversive-specific and three pleasant-specific post-trial sequences. Inset: histogram of  
649 differences between the two proportions. Bottom left/right: using 2/4 valence-specific  
650 sequences, respectively.

651 In all panels error bars and shaded area mark SEM, \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$

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654 **Figure 5. Maximum Entropy (ME) models support structure, consistency, coding and**  
655 **rehearsal in triplets.**

656 (A) Spatial-ME model. Left: a quadruplet with triple-wise correlations. Right: a quadruplet  
657 with pairwise but not triple-wise correlation. The probability of each word (Extended Data  
658 Fig.6) in each time segment ( $n=30$ ) is plotted for the independent (blue), pairwise (orange)  
659 and triple-wise (yellow) models as a function of the probabilities in the real data of the  
660 quadruplet. In the left panel, the triple-wise model probabilities are proximate to the black  
661 dashed identity line while the others are scattered, indicating that only the triple-wise model  
662 is a good predictor of the data. Accordingly, the proportion of reduction of entropy due to  
663 the triple-wise interactions ( $I_{(3)} / I_N$ ) is high. In the right panel, the independent model  
664 probabilities are scattered while the pairwise and triple-wise are proximate to the identity  
665 line, as both are good predictors of the data. Accordingly, the proportion of reduction of  
666 entropy due to the triple-wise interactions ( $I_{(3)} / I_N$ ) is low. Insets: JSD dissimilarity between  
667 the probability distributions of the data and the distributions of each model for each time  
668 segment. The reduction in entropy is calculated as  $I_{(3)} = H_2 - H_3$  and the multi  
669 information,  $I_3 = H_1 - H_3$ , where  $H_k$  is the entropy of the  $k$ 'th order of the model.

670 (B) Sequence-ME model in triplets. Same presentation as in (A).

671 (C) Spatial-ME model ( $n=358$ ). Proportion of reduction in entropy due to the triple-wise  
672 correlations ( $I_3/I_N$ ) for the real data (x-axis) and for surrogate data sampled from the  
673 pairwise ME distribution (pairwise-surrogate control, y-axis). This surrogate data preserves  
674 pairwise correlations, as it is sampled from the pairwise ME model, but any third order  
675 correlations are random. Therefore,  $I_3/I_N$  in the pairwise surrogate is the reduction in  
676 entropy expected by chance. The reduction in entropy due to the triple-wise correlations is  
677 larger for the real data (below the black dashed identity line, paired t test between medians  
678 across trials:  $t_{357} = 25.93, p < 10^{-20}, d = 0.78$ ), indicating that triple-wise correlations  
679 explain the variability beyond expected from pairwise correlations. Inset: means and SEM  
680 over all quadruplets. \*\*\*  $p < 0.001$ .

681 (D) Sequence-ME model in triplets ( $n=291$ ). Same presentation as in C (Paired t test between  
682 medians across trials:  $t_{290} = 14.85, p < 10^{-20}, d = 0.62$ ).

683 (E) Spatial-ME model. Decoding hit rate for single quadruplets based on the pairwise model  
684 (x-axis) and based on the triple-wise model (y-axis), with the histogram of the ratios  
685 between the hit rate of the triple-wise and pairwise models ( $n=119$ , paired one tailed t-test,  
686  $t_{118} = 2.69, p < 0.005, d = 0.25$ ). The higher hit rate based on the triple-wise model  
687 suggest coding in triple-wise correlations.

688 (F) Sequence-ME model in triplets. Same presentation as in (E) ( $n=150$ , paired one tailed t-  
689 test,  $t_{149} = 1.5, p = 0.07$ ; Pink: triplets with significant sequence-decoding taken from  
690 Fig.3A).

691

692

## 693 **Methods**

### 694 ***Behavioral paradigm and Electrophysiological recordings***

695 Two male macaca fascicularis (4 years old) were implanted with a recording chamber above  
696 the right amygdala and the dACC, and an MRI scan was performed to assess chamber  
697 position over dACC and amygdala (Supplementary Fig.1). Images were acquired on a 3T Trio  
698 (Siemens) Scanner, equipped with a 12 channels head matrix coil combined with a knee coil  
699 (Siemens), the primate was lying in prone position. 3D T1 weighted magnetization prepared  
700 rapid acquisition gradient-echo (MPRAGE) pulse sequence was acquired, Cartesian  
701 acquisition, field of view 160 × 130 mm, 192 × 156 matrix and 0.83 mm<sup>3</sup> slice thickness,  
702 resolution tilted from the sagittal plane. TE/TR/TI = 3.36ms/2500ms/1100ms, 8° flip angle, 2  
703 averages. All surgical and experimental procedures were approved and conducted in  
704 accordance with the regulations of the Weizmann Institute Animal Care and Use Committee  
705 (IACUC), following NIH regulations and with AAALAC accreditation. Food, water, and  
706 enrichments (e.g., fruits and play instruments) were available ad libitum during the whole  
707 period, except before medical procedures.

708 In the behavioral paradigm, primates were seated in a dark room and engaged in a classical  
709 conditioning task in which tones (conditioned stimulus, CS) were coupled with odors  
710 (unconditioned stimulus, US)<sup>51, 52</sup>. Each recording day was initiated with a habituation phase  
711 of ten presentations of two conditioned stimuli (CS), pure (sinus wave) tones chosen  
712 randomly in the range between 1000-2500 Hz to induce new learning in each session. The  
713 acquisition session that followed included 30 intermixed presentations of the two CS tones  
714 paired with an aversive (Propionic acid) or pleasant (a mixture of banana and melon organic  
715 extract) odor. Odor presentation was locked to the first breath after the CS tone, but not less  
716 than 1 second (s) after tone onset.

717 Each day, 3–4 microelectrodes were lowered inside a metal guide into the brain using a  
718 head-tower and electrode-positioning-system (Alpha Omega). The electrodes were then  
719 moved independently further into the amygdala and dACC. Electrode signals were pre  
720 amplified, 0.3 Hz-6 KHz band-pass filtered and sampled at 25 KHz. At the end of the  
721 recording period, off-line spike sorting was performed (offline sorter, Plexon Inc).

722 Number of monkeys, number of recording days (sessions), and overall number of recorded  
723 neurons is similar to those reported in previous publications and as customary in the field<sup>51,  
724 52</sup>.

### 725 ***Data analysis***

#### 726 ***Behavioral conditioned response***

727 Breath duration was quantified as full width at half maximum (FWHM) of inhale pressure.  
728 Conditioned response (CR) was quantified as inhale FWHM following the CS, normalized by  
729 the inhale FWHM in the 3 baseline breathes prior to CS:

$$CR = \frac{FWHM_{CS} - \overline{FWHM}_{baseline}}{FWHM_{CS} + \overline{FWHM}_{baseline}}$$

730 ,where  $\bar{x} = \sum_i \frac{X_i}{N}$ .

731 To examine the change of the CR along the day, the difference between each CR and the CR  
732 of the first trial (prior to any feedback) was evaluated. This response was quantified only in  
733 days with reliable pressure measurement and inhale onset detection (requiring peak  
734 amplitude  $> 0$ , time to peak  $< 500\text{ms}$  and FWHM  $< 800\text{ms}$  but  $> 50\text{ms}$ ) in at least 2/3 of the  
735 trials (n=42).

736 Differential aversive and pleasant CR was identified by performing 2-way ANOVA (valence X  
737 trials) for each day, taking days with significance effect of valence. For these days, the  
738 difference between CRs was quantified, taking  $\Delta\text{CR} = \text{CR}_{\text{pl}} - \text{CR}_{\text{av}}$  for days with  $\overline{\text{CR}}_{\text{pl}} >$   
739  $\overline{\text{CR}}_{\text{av}}$  (n=16) and  $\Delta\text{CR} = \text{CR}_{\text{av}} - \text{CR}_{\text{pl}}$  for days with  $\overline{\text{CR}}_{\text{pl}} < \overline{\text{CR}}_{\text{av}}$  (n=15). The development  
740 of this response along the day, namely learning, was verified by testing for the difference  
741 between the  $\Delta\text{CR}$  in the initial stage of learning (trials 1-10) and later stages (trials 11-20,  
742 21-30).

743 Whereas the unconditioned-response (UR, the response to the odor) shows the expected  
744 lower-shorter inhale for aversive odor and higher-longer inhale for pleasant odor<sup>52, 53</sup>, the  
745 conditioned-responses (CR) reflects a coping strategy and varies between animals and  
746 sessions. One can observe the two typical behaviors described in classical conditioning  
747 literature: either the CR and UR are in the same direction, as in early classical-conditioning  
748 theories, or they have opposite direction, as can be expected from 'naïve' reasoning (a  
749 longer inhale for the CS to prepare for the shorter inhale for the aversive odor), or as  
750 observed in electric-shock studies that show opposite direction between CR and UR of  
751 evoked autonomic measures. To measure learning and the development of the CR  
752 independent of this and in-line with our previous studies that found different strategies  
753 between animals and sessions, we tested for a difference in the half-width as long as it is  
754 consistent within a session.

755

### 756 ***Neuronal analyses***

757 Baseline activity was taken from a 30 segments X 10s time period prior to any paradigm-  
758 related stimulus. Post-trial activity was taken as 30 trials X 10s periods starting 2s after US  
759 offset.

760 Sequence distributions (below) were estimated for all possible triplets of neurons that were  
761 recorded simultaneously. In addition, the results were compared to triplets based on  
762 independent neurons that were recorded in different days (across-days-triplets), preserving  
763 independent neurons activity and the dynamic of each neuron along time. The results were  
764 also compared to shuffling of two of the neurons across trials (trial-shuffle), preserving  
765 independent neurons activity and single neuron identity.

### 766 ***Estimation of sequence probability distributions***

767 Sequences were defined as a sequence of three spikes from the activity of three neurons  
768 that occurred within a time lag (10-250ms). Sequences were counted by using an  
769 overlapping running window and calculating the probability of each sequence.

$$\hat{p}^{\text{data}}(\text{seq}_i) = \frac{\#(\text{seq}: \text{seq} = \text{seq}_i)}{N}$$

770 Where  $\text{seq}_i$  is a specific sequence of three spikes (from any of the three neurons) and  $N$  is  
771 the total number of recorded sequences.

772 ***Shuffling methods***

773 To test for differences between the spatiotemporal-structured triplet and that expected  
774 from firing-rate (FR) correlations and single-neuron firing patterns (FP) of the same three  
775 neurons, shuffled data sets ( $n=500$ ) were created by circularly shuffling the entire spiking  
776 patterns of two neurons in a random duration between  $\pm 150\text{-}300\text{ms}$  (Extended Data  
777 Fig.1A). Circular shuffling was performed on each time segment separately, i.e. on 10s time  
778 epochs during baseline activity, or on individual 10s post-trial activity.

779 Analyses were repeated with three additional shuffles that were applied on two of the  
780 neurons: unbounded circular shift (rather than 150-300ms), shuffling across trials, and  
781 Poisson shuffle. In trial shuffle, the order of the 30 time segments (post-trial or time  
782 segments of baseline activity) was randomly shuffled. In Poisson shuffle the number of  
783 spikes within a predetermined non-overlapping time window (150-500ms) was counted and  
784 randomly assigned back<sup>54</sup>. The Poisson shuffle was highly sensitive to single neuron firing  
785 patterns such that the structure and consistency analyses appeared significant even for  
786 independent across-days-triplet. These shuffles showed to be generally less stringent and  
787 are therefore not reported in the main text.

788 For each shuffled data instance the sequence probability distributions were estimated as:

$$\hat{p}_j^{\text{sh}}(\text{seq}_i) = \frac{\#(\text{seq}: \text{seq} = \text{seq}_i)}{N}$$

789 Where  $\hat{p}^{\text{sh}}$  is the estimated sequence distribution of a shuffled data set,  $\text{seq}_i$  is a specific  
790 sequence of three spikes (from any of the three neurons),  $N$  is the total number of recorded  
791 sequences,  $j$  is shuffle index.

792 ***Shuffling to test for pairwise activity for all 3 pairs***

793 To control for pairwise activity, the same shuffling method was performed only on one of  
794 the neurons, thereby preserving the joint activity of the unshuffled pair and destroying the  
795 relation to the shuffled neuron (Supplementary Fig.2A). Thus, each triplet was tested against  
796 three shuffled data sets ( $n=200$  instances for each shuffled neuron). Tests for pairwise  
797 activity were performed for all three shuffled data sets and determined significant for  
798  $p \leq 0.05$  for all three tests. This tests if the sequence activity is different from expected  
799 from either pairwise activity separately. Since all three tests are required to ascribe  
800 significance, the probability of type-1 error for all three tests is bounded by  $\alpha = 0.05$ :

801 If  $H_0$  is true for all three tests:  $\alpha_{\text{group}} = \alpha^3$

802 If  $H_0$  is true only for the first test:  $\alpha_{\text{group}} = \alpha * (1 - \beta_2)(1 - \beta_3) \leq \alpha$ , where  $(1 - \beta_i)$  is the  
803 power of the test for the  $i$ 'th shuffled unit.

804 **Jensen–Shannon divergence (JSD) - probability distribution dissimilarity measure**

805 JSD was used as a measure of dissimilarity between probability distribution. JSD is symmetric  
806 and bounded in the range [0,1].

$$\text{JSD}(P||Q) = \frac{D_{\text{KL}}(P||M) + D_{\text{KL}}(Q||M)}{2}, \quad M = \frac{P + Q}{2}, \quad D_{\text{KL}}(P||M) = \sum_x P(x) \log \frac{P(x)}{Q(x)}$$

807 **Benjamini–Hochberg (BH) correction for false discovery rate (FDR) in multiple comparisons**

808 The largest p-value for which  $P_i < \frac{i}{m} * \alpha$  was detected, where m is the total number of  
809 comparisons and  $\alpha = 0.05$  is the maximal expected proportion of errors. The critical p-value  
810 was set as  $P_i$ , guarantying  $\text{FDR} \leq \alpha$ .

811 **Structure analysis scheme**

812 The probability of each sequence was estimated for the shuffled data sets and for the real  
813 data using the entire 300s time period or 30 acquisition post-trial epochs (30 trials X 10s).

814 The mean JSD between the shuffled sequence distribution and the individual sequence  
815 distribution was estimated as a measure of dissimilarity for both the data and the shuffled  
816 data sets:

$$\bar{D}_{\text{data}} = \frac{\sum_{j=1}^n \text{JSD}(\hat{P}_j^{\text{Sh}} || \hat{P}^{\text{data}})}{n}$$

$$\bar{D}_{\text{shuffle}}^k = \frac{\sum_{j=1}^n \text{JSD}(\hat{P}_j^{\text{Sh}} || \hat{P}_k^{\text{Sh}})}{n}$$

817 , where n is the number of shuffled data instances.

818 Large dissimilarity between data and shuffled data would suggest a structured probability  
819 distribution (Extended Data Fig.1B), so a right tailed Monte Carlo p-value for the structure  
820 measure and a structure score ( $\bar{D}_{\text{idx}}$ ) were estimated based on shuffled data instances:

$$P_{\text{val}}(\bar{D}_{\text{data}}) = \frac{1 + \sum_{k=1}^n I\{\bar{D}_{\text{shuffle}}^k \geq \bar{D}_{\text{data}}\}}{1 + n}$$

821

$$\bar{D}_{\text{idx}} = \frac{\bar{D}_{\text{data}} - \bar{D}_{\text{sh}}^{1,2,\dots,n}}{\bar{D}_{\text{data}} + \bar{D}_{\text{sh}}^{1,2,\dots,n}}$$

822 , where n is the number of shuffled data instances and  $\bar{x} = \sum_i \frac{x_i}{N}$

823 **Consistency analysis scheme**

824 The probability of each sequence was estimated on L=100 semi-randomized subdivisions of  
825 the 30 time segments into two groups (15 segments of 10s each). Sets of subdivisions were  
826 randomly selected 1000 times and the chosen set was the one that maximized the Hamming  
827 distance between the different subdivisions. The JSD between the probability distributions  
828 of the two data segments was averaged over subdivisions ( $\bar{C}_{\text{data}}$ ) and compared to the  
829 average JSD between one data segment and one shuffled data segment ( $\bar{C}_{\text{shuffle}}^k$ ).

$$\bar{C}_{\text{data}} = \frac{\sum_{l=1}^L \text{JSD}(\widehat{P}_{1,1}^{\text{data}} || \widehat{P}_{1,2}^{\text{data}})}{L}$$

$$\bar{C}_{\text{shuffle}}^k = \frac{\sum_{l=1}^L \frac{\text{JSD}(\widehat{P}_{1,1k}^{\text{sh}} || \widehat{P}_{1,2}^{\text{data}}) + \text{JSD}(\widehat{P}_{1,2k}^{\text{sh}} || \widehat{P}_{1,1}^{\text{data}})}{2}}{L}$$

830 , where  $\widehat{P}_{1,1}^{\text{data}}$  is the sequence probability distribution of the first group of the  $l$ 'th  
831 subdivision of the data and  $\widehat{P}_{1,1k}^{\text{sh}}$  is the sequence probability distribution of the first group  
832 of the  $l$ 'th subdivision of the  $k$ 'th shuffle.

833 Large similarity between data segments would suggest a consistent distribution (Extended  
834 Data Fig.3), so left-tailed Monte Carlo p-values and consistency scores ( $\bar{C}_{\text{idx}}$ ) were  
835 estimated:

$$P_{\text{val}}(\bar{C}_{\text{data}}) = \frac{1 + \sum \mathbb{1}\{\bar{C}_{\text{shuffle}}^k \leq \bar{C}_{\text{data}}\}}{1 + n}$$

836

$$\bar{C}_{\text{idx}} = \frac{\bar{C}_{\text{shuffle}}^{1,\dots,n} - \bar{C}_{\text{data}}}{\bar{C}_{\text{shuffle}}^{1,\dots,n} + \bar{C}_{\text{data}}}$$

837 , where  $n$  is the number of shuffled data instances.

### 838 **Consistency within versus across comparisons**

839 For each triplet, we found the sequence duration that produced the maximal mean  
840 consistency score for between and within stimulus valence ( $\text{Seq}_{\text{lag}}^*(\text{pl})$ ,  $\text{Seq}_{\text{lag}}^*(\text{av})$ ):

$$\text{Seq}_{\text{lag}}^*(\text{pl}) = \underset{\text{Seq}_{\text{lag}} \in \{10,25,50,100,150,200,250\}}{\text{argmax}} \quad \bar{C}_{\text{idx}}^{\text{pl-pl}} + \bar{C}_{\text{idx}}^{\text{pl-av}}$$

$$\text{Seq}_{\text{lag}}^*(\text{av}) = \underset{\text{Seq}_{\text{lag}} \in \{10,25,50,100,150,200,250\}}{\text{argmax}} \quad \bar{C}_{\text{idx}}^{\text{av-av}} + \bar{C}_{\text{idx}}^{\text{pl-av}}$$

841

842 , where  $\bar{C}_{\text{idx}}^{\text{pl-pl}}$ ,  $\bar{C}_{\text{idx}}^{\text{av-av}}$  are calculated as  $\bar{C}_{\text{idx}}$ , where  $\widehat{P}_{1,1}$  and  $\widehat{P}_{1,2}$  are estimated from  
843 pleasant or aversive post-trial epoch, respectively.  $\bar{C}_{\text{idx}}^{\text{pl-av}}$  is calculated as  $\bar{C}_{\text{idx}}$  where  $\widehat{P}_{1,1}$  is  
844 estimated from pleasant and  $\widehat{P}_{1,2}$  is estimated from aversive post-trial epoch.

845 Taking the relevant sequence duration for each triplet, the JSD between the post-trial  
846 sequence distributions of the same stimulus was estimated and averaged over 100  
847 subdivisions (as in Consistency analysis scheme):

$$\text{JSD}(\text{pl}||\text{pl}) = \frac{\sum_{l=1}^L \text{JSD}(\widehat{P}_{1,1}^{\text{pl}} || \widehat{P}_{1,2}^{\text{pl}})}{L}$$

$$\text{JSD}(\text{av}||\text{av}) = \frac{\sum_{l=1}^L \text{JSD}(\widehat{P}_{1,1}^{\text{av}} || \widehat{P}_{1,2}^{\text{av}})}{L}$$

848 These were compared to the JSD between the post-trial sequence distributions of the  
849 different stimuli, averaged over the two possibilities:



$$\text{JSD}(\text{av}||\text{pl}) = \frac{\sum_{l=1}^L \text{JSD}(\widehat{P}_{1,1}^{\text{av}} || \widehat{P}_{1,2}^{\text{pl}}) + \text{JSD}(\widehat{P}_{1,1}^{\text{pl}} || \widehat{P}_{1,2}^{\text{av}})}{2L}$$

850 Next, consistency scores were evaluated for post-trial sequence distributions of the same  
 851 stimulus,  $\bar{C}_{\text{idx}}^{\text{pl-pl}}$  and  $\bar{C}_{\text{idx}}^{\text{av-av}}$  and compared to the consistency score between the post-trial  
 852 sequence distributions of the different stimuli,  $\bar{C}_{\text{idx}}^{\text{pl-av}}$ .

### 853 ***Inter-spike interval (ISI) distribution estimation***

854 First, a naïve estimation of the inter spike interval (ISI) distribution was estimated

$$P(\text{ISI} = x) = \frac{\#(\text{isi: isi} = x)}{N}$$

855 Where N is the total number of counted inter spike interval. This distribution was smoothed  
 856 using Kernel density estimation with a normal kernel evaluated at 100 equally spaced points.

### 857 ***Firing rate (FR) distribution estimation***

858 Firing rates (FR) were counted on non-overlapping 250ms time bins and the probability  
 859 distribution was estimated naïvely, without accounting for the timing of the FR.

### 860 ***Likelihood ratio decoding from post-trial activity***

861 According to Neyman–Pearson lemma, the log-likelihood ratio is the most powerful test to  
 862 discriminate between two hypotheses. Therefore, it can be used to test how well a readout  
 863 mechanism can discriminate between the previously presented stimulus and the current  
 864 stimuli.

$$\begin{aligned} 865 \quad L_{\{s_1\}}(r) &= \log \frac{P(s_1|r_1, \dots, r_n)}{P(s_2|r_1, \dots, r_n)} = \log \frac{P(r_1, \dots, r_n|s_1) \cdot \frac{p(s_1)}{p(r_1, \dots, r_n)}}{P(r_1, \dots, r_n|s_2) \cdot \frac{p(s_2)}{p(r_1, \dots, r_n)}} = \dots \\ 866 \quad &= \log \frac{P(r_1, \dots, r_n|s_1)}{P(r_1, \dots, r_n|s_2)} + \log \frac{p(s_1)}{p(s_2)} = \sum \log \frac{P(r_i|s_1)}{P(r_i|s_2)} \end{aligned}$$

867 Where r is the neural response (sequences, ISI or FR), s1 and s2 are the pleasant and  
 868 aversive stimuli. The last equality holds for balanced stimulus presentation ( $p(s_1) = p(s_2)$ )  
 869 and independent responses.

870 The conditioned probability distributions,  $P(r = r_i|s)$ , were estimated in the post-trial  
 871 activity consecutive to the stimulus s (pleasant or aversive) of all acquisition trials except the  
 872 j'th trial and  $r_1, \dots, r_n$  are the responses in the post-trial of trial j (*Leave one out cross*  
 873 *validation*).

874 If the log likelihood ratio of the test set was smaller than zero, the decoder classified the  
 875 stimulus as  $s_2$  and vice versa. Hit rates were calculated as  $\frac{\# \text{correct classification}}{\# \text{trials}}$ . Significance  
 876 level of decoding performance of single triplets was tested by a binomial or  $\chi^2$  test under the  
 877 null hypothesis that  $p(\text{correct}) = p(\text{error}) = 0.5$ .

878 Decoding performance as a function of time in the post-trial was assessed by decoding on 5s  
 879 running window with 4s overlap, starting from US onset (-3s).

880 For the ISI and FR based decoding independence between the three neurons was assumed  
 881 so likelihood ratios were summed over all three neurons and classified:

882  $L_{\{s_1\}}(r) = \log \frac{P(s_1|r_{11}, \dots, r_{n1}, r_{12}, \dots, r_{n2}, r_{13}, \dots, r_{n3})}{P(s_2|r_{11}, \dots, r_{n1}, r_{12}, \dots, r_{n2}, r_{13}, \dots, r_{n3})} = \dots = \sum_{j=1}^3 \sum \log \frac{P(r_{ij}|s_1)}{P(r_{ij}|s_2)}$ , where  $r_{ij}$  is the  $i$ 'th  
 883 response of neuron  $j$ .

884 As not all triplets work together to produce sequences and the ISI/FR distributions hold  
 885 more information on single units, the average decoding performance of all possible triplets  
 886 was expected to be smaller for sequences. To compare decoding performance on putative  
 887 sequence-coding triplets, the hit rate of the best performing triplets was evaluated as a  
 888 function of proportion of triplets included, taking triplets from best to worst performance.  
 889 To avoid selection bias, best performing triplets were taken separately for each method,  
 890 enabling an unbiased comparison between sequence-best triplets and ISI/FR-best triplets.

### 891 ***Likelihood ratio decoding between post-trial and pre-task activity***

892 To verify that valence-specific sequences did not exist in pre-task activity, post-trial pleasant  
 893 and aversive activity was decoded from pre-task activity. To this end, pre-task activity was  
 894 divided into 30 segments of 10 second each (matched to the post-trial activity) and  
 895 likelihood ratio decoding was performed between pre-task and aversive, as well as between  
 896 pre-task and pleasant, post-trial activity using *Leave one out cross validation*.

### 897 ***Correlation between trial-by-trial decoding performance and CR***

898 Trial by trial decoding performance was assessed by quantifying the proportion of triplets  
 899 that correctly classified the  $i$ 'th pleasant and aversive trials:

$$900 \text{ proportion}(\text{trial } i) = \frac{\sum_{j=1}^{n_{\text{triplets}}} \text{correct}_{\text{av}}(i) + \text{correct}_{\text{pl}}(i)}{2 * n_{\text{triplets}}}$$

901 Conditioned responses were estimated as  $\Delta\text{CR}$  above. As these measures are noisy, we used  
 902 two trial temporal smoothing (two trials running window with 1 trial overlap). The  
 903 correlation was tested by resampling procedure, where trials were first shuffled, then  
 904 smoothed (as the original data) and correlated. This was repeated  $n = 10,000$  times to get:

$$905 P_{\text{resampling}} = \frac{1 + \sum \{r_{\text{data}} \leq r_{\text{resampled}}\}}{1 + n}$$

906 This was further multiplied by 2 to account for the comparisons with no smoothing.

### 907 ***FR response***

908 CS and US FR were evaluated in a 1 sec time window after stimulus onset and baseline  
 909 activity was evaluated in a 1 sec time window prior to CS onset. For each neuron and each  
 910 valence (pleasant or aversive), a paired two tailed t-test was performed on the FR response  
 911 across 30 trials comparing baseline activity to CS response and baseline activity to US  
 912 response. In addition, differential FR response was evaluated by comparing (paired two  
 913 tailed t-test) pleasant and aversive responses to the CS or the US, normalized by baseline  
 914 activity  $\left( \frac{\text{FR}_{\text{stimulus}} - \text{FR}_{\text{baseline}}}{\text{FR}_{\text{stimulus}} + \text{FR}_{\text{baseline}}} \right)$ .

### 915 ***Local field potential (LFP)***

916 LFP signals were sampled at 781.25Hz, filtered with high-pass Butterworth filter with a cutoff  
 917 of 3 Hz and a low-pass Butterworth filter with a cutoff at 90 Hz. After filtering, individual

918 electrodes were Z-scored and power spectral analysis and spike triggered average of the LFP  
919 signal were computed on the normalized signals<sup>55</sup>.

920 **Mutual information (MI)**

921 Mutual information (MI) between sequences of individual triplets and stimulus valence  
922 (pleasant vs. aversive) was calculated by sampling the sequence distribution for all 30 trials  
923 of each valence or for ten trials along learning.

$$MI_{naive}(R|S) = H(R) - H(R|S) = H(\text{sequences}) - H(\text{sequences|valence})$$

924 , where  $H(R)$  is the entropy and  $H(R|S)$  is the conditioned entropy.

925 To decrease under-sampling bias, MI was calculated only for sequence distributions with  
926 sufficient sampling, taking a sampling criterion:  $\frac{N_s}{R} \geq 12$ , where  $N_s$  is the total number of  
927 observed sequences in all pleasant and in all aversive trials and  $R$  is the size of the sampled  
928 space of sequences in either stimuli ( $\leq 27$ )<sup>56</sup>. The under-sampling bias<sup>56</sup> was estimated by:

929  $\text{bias}[MI(R|S)] = \frac{1}{2N \ln(2)} \{ \sum_{s=pl,av} [R_s - 1] - [R - 1] \}$ , where  $N$  is the total number of  
930 sequences and  $R_s$  is the size of the sampled space of sequences for the pleasant or aversive  
931 stimulus.

932 The presented MI are corrected such that:

$$MI = MI_{naive}(R|S) + \text{bias}[MI(R|S)]$$

933 The MI estimates the average information (i.e. reduction in uncertainty) between sequences  
934 and valence in a single event, namely a single sequence. To estimate the average  
935 information transmitted by sequences in one second, we multiplied the MI of individual  
936 triplets in each time segment by the sequence rate in that time segment.

937 To test the significance of the MI we performed a 1000-iterations permutation test where  
938 post-trial activity segments were randomly assigned (without replacement) to pleasant or  
939 aversive groups and the same sufficient sampling criterion and bias correction were applied.

940 **CS-US by post-trial Likelihood ratio decoding**

941 Valence (pleasant vs. aversive) was decoded from CS-US activity based on post-trial  
942 probability distributions. CS-US sequences were counted in a 2s window starting from CS  
943 onset, where US onset was set to the next breath onset ( $\geq 1s$  and  $< 3s$  after CS onset).

$$L_{CS-US \{s_1\}}(r) = \sum \log \frac{P(r_i|s_1)}{P(r_i|s_2)}$$

944 The decoder was trained on post-trial epochs of all trials (estimating the conditioned  
945 distribution  $P(r = r_i|s)$ ). To ensure proper sampling of CS-US activity, the decoder was  
946 tested on 30 sets of CS-US sequences from 15 randomly chosen trials  $J = \{j_1, \dots, j_{15}\}$   
947 (summing over all sequences in CS-US responses of all trials in  $J$ ).

948 **Proportion of post-trial valence-specific sequences in CS-US evoked activity**

949 Valence-specific sequences were categorized by examining the ratio  $\frac{P(\text{seq}_i|av)}{P(\text{seq}_i|pl)}$ , evaluated  
950 from post-trial epoch of all trials. Aversive/pleasant sp\ecific sequences were taken as m

951 sequences with maximal/minimal ratio, respectively, while ignoring single neuron sequences  
 952 (e.g. [1,1,1]). The proportion of valence-specific sequences was evaluated during aversive  
 953 and pleasant CS-US activity:  $\frac{\# \text{aversive specific sequences}}{\# \text{sequences}}$  and  $\frac{\# \text{pleasant specific sequences}}{\# \text{sequences}}$ , for  
 954  $m = 2,3,4$ .

955 Pleasant rehearsing triplet were defined as triplets with a larger proportion of pleasant  
 956 specific sequences in the pleasant CS-US activity than the proportion in aversive CS-US  
 957 activity:

$$\frac{\# \text{pleasant specific sequences (pleasant)}}{\# \text{sequences (pleasant)}} > \frac{\# \text{pleasant specific sequences (aversive)}}{\# \text{sequences (aversive)}}$$

958 Aversive rehearsing triplets were defined as triplets with a larger proportion of aversive  
 959 specific sequences in aversive CS-US activity:

$$\frac{\# \text{aversive specific sequences (aversive)}}{\# \text{sequences (aversive)}} > \frac{\# \text{aversive specific sequences (pleasant)}}{\# \text{sequences (pleasant)}}$$

960 To test if pleasant- and aversive-rehearsed sequences were present in pre-task activity, the  
 961 proportion of valence-specific sequences was compared between CS-US response and pre-  
 962 task activity.

### 963 **Maximum entropy (ME) models**

964 The Maximum Entropy Toolbox for MATLAB, version 1.0.2. 2017<sup>57</sup> was used to fit exact  
 965 solutions to the models described below.

966 Unless stated otherwise, models were fit with a threshold of  $th = 10^{-4}$  standard deviation  
 967 of the expected measurement noise.

968 Spatial-ME model: The ME model for triple-wise spatial connection is of the form:  
 969  $P(x) = \frac{1}{z} \exp(\sum_{i=1}^N h_i x_i + \sum_{i<j} j_{ij} x_i x_j + \sum_{i<j<k} m_{ijk} x_i x_j x_k)$ , where  $z$  is a scaling factor,  $N = 4$  is the  
 970 number of neurons in each group and  $i, j, k$  are indexes for neurons.

971 It is fitted to the data based on three groups of constraints:

972 (independent spike rate)  $\langle \theta_i \rangle = \frac{1}{T} \sum_{t=1}^T \theta_i(t)$

973 (pairwise correlations)  $\langle \theta_{ij} \rangle = \frac{1}{T} \sum_{t=1}^T \theta_i(t) \theta_j(t)$

974 (triple-wise correlations)  $\langle \theta_{ijk} \rangle = \frac{1}{T} \sum_{t=1}^T \theta_i(t) \theta_j(t) \theta_k(t)$

975 The pairwise model is only constrained by the independent and pairwise constraints and  
 976 takes  $m_{ijk} = 0$ , and the independent model is only constrained by the independent  
 977 constraint and takes also  $j_{ij} = 0$ . This model was fitted to all groups of 4 neurons, binned  
 978 into 50ms binary words (Extended Data Fig.6, cases where  $n_{\text{spikes}} > 1$  were taken as  
 979  $n_{\text{spikes}} = 1$ ). The 50ms was taken due to the sequences structure found in this time  
 980 duration (Fig.11).

981 Sequence-ME model: To capture the temporal characteristics of the sequences in three  
 982 neurons while using the ME model, a reduced data set was generated with 1ms bins,  
 983 neglecting all time bins where none of the neurons spiked or more than one neuron spiked  
 984 (Extended Data Fig.6). Triplets with time segments of less than 20 samples were disqualified.

985 The ME model for three steps spatiotemporal model is of the form  $P(x_{T,T+1,T+2}) =$   
 986  $\frac{1}{z} \exp(\sum_{i=1}^N \sum_{t=T}^{T+2} h_{i(t)} x_i(t) + \sum_{i<j} \sum_{t=T}^{T+2} h_{ij(t)} x_i(t)x_j(t) + \sum_{i,j} \sum_{t=T}^{T+1} j_{i(t)j(t+1)} x_i(t)x_j(t+1) +$   
 987  $\sum_{i,j,k} m_{i(t)j(t+1)k(t+2)} x_{i,t}x_{j,t+1}x_{k,t+2})$ , where  $z$  is a scaling factor,  $N = 3$  is the number of neurons  
 988 in each group,  $i, j, k$  are indexes for neurons and  $t$  is time index.

989 It was fitted based on three groups of constraints:

990 (independent)  $\langle \theta_i \rangle = \frac{1}{T} \sum_{t=1}^T \theta_i(t)$  ;  $\langle \theta_{ij} \rangle = \frac{1}{T} \sum_{t=1}^T \theta_i(t)\theta_j(t)$

991 (pairwise spatiotemporal correlations)  $\langle \theta_{i(t)j(t+1)} \rangle = \frac{1}{T} \sum_{t=1}^T \theta_i(t)\theta_j(t+1)$

992 (triple-wise spatiotemporal correlations)  $\langle \theta_{i(t)j(t+1)k(t+2)} \rangle = \frac{1}{T} \sum_{t=1}^T \theta_i(t)\theta_j(t+1)\theta_k(t+2)$

993 The pairwise model is only constrained by the independent and pairwise constraints and  
 994 takes  $m_{i(t)j(t+1)k(t+2)} = 0$ , while the independent model is only constrained by the  
 995 independent constraints and takes also  $j_{i(t)j(t+1)} = 0$ .

996 Notice that the independent constraint in this model includes pairwise spatial correlations  
 997 (but not temporal), as these are bound from model construction (simultaneous spikes from  
 998 two neurons were not allowed) and tends to be severely overestimated. Namely, when the  
 999 sparse 1ms spike matrix is taken without no-spikes time bins, it becomes very abundant in  
 1000 spikes, but there are no events where two neurons spike simultaneously. This is very  
 1001 unpredictable based on the rates of the neurons, as many simultaneous spiking events are  
 1002 expected, such that it creates biased probability distributions compared to the data. This  
 1003 bias is fixed by the learning of pairwise connections, as a spike of one neuron predicts that  
 1004 there is no spike of the others and low co-firing is predicted.

#### 1005 **Testing structure using the ME model**

1006 For each group the spatial-ME and sequence-ME models were fitted to 30 time segments of  
 1007 10sec each from the pre-task data.

1008 To quantify the contribution of the pairwise and triple-wise correlation to the uncertainty in  
 1009 the data (i.e. pairwise and triple-wise structure), the proportion of reduction in entropy by  
 1010 each order was calculated as the ratio between  $I_{(k)} = H_{k-1} - H_k$  and the multi information,  
 1011  $I_N = H_1 - H_N$ , where  $H_k$  is the entropy of the model with  $k$ 'th order correlations and  $H_N$  is  
 1012 the entropy of the data<sup>32</sup>.

1013 Since by definition the data is better explained by higher order models, this measure was  
 1014 compared to a surrogate data set (matching in the number of samples to the real data),  
 1015 sampled from the independent model ( $p_1$ ) or from the pairwise model ( $p_2$ ). New models  
 1016 were fitted to these generated data sets and the same measures were calculated. These  
 1017 comparison guarantees that the contribution of the pairwise and triple-wise correlations is  
 1018 not a result of chance or overfitting the model to the data.

#### 1019 **Consistency account using the ME model**

1020 For each group of neurons, the spatial-ME and sequence-ME models were fitted to 30 time  
 1021 segments of 10sec each from the pre-task data. 200 sets of train-test subdivisions were  
 1022 created, with 90% train segments ( $n_{\text{train}} = 27$  segments of 10sec each) and 10% test  
 1023 segments ( $n_{\text{test}} = 3$  segments). For each train-test subdivision the probabilities of the model

1024 fitted to individual trials were averaged and the JSD between the model train distribution  
1025 ( $P_i^{\text{train}}$ ) and the data test distribution ( $p_{\text{data}}^{\text{test}}$ ) were calculated:

$$\text{JSD}_{\text{model order}} = \text{JSD}(P_i^{\text{train}} || p_{\text{data}}^{\text{test}})$$

1026 Where  $p_i$  ( $i = 1,2,3$ ) is the probability distribution corresponding to the model order.

1027 For each triplet/quadruplet the JSD of low and high model orders were compared using a  
1028 paired t-test. Pairwise consistent triplets/quadruplets had significantly lower JSD in the  
1029 pairwise compared to the independent model. Triple-wise consistent triplets/quadruplets  
1030 had significantly lower JSD in the triple-wise compared to the pairwise model.

### 1031 ***Likelihood ratio decoding from ME models***

1032 For each group of neurons, the spatial-ME and sequence-ME model  
1033 ( $\theta = 0.1$  standard deviations, to reduce over-fitting) were fitted to post-trial activity of all  
1034 pleasant and aversive trials (30 trials of 10sec from each stimulus). For each order of the ME  
1035 model, the ME probability distribution were used to train the decoder and it was tested on  
1036 the real data using *Leave one out cross validation*:

1037  $L_{\{s_1\}}(r) = \sum \log \frac{P_{\text{ME}}(r_i|s_1)}{P_{\text{ME}}(r_i|s_2)}$ , where  $P_{\text{ME}}(r|s)$  is taken as the average probability of the  
1038 maximum entropy model of all trial but trial  $j$ , and  $r$  are taken from the data of trial  $j$ . For the  
1039 sequence-ME, triplets were included if the ME model was valid in at least 75% of the trials.

1040 To avoid overfitting in the case of triplets and quadruplets that only code the stimulus  
1041 independently, the comparison between the pairwise and triple-wise models were done  
1042 only on groups that were not clearly coding independently:

$$\text{hit rate}_{\text{pairwise}} > \text{hit rate}_{\text{independent}} \cup \text{hit rate}_{\text{triple-wise}} > \text{hit rate}_{\text{independent}}$$

1043 These preconditions do not create selection bias, as they are symmetric with respect to the  
1044 pairwise and triple-wise orders.

1045 To test CS-US decoding from post-trial activity, the decoder was trained on post-trial epochs  
1046 of all trials but trial  $j$ . The sequence-ME was tested on 30 sets of CS-US sequences from 15  
1047 randomly chosen trials  $J = \{j_1, \dots, j_{15}\}$ , to ensure sufficient sampling (as the number of sample  
1048 was dependent on the activity). The spatial-ME model was tested only on trial  $j$  (as the  
1049 number of samples was fixed,  $n=40$ ).

### 1050 ***Putative interneurons and projection cells***

1051 Spike durations were measured on unfiltered voltage traces and defined as the interval  
1052 between trough and peak for the negative spikes and the interval between peak and trough  
1053 for positive spikes. To minimize misclassification, we applied two criteria for putative  
1054 interneurons:  $\text{FR} \geq 7\text{Hz}$  and spike duration  $\leq 0.5\text{ms}$ , and two criteria for putative  
1055 projection cells:  $\text{FR} \leq 1\text{Hz}$  and spike duration  $\geq 0.7\text{ms}$ . Since the number of neurons that  
1056 were classified using this method was low, the firing rates and spike duration of all neurons  
1057 pertaining to significant and non-significant triplets in different criteria were also examined  
1058 (Supplementary Fig.11).

1059 Since each neuron can take part in more than one triplet, comparison of the groups was  
1060 done by permutation tests that preserve neurons identity. Thus, the FR and spike durations

1061 were shuffled across the neurons, but the composition of triplets from neurons was  
 1062 preserved, thereby preserving dependencies between triplets. This shuffling approach was  
 1063 repeated 10000 times, and MC p-value was extracted by comparing the mean difference in  
 1064 FR or spike durations in the real data to the mean difference in FR or spike durations in the  
 1065 shuffled data.

1066 Similarly, the difference in the probabilities of putative interneurons and projection cells to  
 1067 have significant structure, decoding and rehearsal were tested by shuffling the identity of  
 1068 the interneurons and projection cells across all classified neurons. Here again, the shuffling  
 1069 approach was repeated 10000 times, and MC p-value was extracted by comparing the mean  
 1070 difference in probabilities between interneurons and projection cells based on the real  
 1071 classification of the neurons to that of the shuffled classification.

1072 **FR Stationarity**

1073 Two tests for stationarity were employed: 1. Two tailed t-test comparing the average firing  
 1074 rate in the first and last 150 seconds of the pre-task activity (FR t-test). 2. Runs-test  
 1075 examining if inter-spike-intervals (ISI) along the pre-task activity were drawn randomly from  
 1076 a single distribution. The proportion of structured and consistent triplets in stationary and  
 1077 non-stationary triplets was compared and the reduction in entropy analysis that  
 1078 demonstrated 3-wise sequence activity was repeated.

1079 **Isolation score (unit isolation)**

1080 Isolation scores<sup>58</sup> were calculated as the isolation between unit<sub>1</sub> and unit<sub>2</sub>:

$$P_X(Y) = \frac{\exp\left(-d(X, Y) \left(\frac{\lambda}{d_0}\right)\right)}{\sum_{Z \neq X} \exp\left(-d(X, Z) \left(\frac{\lambda}{d_0}\right)\right)}$$

1081 Where  $d(X, Y)$  is the Euclidian distance,  $X, Y, Z$  are spike shapes of unit<sub>1</sub> and unit<sub>2</sub>,  $\lambda = 10$  is  
 1082 a scaling factor and  $d_0$  is the average Euclidian distance between all spike shapes of the two  
 1083 units.

$$P(X) = \sum_{Y \in \text{unit}_1} P_X(Y)$$

$$\text{Isolation score (unit}_1) = \frac{1}{|\text{unit}_1|} \sum_{X \in \text{unit}_1} P(X)$$

1084 Where  $|\text{unit}_1|$  is the number of spike shapes in unit<sub>1</sub> cluster.

1085 This quantifies a measure of similarity between each spike shape and all other spike shapes,  
 1086 normalized as probability of similarity in the two units, and summed over shapes within the  
 1087 same unit. This measure can be intuitively viewed as the average probability that an event  
 1088 that was classified as a spike belongs to the neuron it was classified to and not to the other  
 1089 neurons from the same electrode<sup>58</sup>.

1090 **Error bars**

1091 All error bars represent standard error of the mean (SEM), unless specifically stated  
1092 otherwise.

### 1093 **Effect size**

1094 Cohen's d was calculated as:  $d = \frac{\bar{X}_1 - \bar{X}_2}{S_{\text{pooled}}}$  for two samples ;  $d = \frac{\bar{X}_1 - \mu_0}{S_1}$  for one sample.

1095  $r_{rb}$  is the rank-biserial correlation coefficient.

### 1096 **Statistical tests**

1097 Statistical testing was done using t-test, ANOVA, Wilcoxon rank-sum, sign-rank test,  
1098 permutation testing and Monte-Carlo p-value with resampling procedures. Significance level  
1099 was set to  $p < 0.05$  unless otherwise mentioned. Correction for multiple comparison was  
1100 done using Tukey correction for family wise error or using Benjamini–Hochberg (BH)  
1101 correction for FDR.

1102 All statistical tests were two sided, unless specifically stated otherwise.

1103 In some statistical tests, data distributions were assumed to be normal and/or with equal  
1104 variances but this was not formally tested.

### 1105 **Randomization**

1106 Pleasant and aversive trials were pseudorandomly presented to the monkeys but equalized  
1107 in total number. Tones were randomly selected daily for pleasant and aversive CS. As  
1108 randomization is irrelevant to triplets of neurons (all simultaneously recorded triplets were  
1109 analyzed in this study), randomization was achieved by randomizing the control groups.  
1110 Thus, shuffling lags were randomly chosen to the shuffled data sets and trials were randomly  
1111 matched for the trial shuffle controls.

### 1112 **Blinding**

1113 Blinding is done as spike sorting is blind to the timing of the stimuli.

### 1114 **Data exclusions**

1115 Data was not excluded from the analysis.

### 1116 **Reporting Summary**

1117 Further information is available in the Nature Research Life Sciences Reporting  
1118 Summary linked to this article.

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### 1123 **Code availability**

1124

1125 Custom code for behavioral and electrophysiological tests is available from the  
1126 corresponding author upon reasonable request.

### 1127 **Data availability**



1128 All data supporting the findings of this study are available from the corresponding author  
1129 upon reasonable request.  
1130  
1131  
1132  
1133  
1134

1135 **Methods-only References**

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1157

# Figure 1

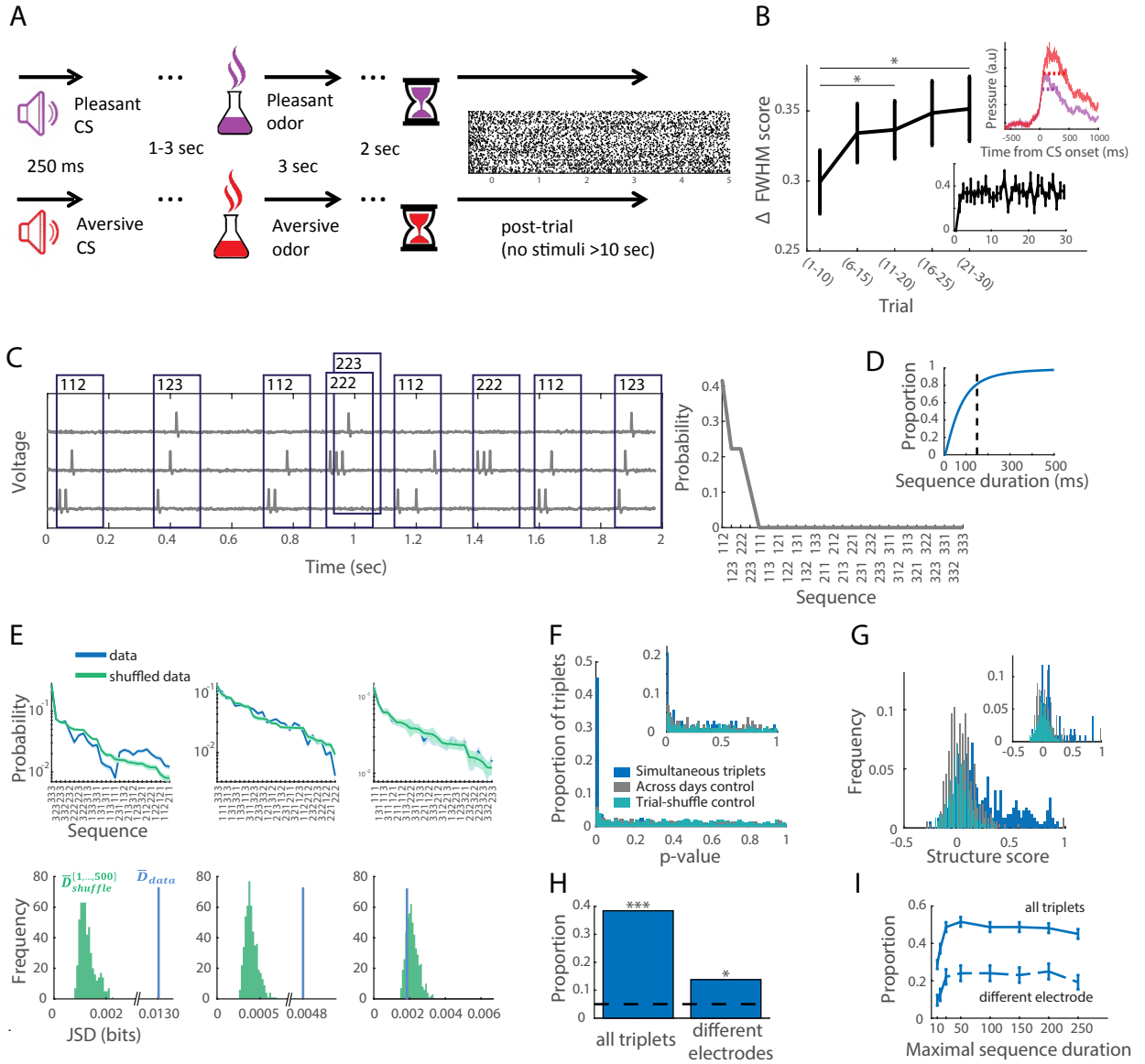


Figure 2

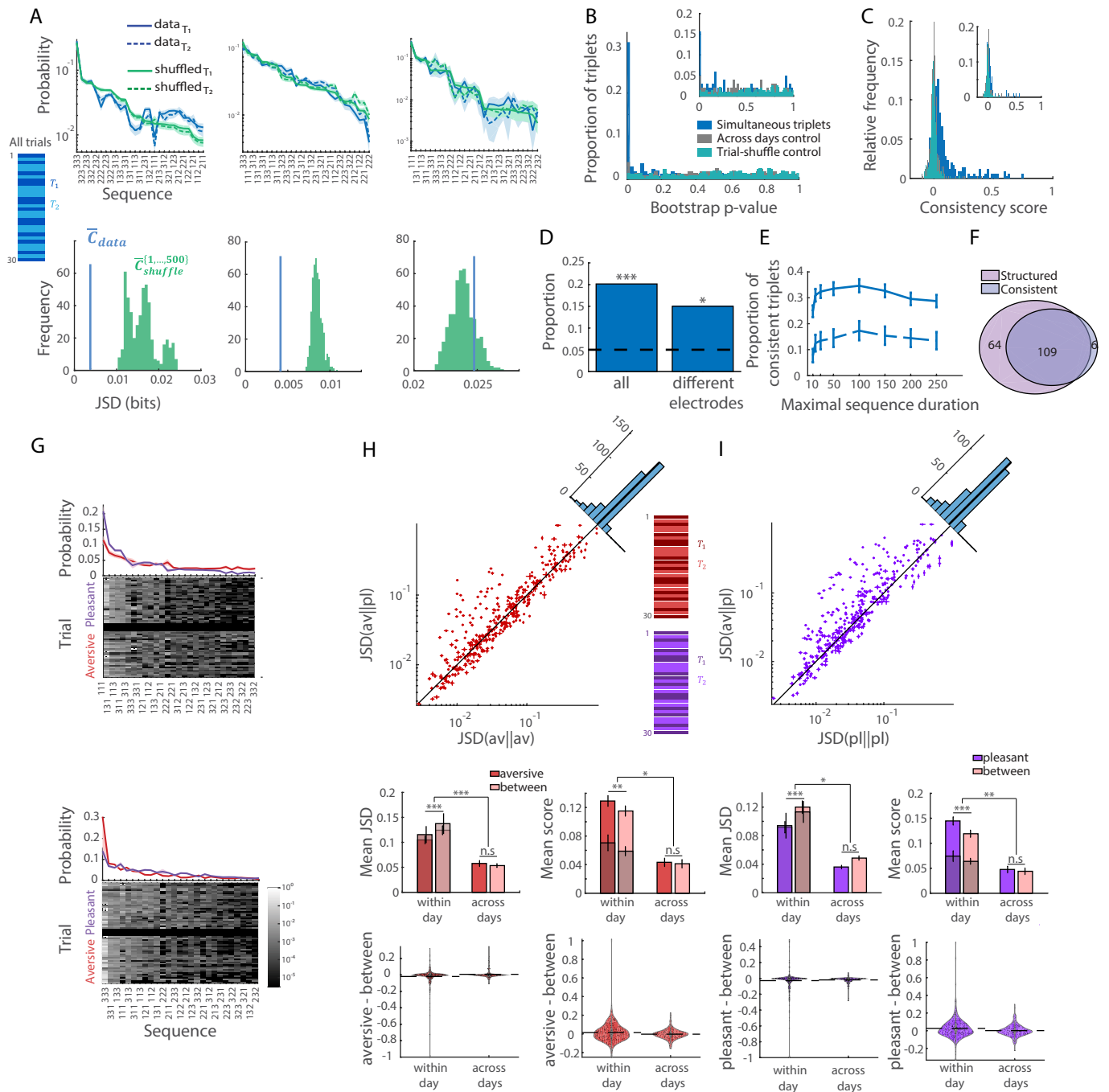


Figure 3

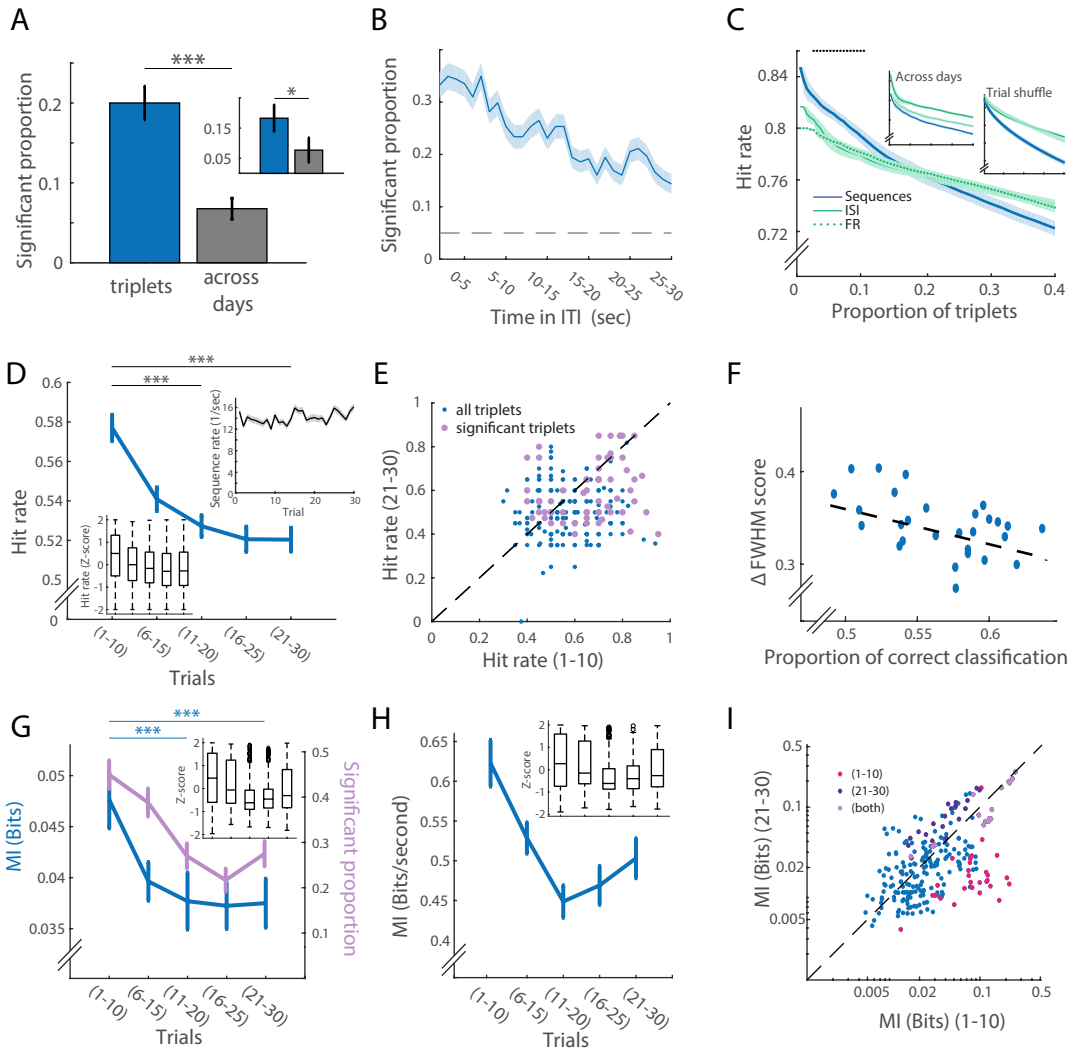


Figure 4

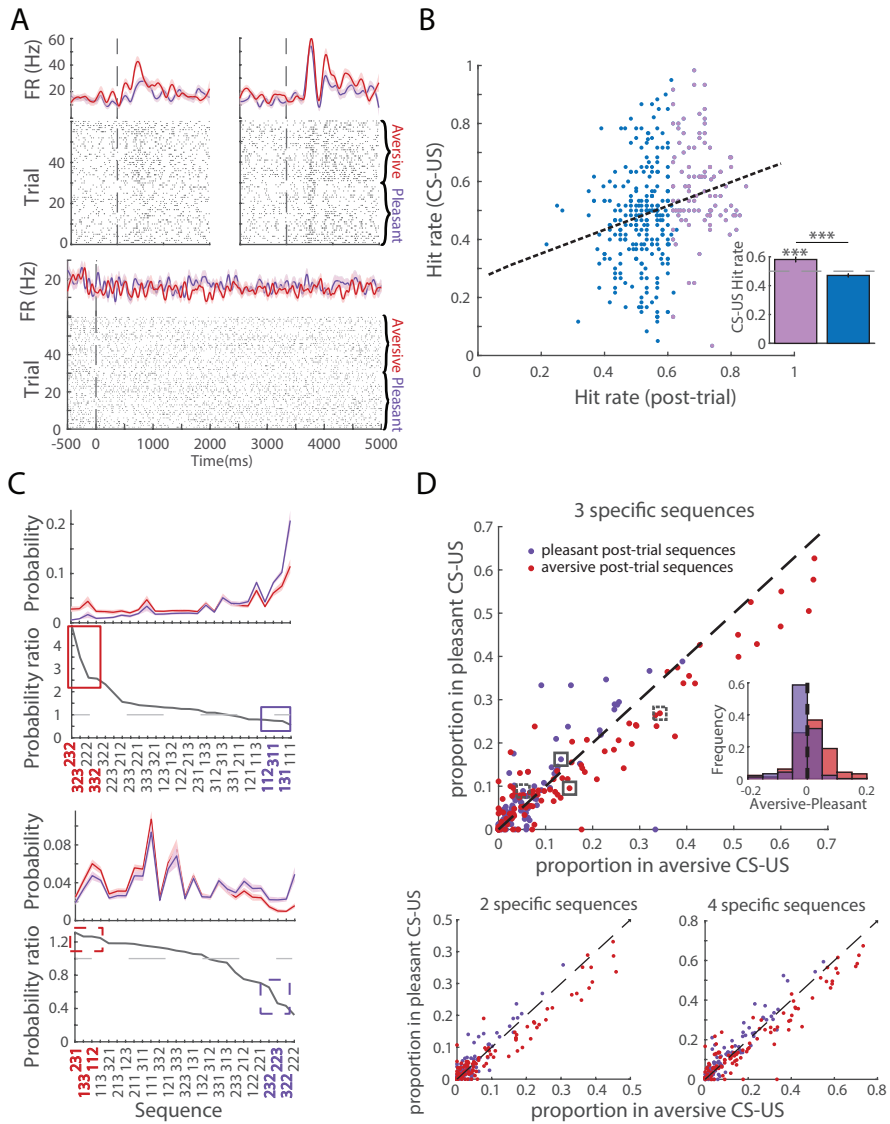
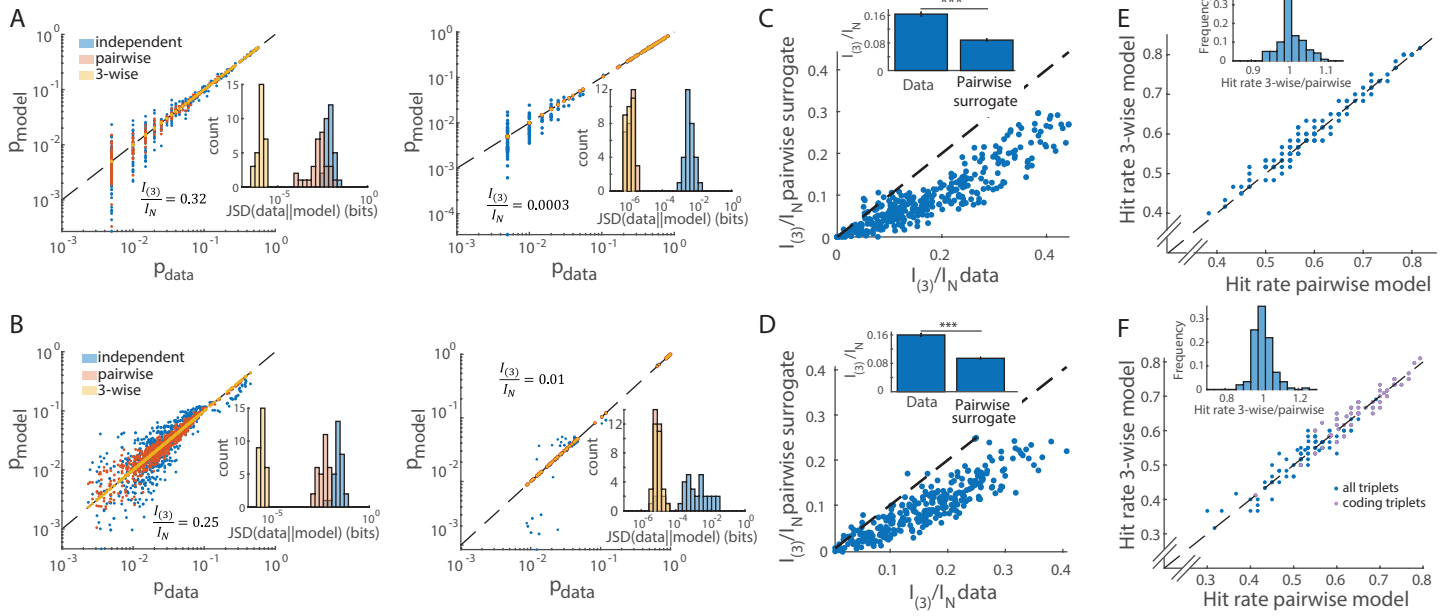
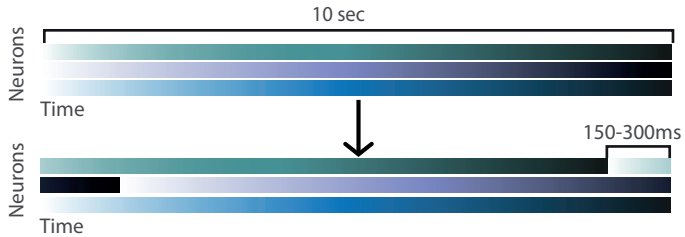


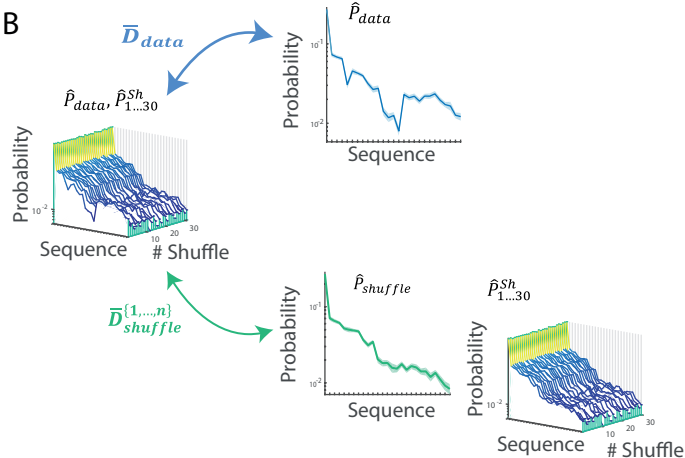
Figure 5



A



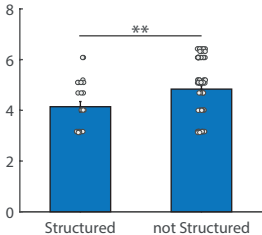
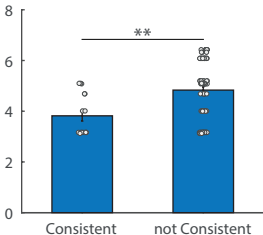
B





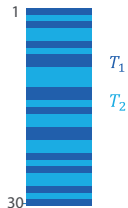
**A**

Max distance (mm)

**B**

# Trials bisection example

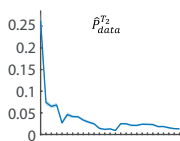
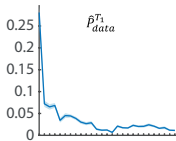
All trials



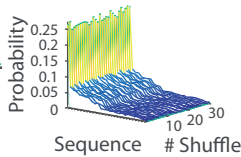
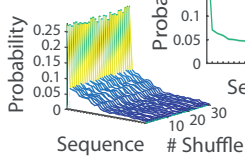
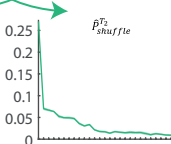
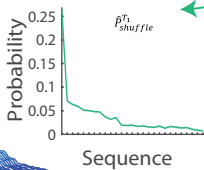
$C_{data}$   
 $\times 100$

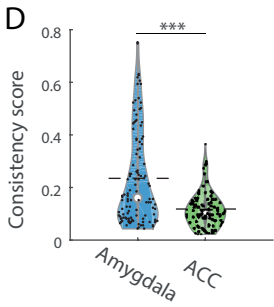
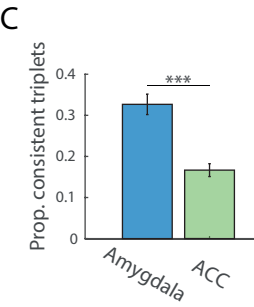
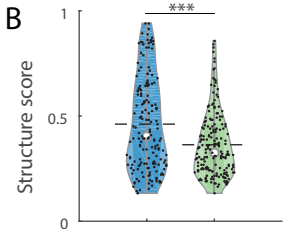
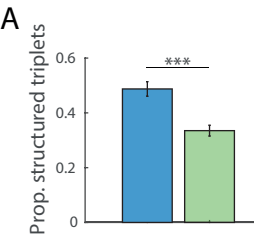
$$T_1: \{t \in t_1, \dots, t_{30} \mid |T|=15\}$$

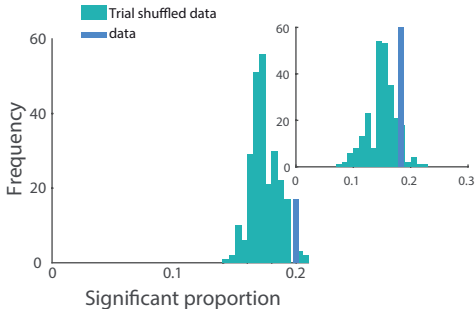
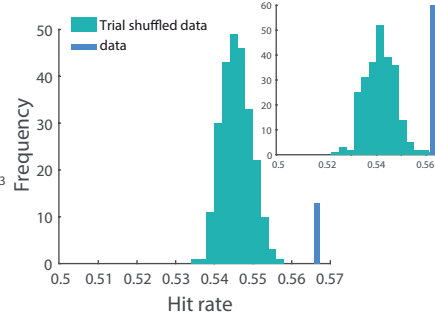
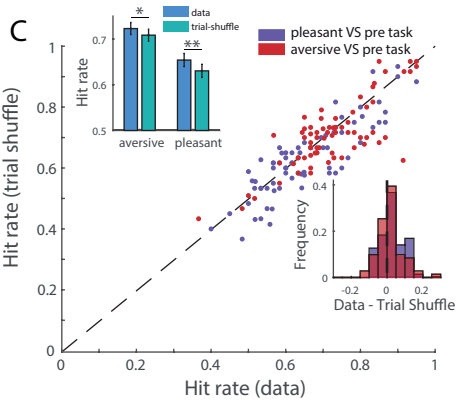
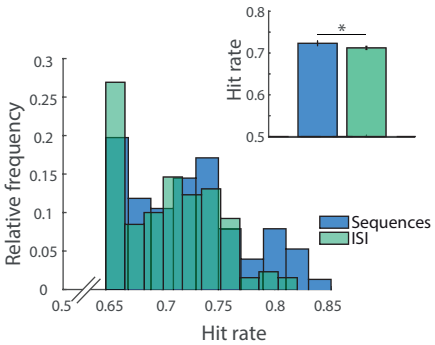
$$T_2 = \bar{T}_1$$



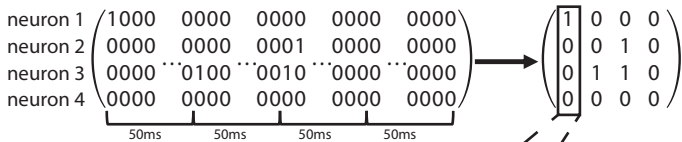
$\bar{C}_{shuffle}^{(1, \dots, n)}$





**A****B****C****D**

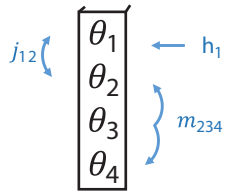
**A**



(independent)  $\langle \theta_i \rangle = \frac{1}{T} \sum_{t=1}^T \theta_i(t)$

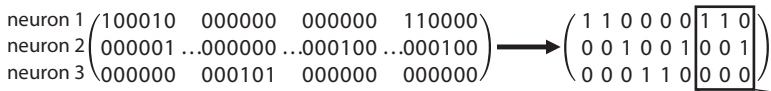
(pairwise)  $\langle \theta_{ij} \rangle = \frac{1}{T} \sum_{t=1}^T \theta_i(t) \theta_j(t)$

(triple-wise)  $\langle \theta_{ijk} \rangle = \frac{1}{T} \sum_{t=1}^T \theta_i(t) \theta_j(t) \theta_k(t)$



$$P(x) = \frac{1}{Z} \exp(\sum_{i=1}^N h_i x_i + \sum_{i<j} j_{ij} x_i x_j + \sum_{i<j<k} m_{ijk} x_i x_j x_k)$$

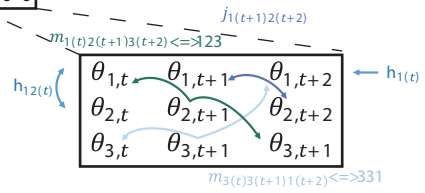
**B**



(independent)  $\langle \theta_i \rangle = \frac{1}{T} \sum_{t=1}^T \theta_i(t)$  ;  $\langle \theta_{ij} \rangle = \frac{1}{T} \sum_{t=1}^T \theta_i(t) \theta_j(t)$

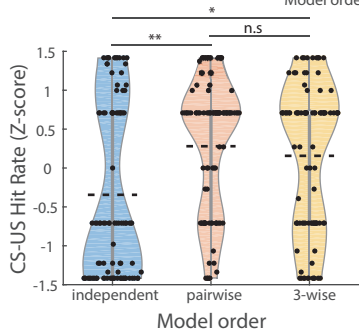
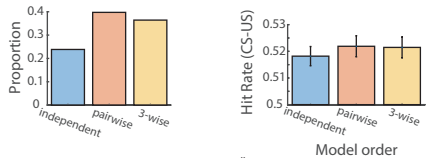
(pairwise)  $\langle \theta_{i(t)j(t+1)} \rangle = \frac{1}{T} \sum_{t=1}^T \theta_i(t) \theta_j(t+1)$

(triple-wise)  $\langle \theta_{i(t)j(t+1)k(t+2)} \rangle = \frac{1}{T} \sum_{t=1}^T \theta_i(t) \theta_j(t+1) \theta_k(t+2)$



$$P(x_{T,T+1,T+2}) = \frac{1}{Z} \exp(\sum_{i=1}^N \sum_{t=T}^{T+2} h_{i(t)} x_i(t) + \sum_{i<j} \sum_{t=T}^{T+2} h_{ij(t)} x_i(t) x_j(t) + \sum_{i,j} \sum_{t=T}^{T+1} j_{i(t)j(t+1)} x_i(t) x_j(t+1) + \sum_{i,j,k} m_{i(t)j(t+1)k(t+2)} x_i(t) x_j(t+1) x_k(t+2))$$

A



B

