



**HAL**  
open science

# Activity of tonically active neurons in the primate striatum reflects interaction between time processing and reward prediction

A.-C Martel, P. Apicella

► **To cite this version:**

A.-C Martel, P. Apicella. Activity of tonically active neurons in the primate striatum reflects interaction between time processing and reward prediction. 2022. hal-03845330

**HAL Id: hal-03845330**

**<https://hal.science/hal-03845330v1>**

Preprint submitted on 9 Nov 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## **Activity of tonically active neurons in the primate striatum reflects interaction between time processing and reward prediction**

Martel A.-C <sup>1,2</sup>, Apicella P. <sup>1</sup>

<sup>1</sup> Institut de Neurosciences de la Timone, UMR 7289, Aix Marseille Université, CNRS, Marseille, France

<sup>2</sup> Present address: Emory National Primate Research Center, Emory University, Atlanta, Georgia, USA

*Funding* : This work was supported by Centre National de la Recherche Scientifique and the Association France Parkinson. Funding for A.-C. Martel was provided by a doctoral fellowship from the French government, Fondation pour la Recherche Médicale, and Fondation des Treilles.

*Author contributions* : ACM, Acquisition of data, Analysis and interpretation of data, Drafting or revising the article; PA, Experimental design, Acquisition of data, Analysis and interpretation of data, Drafting or revising the article;

## **Abstract**

The striatum and its dopaminergic input participate in temporal processing and numerous studies provide evidence that interactions between dopamine and acetylcholine are critical for striatal functioning. However, the role of local cholinergic innervation of the striatum in behaviors requiring precise timing has not been specifically investigated. Here, we recorded from presumed striatal cholinergic interneurons, identified as tonically active neurons (TANs), in two male rhesus monkeys performing self-initiated movements after specified learned time intervals have elapsed since a visual cue. We found that 38% of all recorded TANs responded to the cue with a pause in firing and the strength of these responses could be modulated according to the duration of the interval being timed and the accuracy of time estimates. By examining the TAN response to the reward itself and by recording from TANs during a Pavlovian procedure in which no action was required, we found evidence that TAN activity modulation may potentially reflect differences in the animal's prediction of reward. Thus, besides their well-known role in predicting and detecting rewarding events, TANs may generate signals related to the processing of time. Our findings suggest a role of the local cholinergic circuitry in the representation of time within the striatum.

## 1 **Introduction**

2 Convergent evidence indicates that the striatum, the main recipient of afferents to the basal  
3 ganglia, is a key component in brain networks involved in temporal processing. Apart from  
4 timing deficits observed in striatal-based disorders in humans, such as Parkinson's disease  
5 (Pastor et al., 1992; Parker et al., 2013) and attention-deficit hyperactivity disorder (Rubia et  
6 al., 2009; Noreika et al., 2013), a large body of data supporting the role of the striatum and its  
7 dopaminergic input in the processing of time also comes from brain imaging studies in  
8 humans (Harrington et al., 2004; Coull et al., 2011) and lesion/inactivation studies in animals  
9 (Meck, 1996; 2006; Paton and Buonomano, 2018). In addition, electrophysiological studies in  
10 both rodents and monkeys trained on timing tasks have provided evidence of striatal activity  
11 related to the encoding of time at both single-neuron and population levels (Matell et al.,  
12 2003; Jin et al., 2009; Chiba et al., 2015; Gouvêa et al., 2015; Mello et al., 2015; Bakhurin et  
13 al., 2016; 2017; Wang et al., 2018; Zhou et al., 2020). Until now, hypotheses about the  
14 neuronal basis of striatal timing function have focused on the output neurons and the  
15 modulating influence of dopamine on temporal processing (Coull et al., 2011; Merchant et al.,  
16 2013; Paton and Buonomano, 2018). However, the striatum and its output pathways are  
17 strongly regulated by local circuits, including cholinergic interneurons, which exert a  
18 powerful influence on striatal network activity (Zhou et al., 2002). How the cholinergic  
19 innervation of the striatum, derived from interneurons, contributes to the processing of time  
20 remains unclear. On the other hand, there is considerable evidence that dopaminergic  
21 transmission interacts closely with the intrinsic striatal cholinergic system (Threlfell and  
22 Cragg, 2011; Cachope et al., 2012; Threlfell et al., 2012; Cai and Ford, 2018) and it is  
23 therefore possible that cholinergic signaling may complement DA transmission during timing  
24 behavior.

25 Although a link has been suggested between cholinergic activity and timing behavior  
26 based on lesion and pharmacological studies of cholinergic transmission in animals (Buhusi  
27 & Meck 2005; Meck 1996), none of them were specific enough to determine the contribution  
28 of the local cholinergic innervation of the striatum. Up to now, studies of behavioral effects of  
29 targeted disruption of striatal cholinergic transmission have documented disturbances in the  
30 ability to modify behavior in changing environments (Brown et al., 2010; Aoki et al. 2015),  
31 without specifically addressing a contribution to timing behavior.

32 Cholinergic interneurons of the striatum, presumed to be tonically active neurons  
33 (TANs) extracellularly recorded in behaving animals, are considered as detectors of  
34 motivationally salient stimuli whose timing cannot be precisely predicted. Indeed, in previous

35 work, we showed that TANs are particularly sensitive to the expected time of rewarding  
36 stimuli (Sardo et al., 2000; Ravel et al., 2001), suggesting that they may have access to  
37 temporal prediction information (Apicella et al., 2006). However, no study to date has  
38 specifically investigated the contribution of the cholinergic TAN system to timing, under  
39 conditions which require precise temporal control of behavior.

40 Another open question is whether reward expectation may bias time-related signals in  
41 the striatum. As for dopaminergic neurons, TANs are highly sensitive to reward-predicting  
42 stimuli (Aosaki et al., 1994; Apicella et al., 1997) and some of them are involved in the  
43 encoding of reward prediction error (Apicella et al., 2009; 2011). The intertwining of reward  
44 prediction and temporal processing is the source of an inherent difficulty in disambiguating  
45 effects related to timing processes from those related to expectations about reward (Paton and  
46 Buonomano, 2018; Fung et al., 2021).

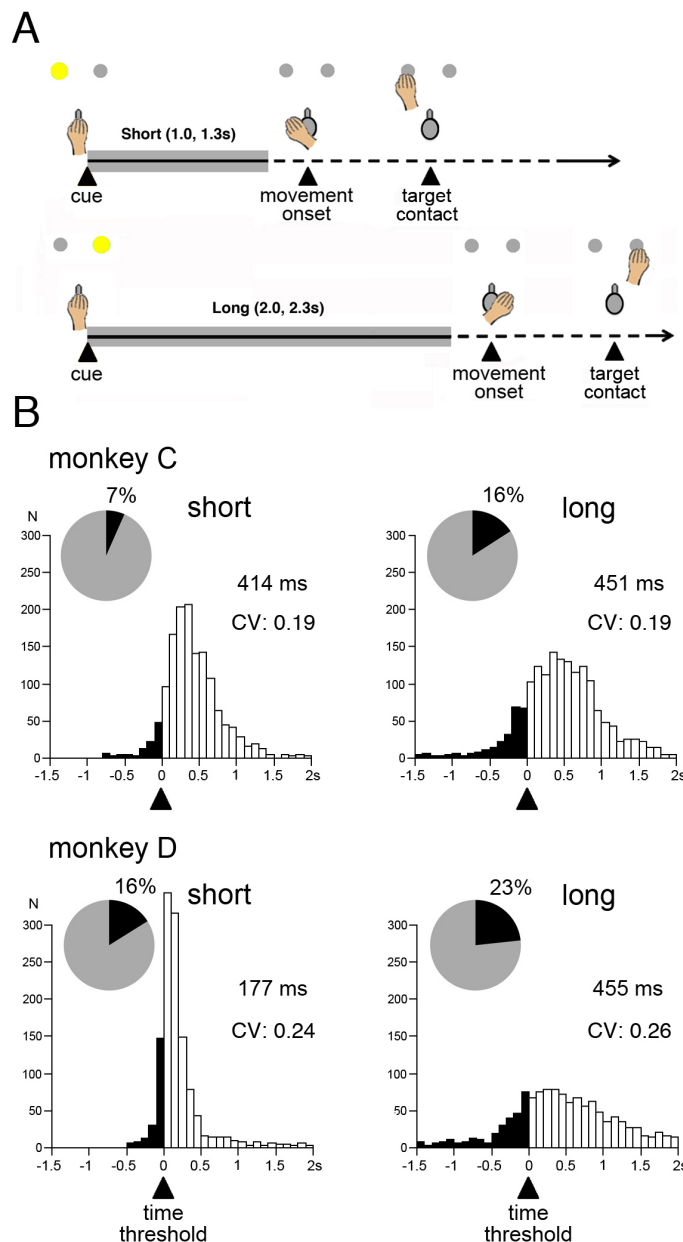
47 In the present study, we analyzed the activity of TANs during a time estimation task  
48 which requires monkeys to produce overt estimates of elapsed time. In this task, a visual cue  
49 is presented to the animals indicating that the reward is delivered for a self-paced movement  
50 executed at a specified time interval since the cue. We found that TANs displayed responses  
51 to the timing cue, some of them being scaled according to the duration of the interval to be  
52 timed and the accuracy of time estimates. Our results also indicate that the observed  
53 modulations in TAN activity may integrate reward prediction, in addition to the processing of  
54 time. Based on these findings, we suggest that the local cholinergic circuitry of the striatum  
55 could provide a neuronal mechanism that detect and process stimuli relevant for timing  
56 behavior and engage the striatal mechanisms more directly involved in the representation of  
57 time. A preliminary account of this work previously appeared in a short review (Martel and  
58 Apicella, 2021).

59

## 60 **Results**

### 61 *Monkey's ability to estimate time intervals*

62 We examined neuronal activity while rhesus monkeys performed a time estimation task  
63 (TET) in which they made self-initiated movements based on time estimates (Figure 1A). In  
64 this task, animals were presented with a visual spatial cue that indicates reward can be  
65 obtained for making reaching movements towards targets after a specified time interval, either  
66 *short* (1.0, 1.3s) or *long* (2.0, 2.3s), has elapsed since the onset of the cue. A schematic  
67 representation of the sequence of events in the TET is given in Figure 1A.



**Figure 1.** Time estimation task and timing performance. **A.** Temporal sequence of events in the task. Monkeys were required to initiate reaching movement after a specified time interval has elapsed. At the beginning of the trial, a visual spatial cue (yellow light), either on the left or the right side, indicated the duration of the interval (*Short* or *Long*) in the range of seconds, each time interval being associated with a particular location. Gray-shaded horizontal lines indicate the minimal waiting period before movement initiation assigned to each location of the cue (time threshold). Correctly timed movements were reinforced with fruit juice immediately after target contact. **B.** Timing performance. Distribution of movement onset times produced by monkeys in the task. CV, coefficient of variation (i.e., the ratio of the standard deviation to the mean). Values correspond to the mean onset time of movement after the criterion time. Black parts of the histograms correspond to movements which did not reach the criterion time (underestimation) and inset pie charts indicate the proportions of these incorrectly timed movements. Number of trials were 1370 and 1594 for monkey C and 1219 and 1146 for monkey D, for short and long intervals, respectively.

68

69

70 Distributions of movement onset times (MOTs) for each interval, indicate that the monkeys’  
71 timing accuracy was lowered in both animals when the duration of the interval was longer, as  
72 reflected by a broader distribution around the time threshold for movement onset (Figure 1B).  
73 The relative constancy of the coefficients of variation in the range of intervals we used  
74 indicates that the extent of the MOT distribution was proportional to the length of the interval  
75 being timed, consistent with the scalar property of interval timing which refers to the  
76 tendency for response time variability to increase with interval duration (Gibbon, 1977). This  
77 supports the idea that both monkeys have learned to adjust the timing of their movements  
78 according to the location of the cue. We also quantified the timing accuracy of movements by  
79 calculating the percentage of trials in which animals correctly awaited the end of the

80 minimum waiting period. Both monkeys responded after the criterion time in more than 80%  
81 of the trials, regardless of interval duration, reflecting their ability to adjust time estimates  
82 according to temporal information from cue. There was a mean 11.6% and 19.7%  
83 underestimation errors in monkeys C and D, respectively (Fig. 1B, insets), this difference  
84 being statistically significant ( $\chi^2 = 66.29$ ,  $df = 1$ ,  $p < 0.0001$ ). In monkey D, the timing of  
85 movements was remarkably accurate for the short interval, the mean MOT being 177 ms after  
86 the time threshold, compared to 414 ms in monkey C. On the other hand, MOTs were  
87 relatively similar between the two animals for the long interval (451 ms and 455 ms in  
88 monkeys C and D, respectively). These interindividual differences may reflect different  
89 timing strategies. Namely monkey C could efficiently use the cue associated with each  
90 interval duration, leading to similar levels of timing accuracy in self-timed movements,  
91 whereas monkey D apparently used a strategy to optimize the estimated onset time of its  
92 movement specifically for the short interval.

93

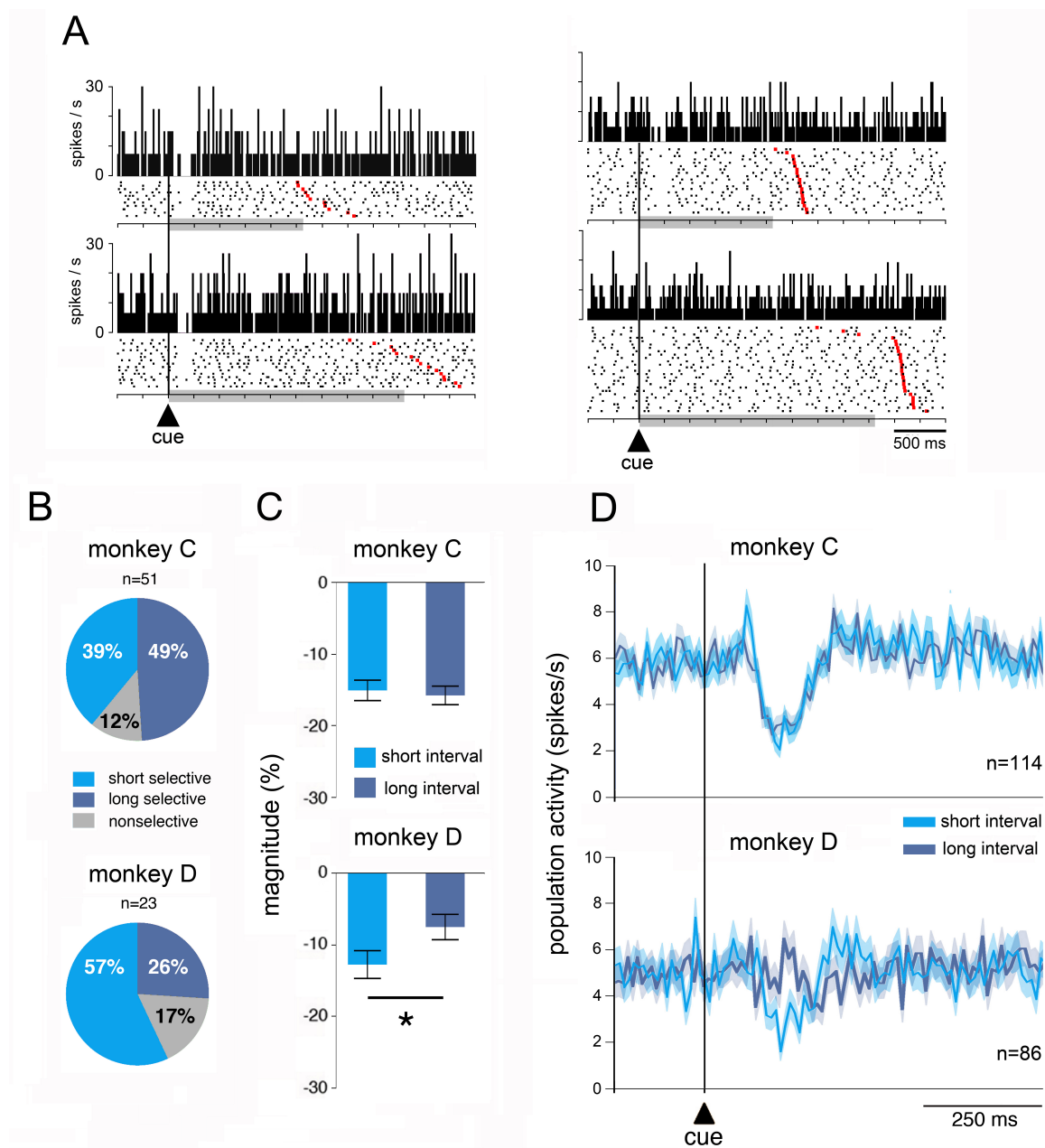
#### 94 *Sensitivity of TANs to the timing cue*

95 We recorded the activity of 200 neurons (114 in monkey C, 86 in monkey D)  
96 electrophysiologically identified as tonically active neurons (TANs). Previous work in  
97 behaving monkeys has shown that TANs constitute a group of neurons that share  
98 characteristic spiking features (Figure 2-figure supplement 1) and display relatively  
99 homogeneous changes in activity related to the detection of motivationally salient stimuli  
100 (Apicella, 2017). In the present study, the predominant task-related modulation of TAN  
101 activity consisted in a short-lasting depression in activity (*pause*) after the presentation of the  
102 timing cue. This pause could be followed by a transient increase in activity (*rebound*) and, in  
103 some rare instances, preceded by a brief excitatory phase. The histological verification of  
104 recording sites in both monkeys showed that most of the recorded TANs were located in the  
105 dorsal putamen, at precommissural and postcommissural levels, as will be reported in detail  
106 later (Figure 7).

107 For each recorded TAN, we analyzed the responsiveness to the timing cue separately  
108 for short and long intervals. Over 38% of all TANs displayed statistically significant  
109 decreases in activity in response to the onset of the cue associated with the short and/or long  
110 interval (monkey C: 51/114, 45%; monkey D: 23/86, 27%), monkey C showing a larger  
111 fraction of responsive neurons than monkey D ( $\chi^2=6.80$ ,  $df=1$ ,  $p=0.009$ ). Example responsive  
112 neurons are shown in Figure 2A. The first neuron (Fig. 2A, left) displayed a pause response to  
113 the cue for the short- and long-interval trials. In the second neuron (Fig. 2A, right), a pause

114 response to the cue was detected for the short-interval trials, whereas a weak nonsignificant  
 115 change occurred for the long-interval trials.

116



117

118

**Figure 2.** Sensitivity of TANs to the timing cue. **A.** Two example neurons responding to the cue. Left, nonselective response. Right, selective response. Each trial is displayed as a row of spikes (dots) aligned at cue onset, with perievent time histogram above each raster plot. Data are separated by interval duration and sorted by MOT. Red markers indicate the times of movement onset. Gray-shaded bars indicate the minimum waiting period before movement onset. **B.** Percentages of different response types evoked by the cue. n, number of responsive neurons. **C.** Boxplot representation of magnitudes of changes in TAN activity after the cue for short and long intervals. Data are indicated as decreases in percentage below baseline activity and expressed as means  $\pm$  SEM (\*  $p < 0.05$ , Wilcoxon rank-sum test). **D.** Population activity of TANs aligned on the cue onset marked by the vertical line. The analysis included rewarded trials (i.e., correctly timed movements) and unrewarded trials (i.e., incorrectly timed movements). Colored curves represent mean activity of TANs separately for short and long interval trials calculated in nonoverlapping time bins of 10 ms. Shading indicates SEM. n, number of neurons included for population curves.



119

120 Of the 74 neurons responding to the cue, 10 (14%) responded regardless of the interval and 64  
121 (86%) responded with only one interval, indicating that most TANs were able to discriminate  
122 between short- or long-interval cues. As illustrated in Figure 2B, in monkey C, there were no  
123 significant differences in the fraction of TANs showing selectivity for one interval or the  
124 other (short-preferring,  $n=20/51$ , 39%; long-preferring,  $n=25/51$ , 49%,  $\chi^2=0.99$ ,  $df=1$ ,  
125  $p=0.318$ ), whereas in monkey D the percentage of selective responses was significantly  
126 higher for the short interval than for the long interval (short-preferring,  $n=13/23$ , 57%; long-  
127 preferring,  $n=6/23$ , 26%,  $\chi^2=4.16$ ,  $df=1$ ,  $p=0.041$ ). It therefore appears, in both animals, that  
128 most TANs showed selective responses to the cue depending on the interval duration, with  
129 monkey D showing an increased responsiveness of TANs to the cue associated with the short  
130 interval, possibly reflecting the higher accuracy of time estimates with this interval.

131 To analyze quantitatively whether the sensitivity of TANs to the timing cue differed  
132 according to interval duration, we rated the magnitude of changes in TAN activity during  
133 specified time windows which we selected on the basis of our analysis of latency and duration  
134 of individual neuronal responses for each monkey and each time interval (see Materials and  
135 Methods). We did this for each neuron of the entire sample of TANs tested in the TET. The  
136 results of this analysis are given in Fig. 2C. The magnitude of pauses in TAN activity varied  
137 insignificantly between short and long intervals in monkey C (Wilcoxon rank-sum test,  
138  $z=0.415$ ,  $p=0.677$ ), whereas it was significantly higher for the short interval than for the long  
139 one in monkey D (Wilcoxon rank-sum test,  $z=2.241$ ,  $p=0.025$ ). We also examined the average  
140 activity from all neurons separately for the short and long intervals. As shown in Figure 2D, a  
141 clear population response after the cue onset was obvious for both intervals in monkey C,  
142 whereas a response occurred for the short interval in monkey D, with comparatively little sign  
143 of activity modulation for the long interval. In this animal, a preference for the short interval  
144 was therefore evident at the level of both the population and the individual neurons, reflecting  
145 the monkey's tendency to be more efficient in using temporal information from short-interval  
146 cues. On the other hand, in monkey C, the magnitudes of the TAN response were not  
147 dependent of the interval duration, suggesting that this animal made use of temporal  
148 information to initiate actions in a more homogeneous way. This indicates that the different  
149 responsiveness of TANs in the two monkeys may potentially reflect differences in strategies  
150 for performing the timing task.

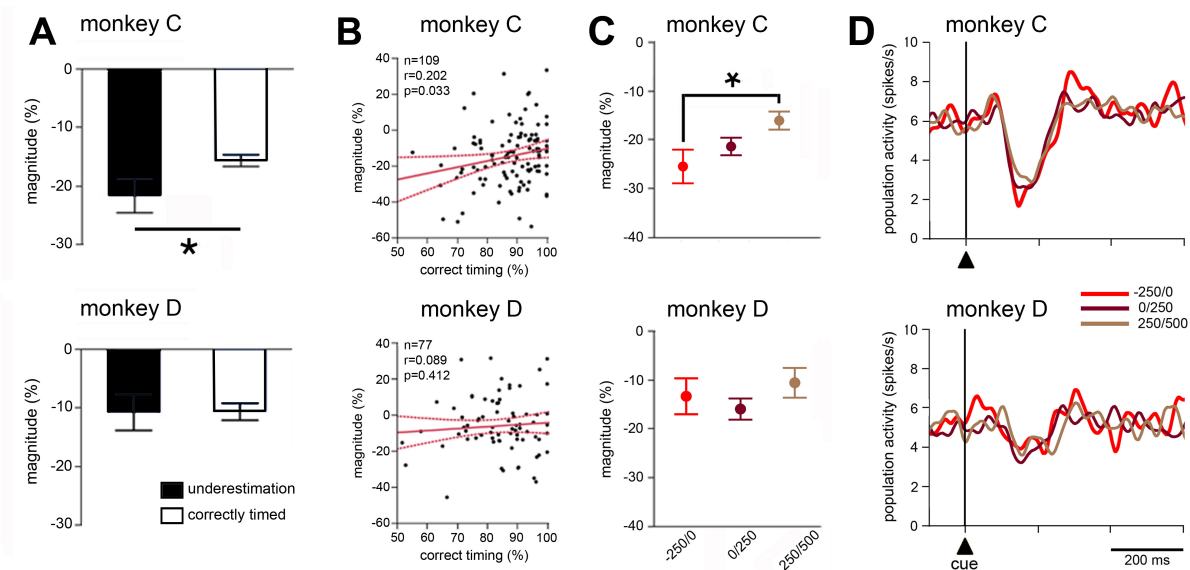
151

152 *Influence of timing accuracy on TAN responsiveness*

153 We next examined whether TAN activity after cue onset may be related to the monkeys'  
154 timing accuracy. To this end, we first compared movements initiated before reaching the time  
155 threshold (i.e., movements with negative MOTs) and movements that meet the time threshold  
156 (i.e., movements with positive MOTs), combining data from both intervals. As shown in  
157 Figure 3A, in monkey C, the magnitude of TAN pauses was stronger for incorrectly timed  
158 movements, compared with correctly timed movements (Wilcoxon rank-sum test,  $z=2.239$ ,  
159  $p=0.025$ ), whereas differences in activity between correctly and incorrectly timed movements  
160 were not statistically different in monkey D (Wilcoxon rank-sum test,  $z=0.177$ ,  $p=0.859$ ) (Fig.  
161 3A).

162 To further assess a possible link between the TAN activity and timing performance,  
163 we performed a correlation analysis across neurons between the magnitude of TAN responses  
164 to the cue and the percentage of trials in which monkeys correctly reached the time threshold.  
165 As shown in Figure 3B, in monkey C, we found a statistically significant linear relationship  
166 between the magnitude of TAN modulations and percentage of correctly timed movements  
167 ( $r=0.202$ ,  $p=0.033$ ), indicating that trials in which the magnitude of the TAN response to the  
168 cue was higher were followed by shorter production time intervals. In contrast, we did not  
169 found any significant correlation between the strength of the TAN response to the cue and  
170 timing accuracy in monkey D ( $r=0.089$ ,  $p=0.412$ ).

171



172

**Figure 3.** Sensitivity of TANs to timing accuracy. **A.** Comparison of magnitudes of TAN responses to the cue between correctly and incorrectly timed movements. Same conventions as in Fig. 2C. **B.** Correlations between magnitudes of TAN responses to the cue and levels of timing performance, for each block of trials in which a TAN was recorded. The solid lines (in red) indicate the fit of a linear regression and dashed lines indicate the 95% confidence interval from the regression. *n*, number of neurons. *r*, Pearson correlation coefficient. **C.** Influence of the proximity to the time threshold for movement onset. Magnitudes of TAN responses to the cue were computed separately from trials within different ranges of MOT (three consecutive 250 ms periods starting from 250 ms before to 500 ms after the time threshold), \*  $P < 0.05$ . **D.** Population activity of TANs for trials in different MOT ranges. Same conventions as in Fig. 2D, except that curves are smoothed with a Gaussian filter ( $\alpha=0.04$ ).

173

174 We then asked whether the strength of TAN modulation is dependent on proximity to the time  
 175 threshold. To examine this, we sorted the trials by their MOT value and computed the  
 176 magnitude of the pause response to the cue in three ranges of values around the time criterion  
 177 (Fig. 3C). We found that the pause was significantly higher for the trials with the lower  
 178 MOTs than for the trials with the higher MOTs in monkey C. This indicates that TAN  
 179 response magnitude to the cue was influenced by the ability of monkey C at estimating the  
 180 elapsed time before movement onset, with TAN modulations being stronger for movements  
 181 initiated just before the time threshold (i.e., underestimation errors). Such a relationship  
 182 between TAN response and timing accuracy was not observed for monkey D. However, when  
 183 comparing across different MOT ranges in monkey C, there was no visible difference in the  
 184 response of the population of neurons (Fig. 3D), indicating that individual TANs can  
 185 discriminate better between the short- and long-interval cues than the average activity from  
 186 the whole sample of neurons.

187 In summary, we showed, in one of our monkeys, that the strength of TAN response to  
 188 the cue seemed to reflect the animal's intention to move at a particular time, suggesting that

189 TANs are involved in signaling motor initiation in a time-dependent manner. The  
190 interindividual difference in the sensitivity of TANs to timing performance emphasizes a  
191 possible link with the monkey's strategy to determine the onset time of movements.

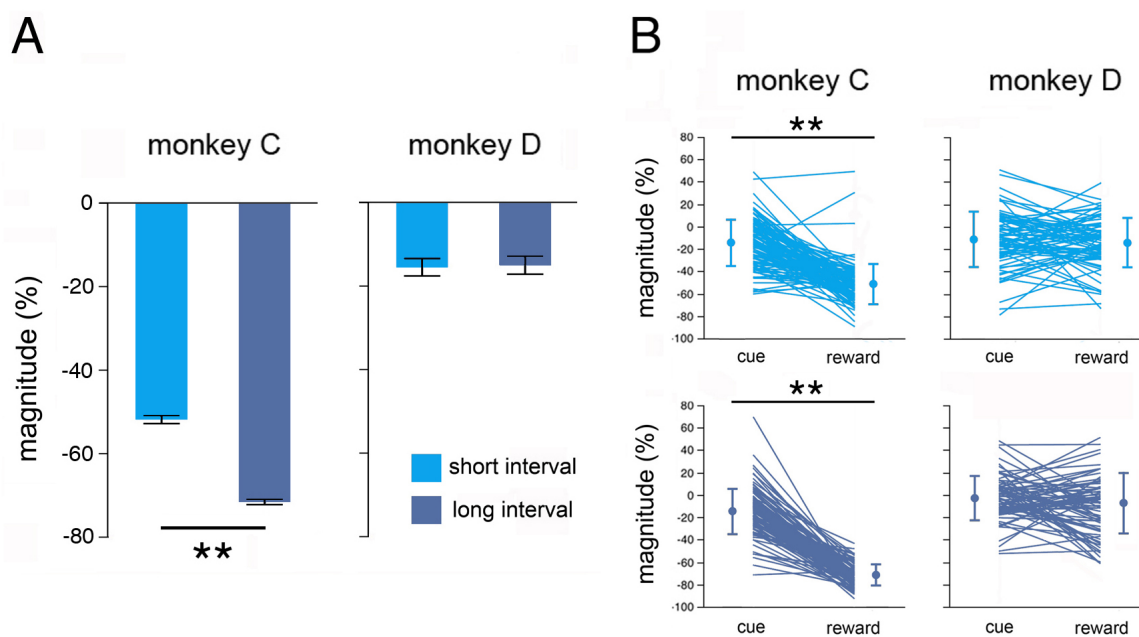
192

### 193 *Influence of reward prediction on TAN responsiveness*

194 In the task employed here, the cue not only contains temporal information, but also predicts  
195 reward after a correctly timed movement. Because of the role TANs play in the detection of  
196 reward-predictive events, we should expect that prediction of the upcoming reward  
197 contributes to TAN sensitivity to the cue. Our behavioral data analysis showed that the  
198 proportion of correctly timed movements was higher in monkey C (88.4%) than in monkey D  
199 (80.3%), and this was paralleled by an increased TAN responsiveness to the cue in monkey C,  
200 suggesting that the increased ability of this animal at producing correctly timed movements  
201 favored predictable rewards and then enhanced the TAN responsiveness to the cue. To test  
202 this suggestion indirectly, we examined the effect of the reward itself on TAN activity. It was  
203 reasoned that a higher reward predictive value of the cue would lead to a decreased TAN  
204 responsiveness to reward, consistent with the inverse relationship between reward prediction  
205 and TAN responses to reward we have reported in previous studies (i.e., the more predictable  
206 the reward, the less strongly TANs respond to it). We then turned to the effects of reward on  
207 TAN activity using a similar time window analysis to that done for assessing the  
208 responsiveness of TANs to the cue. We found statistically significant pause responses to  
209 reward in 28 of 114 neurons (25%) and 31 of 86 neurons (36%) in monkeys C and D,  
210 respectively, the TANs in monkey C showing a tendency to lower, albeit not significant,  
211 responsiveness to reward than did TANs in monkey D ( $\chi^2=3.10$ ,  $df=1$ ,  $p=0.07$ ). This  
212 indicates that reward prediction could potentially be incorporated in TAN responses to the  
213 timing cue in monkey C.

214 To further investigate sensitivity to reward for all TANs, we examined the magnitude  
215 of changes in activity during specified time windows adjusted to latency and duration of  
216 individual TAN responses to reward. As shown in Figure 4A, in monkey C, we found that  
217 TAN responses to reward were stronger for the long interval, compared to the short interval  
218 (Wilcoxon rank-sum test,  $z=16.30$ ,  $p < 0.0001$ ). This is in contrast with what we observed in  
219 monkey D, in which the effect of the delivery of reward on TAN activity was relatively mild  
220 and (did not differ) in magnitude between intervals (Wilcoxon rank-sum test,  $z=0.21$ ,  
221  $p=0.833$ ). In addition, when comparing across neurons the magnitudes of activity changes  
222 following the cue and reward, we found an inverse relationship between cue and reward

223 responses in monkey C (i.e., TANs that responded weakly to the cue responded strongly to  
224 reward) irrespective of interval duration (Fig. 4B) (Wilcoxon rank-sum test,  $z=0.69$ ,  $p=0.487$ ),  
225 providing support to the idea that differences in the responsiveness of TANs to the timing cue  
226 may partly result from differences in reward prediction. This effect was not present in  
227 monkey D (Wilcoxon rank-sum test,  $z=0.33$ ,  $p=0.534$ ). The above results suggested that  
228 changes in reward prediction, in at least one animal, could account for TAN sensitivity to the  
229 timing cue we observed.  
230



231

**Figure 4.** Sensitivity of TANs to reward. **A.** Boxplot representation of magnitude of changes in TAN activity after the delivery of reward separately for short and long intervals. Conventions are the same as in Fig. 2C, except that only rewarded trials were included. \*\*  $P < 0.01$  (Wilcoxon signed-rank test). **B.** Changes in TAN activity after cue and reward separately for short and long intervals for each neuron recorded. Each line indicates the data of one neuron. Thick dots and error bars represent mean and SEM respectively. \*  $P < 0.05$ , \*\*  $P < 0.01$  (Wilcoxon signed-rank test).

232

233 In the TET, the movement was triggered by an internal decision process based on the  
234 estimation of elapsed time from the cue onset and there may be a possibility, in the absence of  
235 an external movement-triggering stimulus, that the movement itself serves as an event that  
236 predicts reward. We then focused on the influence of the movement onset on TAN activity.  
237 By analyzing TAN activity aligned on the initiation of movements in both monkeys, we  
238 showed that TANs, as a population, did not display activity modulation around the onset of  
239 movement, regardless of the interval duration (Figure 4-figure supplement 1).

240

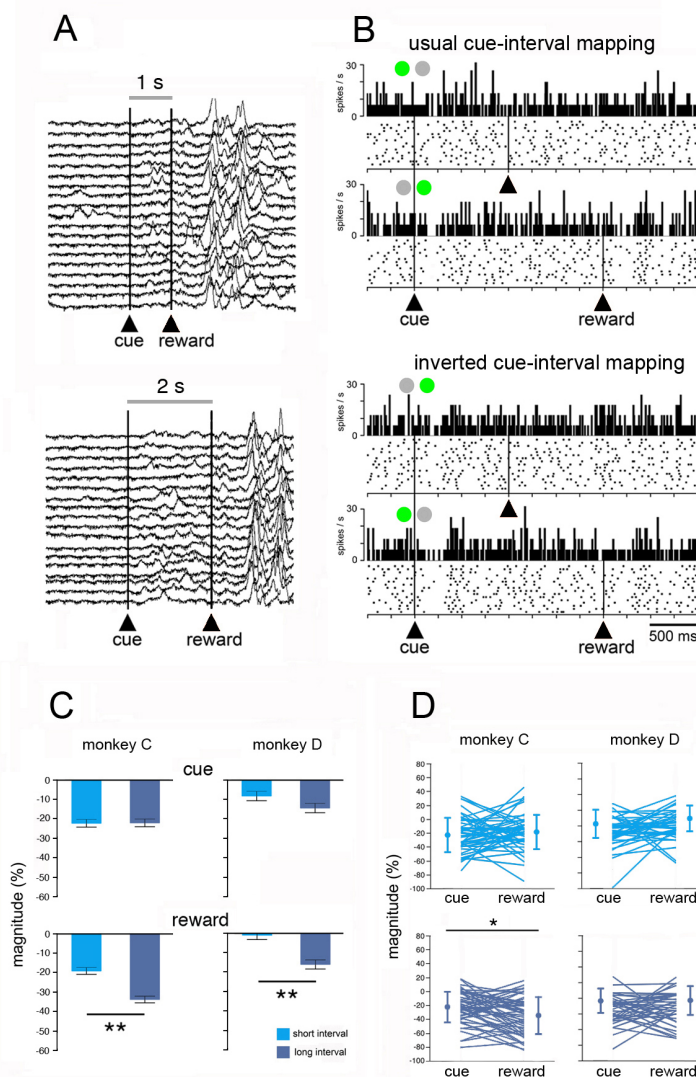
241 *Influence of interval duration in the Pavlovian protocol*

242 To assess more specifically the possible contribution of reward prediction in modulating TAN  
243 activity, we examined TAN responses when monkeys were engaged in a Pavlovian  
244 conditioning task (PCT) in which visual cues solely conveyed differences in the time elapsed  
245 until reward delivery, with no requirement for action. Such a condition allowed us to examine  
246 the influence of elapsed time while reward was invariably delivered on every trial. By using  
247 two time intervals in the same range as those in the TET and same relationships between cue  
248 location and interval duration, we found that both monkeys showed anticipatory licking  
249 movements prior to the delivery of reward indicating that the cue served as a predictor of  
250 reward (Fig. 5A).

251         Among 90 TANs tested in the PCT, responses to the cue and reward were detected in  
252 30 neurons (monkey C: 14/52, 27%; monkey D: 16/38, 42%) and 52 neurons (monkey C:  
253 32/52, 62%; monkey D: 20/38, 53%), respectively. In contrast with what we observed in the  
254 TET, the proportion of TANs responding to the cue did not vary significantly between the  
255 two monkeys ( $\chi^2 = 2.27$ ,  $df = 1$ ,  $p = 0.131$ ). Fig. 5B shows an example of a neuron sensitive  
256 to the interval duration in the PCT. In a first block of trials (*usual cue-interval mapping*), the  
257 neuron displayed a response to the cue which was stronger for long interval trials than for  
258 short interval trials. Then, the same neuron was tested in another block of trials (*inverted cue-*  
259 *interval mapping*) in which we reversed the assignments of the spatial cues for the short and  
260 long interval. As can be seen, the response selectivity was maintained, suggesting that the  
261 observed modulations in TAN activity did not appear to be related to the location of the cue.  
262 This neuron also responded selectively to reward delivered at the end of the long interval in  
263 both trial blocks. We examined the sensitivity to cue location in seven TANs by using  
264 standard and inverted cue-interval mappings and found that magnitudes of response to the cue  
265 did not depend on the spatial location of the cue (Figure 5-figure supplement 1).

266         As a next step, we undertook in the PCT a similar analysis to that done in the TET to  
267 assess quantitatively the effects of interval duration on changes in TAN activity within time  
268 windows determined for the cue and reward. As shown in Fig. 5C, the magnitude of TAN  
269 response to the cue failed to show any significant difference between time intervals, whereas  
270 a clear time-dependent modulation of reward response emerged in both monkeys, with  
271 stronger responses to long intervals compared to short intervals. This finding is consistent  
272 with results from our previous studies showing that a lack of prediction about the time of  
273 reward delivery has an enhancing effect on the TAN response to this event (Sardo et al., 2000;  
274 Ravel et al., 2001), which has been interpreted as reflecting a lowered monkey's time-keeping

275 ability with increased duration of the cue-reward interval. Contrary to what we observed in  
 276 TET in monkey C, we did not find a clear relationship between cue and reward responses in  
 277 PCT (Fig. 5D), with the exception of a weak, albeit significant, effect for the long interval  
 278 (Wilcoxon rank-sum test,  $z=2.29$ ,  $p=0.021$ ). It therefore appears that interval duration exerts a  
 279 more homogeneous effect on the activity of TANs in the PCT with the presence of a time-  
 280 dependent effect on the reward response and the absence of relationship between cue and  
 281 reward responses.  
 282



**Figure 5.** Effects of interval duration on TAN activity in the Pavlovian conditioning task (PCT). **A.** Licking movements after the onset of the reward predictive cue. For each interval duration are shown traces of lick records aligned on the cue onset. Licking started immediately after the presentation of the cue, indicating that animals have learned the reward predictive value of this signal. **B.** Raster plots of the activity of a single TAN recorded in the PCT. On this example, two blocks of trials were administered, corresponding to two cue-interval configurations. Rasters are separated by interval duration, with the upper two panels showing the TAN activity in the usual cue-interval configuration, and the lower two when the configuration has been inverted. The two circles at the top of each histogram indicate the left or right location of the cue (in green). Same conventions as in Fig. 2A. **C.** Magnitude of changes in TAN activity after cue and reward separately for short and long intervals. Same conventions as in Fig. 2C. **D.** Changes in TAN activity after cue and reward separately for short and long intervals for each neuron recorded. Same conventions as in Fig. 4B.

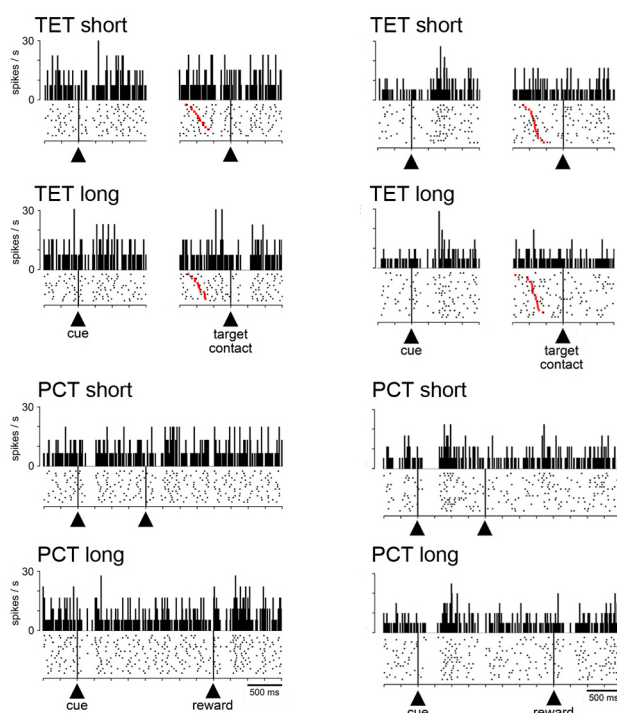
283

284

285

286 Lastly, we compared activity changes in a subset of 31 TANs recorded in both the TET and  
 287 PCT. This comparison allowed us to differentiate the sensitivity of TANs to the timing cue  
 288 when a similar underlying temporal representation in the seconds range was used either to

289 produce an overt estimate of elapsed time (TET) or to simply predict reward after a time  
290 interval has elapsed (PCT). For some neurons, the strength of TAN modulation after the cue  
291 onset was different when passing from the TET to the PCT, while for other neurons cue  
292 responses remained constant in the two conditions. Examples of neurons responding to the  
293 cue are shown in Figure 6. The first neuron (Fig. 6, left) showed a weak response to the cue in  
294 the TET, while the cue response was more pronounced when the animal was shifted to the  
295 PCT, indicating that the response may vary with respect to changes in the learning context. As  
296 is apparent in the figure, the same neuron also responded to reward regardless of the  
297 condition, this response being greatest for the long interval. The second neuron (Fig. 6, right)  
298 showed a response to the cue that was clearly invariant to the condition. Although there was  
299 no modulation of activity following reward delivery in the TET, a response to reward was  
300 present only for the long-interval trials in the PCT. These findings indicate that some TANs  
301 showed different degrees of modulation of their activity upon variation of the task context in  
302 which timing cues were presented.  
303



**Figure 6.** Influence of task condition on TAN responses to the cue and reward. Two example neurons tested in the two conditions. Rasters are separated by interval duration and task condition. Same conventions as in Fig. 2A, except that activity of each neuron is aligned separately to the cue onset and target contact in the TET. A few unrewarded trials are visible at the bottom of each raster aligned on reward delivery (absence of red marker) corresponding to monkey's responses before the criterion time. TET, time estimation task; PCT, pavlovian conditioning task.

304

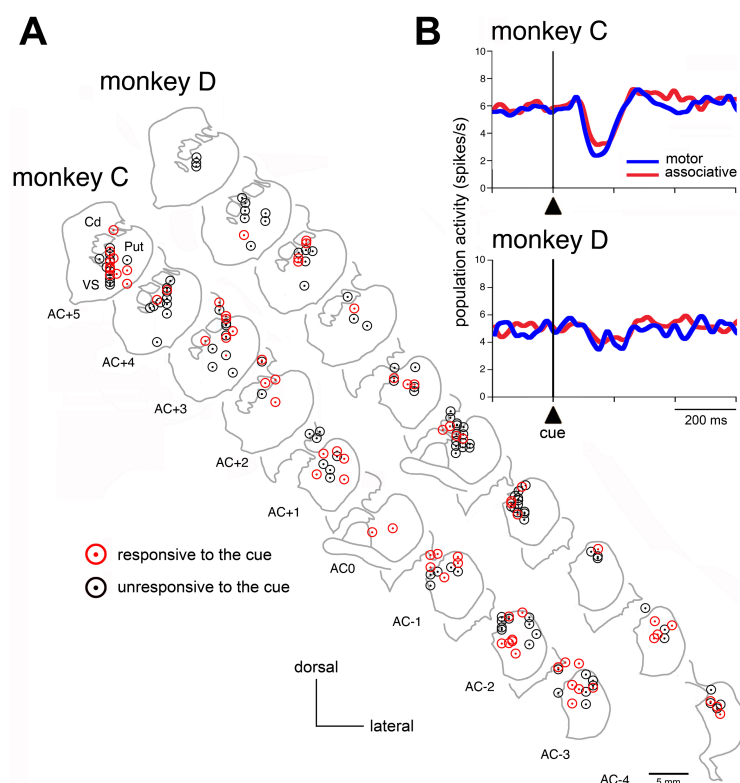
305

### 306 *Sensitivity of TANs to the timing cue among striatal regions*

307 In primates, the posterior putamen is related to sensorimotor functions while dorsal and  
308 ventral parts of the anterior striatum are involved in associative and limbic functions  
309 respectively. In order to look for region-specific signals in TANs, the location of the recorded



310 neurons was identified histologically in both monkeys. As illustrated in Figure 7A, TANs  
311 were mostly sampled from the dorsal parts of the precommissural and postcommissural  
312 putamen and were distributed across the same regions of the nucleus explored in the two  
313 animals. Only a small number of neurons (15 and 3 neurons in monkeys C and D,  
314 respectively) were recorded in the ventral striatum (i.e., the ventral part of the precommissural  
315 caudate nucleus and putamen). They were not assessed in detail because the sample size was  
316 too small for statistical analysis. We then compared the sensitivity of TANs to the timing cue  
317 in the two parts of the dorsal striatum (i.e., the motor and associative striatum). Responses to  
318 the cue were found in 37 neurons of the associative striatum (monkey C: 23/53, 43%; monkey  
319 D: 14/49, 29%) and 33 neurons of the motor striatum (monkey C: 22/46, 48%; monkey D:  
320 11/34, 32%). Frequencies of cue responses did not vary significantly between the two regions  
321 (monkey C:  $\chi^2 = 0.195$ ,  $df = 1$ ,  $p = 0.658$ ; monkey D:  $\chi^2 = 0.136$ ,  $df = 1$ ,  $p = 0.711$ ),  
322 suggesting that response modulations related to the timing cue were not significantly different  
323 for the two regions of the striatum. Data from the two monkeys were also analyzed separately  
324 at the level of the population of TANs sampled in each dorsal striatal region, regardless of the  
325 responsiveness of individual neurons (Fig. 7B). The population responses to the timing cue in  
326 the motor and associative striatum were relatively weak in monkey D, compared with monkey  
327 C, and they did not appear to differentiate short- and long-interval trials.  
328



**Figure 7.** Sensitivity of TANs to the timing cue among striatal regions. **A.** Recording sites of TANs in the striatum of both monkeys. The location of all recorded TANs is plotted in the rostrocaudal direction on coronal sections from 5 mm anterior to 3-4 mm posterior to the anterior commissure (AC), with 1 mm intervals. Cd, caudate nucleus, Put, putamen, VS, ventral striatum. **B.** Comparison of population activities of TANs aligned on cue onset, grouped for short and long intervals, between motor and associative striatum. Same conventions as in Fig. 3D.

## 330 **Discussion**

331 Previous experimental studies and theoretical models assessing the role of the striatum in the  
332 processing of temporal information have focused on the output neurons and did not outline  
333 any contribution of local circuit neurons which are critical regulators of the striatal network  
334 activity (Buhusi and Meck, 2005; Merchant et al., 2013). In previous work, we have  
335 documented that TANs, presumed cholinergic interneurons in the striatum, display strongest  
336 activity changes when rewarding stimuli are presented at unexpected times (Sardo et al.,  
337 2000; Ravel et al., 2001), suggesting that the integration of temporal information interacts  
338 with TAN signaling of reward-related events (Apicella et al., 2006). Here, we specifically  
339 addressed the role of TANs in temporal processing by analyzing their activity in monkeys  
340 performing self-paced movements based on time estimates in the range of seconds. Our  
341 results indicate that the responsiveness of TANs to the timing cue was influenced not only by  
342 temporal variables, but also by predictable rewards. This study provides the first account in  
343 behaving monkeys of modulation of TAN activity related to the animal's ability to react in a  
344 temporally controlled manner, suggesting that the intrinsic striatal cholinergic system may  
345 contribute to timing function.

346

### 347 *TAN activity in relation to time estimation*

348 To perform the TET, our monkeys were required to retrieve temporal information from visual  
349 cues, wait for a specified time interval, and then initiate a self-paced movement. Over 38 % of  
350 the TANs we recorded were responsive to the cue that marked the beginning of the time  
351 interval prior to movement initiation. Behavioral data confirm that the onset time of self-  
352 paced movements became more variable as interval duration increased, reflecting the  
353 increased variability in time estimates with the duration being timed, termed the scalar  
354 property of interval timing (Gibbon, 1977). Although both monkeys were trained extensively  
355 prior to recordings, our results revealed a difference in the degree of timing accuracy between  
356 the two animals, suggesting that they did not perform the task in the same way. On average,  
357 monkey D made more premature responses than monkey C and demonstrated an optimized  
358 timing performance for short-interval trials in terms of proximity to the time threshold. On the  
359 other hand, the timing strategy of monkey C did not change according to the interval duration,  
360 achieving approximately similar levels of timing accuracy for both intervals. Interestingly,  
361 these interindividual differences in timing performance were associated with differential  
362 responsiveness of TANs to the timing cue. The neurons in monkey C showed higher  
363 responsiveness to the timing cue (45%) than did neurons in monkey D (27%), suggesting that

364 the timing cue was better taken into account, regardless of interval duration, for the former  
365 animal. Also, in monkey C, the strength of TAN modulation was similar for both short- and  
366 long-interval trials, whereas, in monkey D, TAN activity showed selectivity for the short  
367 interval compared to the long one, in terms of fraction of responsive neurons and the  
368 magnitude of responses. This may reflect, in this latter animal, the development of a spatial  
369 bias favoring the short-interval cue over the other. An intriguing result in monkey C was that  
370 TAN responses to the timing cue were stronger in trials with movements initiated before  
371 reaching the time threshold than in trials with correctly timed movements. We also found a  
372 relationship between the overall magnitude of TAN responses to the cue and the accuracy of  
373 timing (i.e., the stronger the neuronal modulation, the sooner was the self-timed movement).  
374 It therefore appears, at least for this animal, that the TAN response to the cue was specifically  
375 coupled to the onset time of the movement, providing support for the idea that TANs might  
376 participate in the adjustment of timed movements.

377

#### 378 *Contribution of TANs to the encoding of time*

379 The finding that TANs were responsive to the timing cue suggests a role for these presumed  
380 interneurons in initiating the timing process at the beginning of the interval to be timed,  
381 possibly through the shaping of sustained and/or sequential activity of striatal output neurons.  
382 Indeed, several recording studies in behaving animals have reported changes in the activity of  
383 output neurons of the striatum, at both single-neuron and population levels, that could convey  
384 information about how much time has elapsed (Matell et al., 2003; Jin et al., 2009; Chiba et  
385 al., 2015; Gouvêa et al., 2015; Mello et al., 2015; Bakhurin et al., 2016; 2017; Wang et al.,  
386 2018; Zhou et al., 2020). Our work supports the notion that the TAN response to the cue  
387 signals the detection of a stimulus associated with a learned time interval. This role for TANs  
388 may be quite similar to that proposed for midbrain dopaminergic neurons in a theoretical  
389 model of timing control, called the striatal beat frequency model, in which the dopaminergic  
390 response is considered as crucial for initiating the timing process (Matell and Meck, 2000;  
391 2004; Buhusi and Meck, 2005; Meck, 2006). It is possible, based on known interactions  
392 between dopaminergic and cholinergic transmissions within the striatum (Threlfell and Cragg,  
393 2011; Cachepe et al., 2012; Threlfell et al., 2012; Cai and Ford, 2018), that signals arising  
394 from these two neuromodulatory systems provide striatal circuitry with a starting signal to  
395 keep track of elapsed time.

396 Apart from the characteristic brief firing rate changes observed at the level of  
397 individual neurons, TANs are also known to exhibit synchronous activity during behavior

398 (Raz et al., 1996; Kimura et al., 2003; Morris et al., 2004) and one cannot exclude that  
399 synchronized firing among TANs that was not detected by our analyses may also participate  
400 in the encoding of time. Recent work using two-photon calcium imaging and population  
401 recording with cell-type-specific labeling in behaving mice has reported modulations of the  
402 cholinergic tone during spontaneous behavior which may impact striatal output dynamics  
403 (Howe et al., 2019). It is therefore conceivable that other encoding mechanisms across the  
404 cholinergic population may represent temporal information.

405 Our findings show that TANs responsive to the timing cue were distributed across the  
406 anterior caudate and putamen, a region assumed to serve cognitive-related functions, and the  
407 posterior putamen, which is important for motor control. Previous electrophysiological  
408 studies have reported that output neurons of the striatum, which make up the vast majority of  
409 neurons in this structure, convey temporal information both in motor and non-motor striatal  
410 regions in rodents (Matell et al. 2003; Mello et al., 2015; Gouvêa et al. 2015; Bakhurin et al.,  
411 2016; 2017; Zhou et al. 2020) and monkeys (Jin et al., 2009; Chiba et al., 2015; Wang et al.,  
412 2018). Although these studies have focused on dorsal striatal activity, at least one of them has  
413 reported that output neurons located in the ventral striatum, a region related to affective and  
414 motivational states, are involved in encoding time (Bakhurin et al., 2017). In our study, the  
415 responsiveness of TANs in the most ventral parts of the anterior striatum have not been  
416 assessed in detail, because the data set was small, and further recordings from this region are  
417 needed to draw conclusions about the potential contribution of ventral striatal TANs to  
418 temporal processing.

419

#### 420 *Reward prediction and timing processes*

421 In our task, the cue marks the beginning of the interval to be timed and indicates that reward  
422 is delivered for responding after a specified duration has elapsed. It therefore remains unclear  
423 whether TAN responses to the cue reflect time processing, as distinct from expectations about  
424 reward. It is increasingly recognized that timing and motivation processes are concurrently  
425 active during tasks used to elicit timing behavior in animals and humans (Fung et al., 2021).  
426 For example, a link between time estimation and reward expectation has been demonstrated  
427 for midbrain dopaminergic neurons in mice performing a temporal discrimination task  
428 (Soares et al., 2016). In the present study, we have assessed the sensitivity of TANs to reward  
429 prediction via TAN responses to the reward itself, since it is well established that fully  
430 predicted reward elicits little or no TAN response relative to reward delivered in an  
431 unpredictable way (Sardo et al., 2000; Ravel et al., 2001). As we found in monkey C, the

432 magnitude of the cue response was negatively correlated with the magnitude of reward  
433 response across TANs, a stronger response to the cue being paralleled by a weaker response  
434 to reward irrespective of interval duration. This supports the notion, at least in this animal,  
435 that reward prediction could potentially be incorporated in TAN responses to the timing cue.  
436 In contrast, such a correlation was not observed in monkey D indicating little or no  
437 contribution of reward-predictive value of the cue, likely because this animal was less  
438 successful in obtaining rewards in the timing task, compared to monkey C. It is also  
439 noticeable that the overall magnitude of TAN responses to reward in monkey D was relatively  
440 weak, perhaps reflecting a lower level of motivation to perform the task.

441 In an attempt to assess more specifically the influence of reward prediction on the  
442 sensitivity of TANs to the timing cue, we used the PCT in which timing relies on expectation  
443 of when reward will be delivered. In this condition, differences in the magnitude of the TAN  
444 response to reward between short- and long-interval trials were consistent in both monkeys,  
445 response magnitude being higher when the timing of reward became less precise with longer  
446 intervals. Based on these findings, we suggest that TANs show a clear reward prediction  
447 effect in the PCT. On the other hand, the magnitude of the TAN response to the cue did not  
448 show a consistent relationship with the magnitude of the reward response, indicating that the  
449 modulation of the response of TANs to the reward-predicting stimulus was less marked than  
450 that following reward delivery, as previously observed in a study manipulating reward  
451 probability during a Pavlovian task (Apicella et al., 2009).

452 Although a clear-cut dissociation between temporal and motivational variables is  
453 difficult to make in the TET, our analysis of TAN responses to reward indicates that reward  
454 prediction should be considered as a possible confounding effect. This is in line with studies  
455 showing that the motivational state may affect time processing abilities (Gable and Poole,  
456 2012; Avlar et al., 2015; Balci, 2014; Kirkpatrick, 2014; Daniels and Sanabria, 2017). The  
457 few neuroimaging studies in humans that have attempted to dissociate reward and timing  
458 processes, have reported activations in striatal and prefrontal cortical areas that may reflect  
459 the integration of time perception and reward expectation (Tomasi et al., 2015; Apaydin et al.,  
460 2018). In addition, theoretical models incorporating time as a crucial aspect of reward  
461 processing also emphasize the importance of motivational variables on timing processes (Daw  
462 et al., 2006; Galtress et al., 2012; Gershman et al., 2014; Petter et al., 2018; Mikhael and  
463 Gershman, 2019). Further work using behavioral procedures that will allow independent  
464 variation of temporal and reward parameters is required to clarify whether TANs participate  
465 in time processing independently of reward prediction.

466 *Context detection and timing processes*

467 By focusing on a subset of TANs recorded in the TET and PCT, we sought to directly  
468 compare the efficiency of specific behavioral contexts for eliciting TAN responses to a cue  
469 which serves to either estimate a duration or simply predict the expected time of reward using  
470 short and long intervals in the same range. Results suggest that some TANs can adapt their  
471 responsiveness by taking into account the condition in which the timing cue is presented. This  
472 differential sensitivity to the timing cue indicates that the encoded information is likely to  
473 change with the particular learning context in which the cue was experienced, suggesting that  
474 TANs do participate in temporal processing in a context-dependent manner. This is in  
475 agreement with the notion that the local cholinergic circuitry of the striatum could play a role  
476 in the recognition of a learned context for stimulus detection and action generation (Apicella,  
477 2007; Stocco, 2012; Bradfield et al., 2013). Single neuron recordings in behaving animals  
478 have reported in many circumstances an influence of context on TAN responses to salient  
479 events (Shimo and Hikosaka, 2001; Yamada et al., 2004; Lee et al., 2006; Apicella et al.,  
480 2011; Stalnaker et al., 2016).

481         Although dopaminergic neurons and cholinergic TANs are able to transmit signals that  
482 convey temporal information (Martel and Apicella, 2021), their contribution to the processing  
483 of time may not be equivalent. From an anatomical viewpoint, dopaminergic neurons send  
484 information to different brain regions, including the striatum and frontal cortex, thus exerting  
485 a widespread influence on multiple cortical and subcortical targets involved in the production  
486 of reward-guided behaviors, whereas the influence of the TAN signal is confined within the  
487 striatal circuitry. One can speculate that this local signaling system relates to contextual  
488 information that may impact behavior. The fact that the TAN response is modulated by  
489 multiple factors, including possible individual strategies for initiating self-timed movements,  
490 is consistent with the notion that the information encoding capacity of the TAN system might  
491 specify the context upon which timing is applied.

492

493 *Limitations of the study*

494 In the present study, monkeys produced either a short or a long time interval by making  
495 spatially directed movements. An obvious limitation of this design is that the cue not only  
496 provided a temporal reference for self-initiated movements, but also contained information  
497 about which action to select, each time interval being associated with a movement to a right  
498 or left target. Therefore, we cannot rule out an influence of the visuospatial nature of the  
499 timing task on the TAN responses to the cue we observed. Although previous studies have

500 shown that TANs may display changes in activity in response to visuospatial aspects of task  
501 performance (Kimura, 1992; Shimo and Hikosaka, 2001; Ravel et al., 2006), it is generally  
502 acknowledged that TAN activity does not have a consistent relationship with the production  
503 of a specific action (but see Lee et al., 2006 for the observation of TAN modulations related  
504 to self-paced movements). In addition, TANs have been proposed to drive attentional shifts  
505 required for stimulus detection (Matsumoto et al., 2001; Ding et al., 2010; Schulz and  
506 Reynolds, 2013), suggesting that differences in the monkey's allocation of attention across  
507 the two cue locations may bring about differences in the TAN response. However, in the  
508 Pavlovian protocol, we reversed the relationship between the location of the timing cue and  
509 the interval duration and did not find compelling evidence that a spatial effect may account  
510 for observed changes in TAN activity. Additional work is needed to disambiguate possible  
511 effects related to time processing from those related to visuospatial attentional demands.

512 To conclude, our results support a view that TANs appear to act like detectors of  
513 stimuli relevant for initiating a timing process in the seconds range, providing evidence that  
514 the information encoding capacity of TANs extends to cognitive functions, including the  
515 representation of time. Although identifying the involvement of TANs in timing behavior  
516 remains challenging due to the difficulty to distinguish signals specific to temporal processing  
517 from those related to other confounding processes, changes in TAN activity we observed  
518 likely reflect a combination of time perception and reward prediction. An important question  
519 for future studies will be to clarify how the coordination between the TAN system and striatal  
520 output pathways, under the control of dopamine, participates in striatal timing function. Until  
521 now, there is no experimental evidence from inactivation studies in animals that a selective  
522 alteration in intrinsic striatal cholinergic innervation disrupts timing behavior. However,  
523 human studies have pointed to an impaired temporal control of motor behavior in patients  
524 with Tourette syndrome (Vicario et al., 2010; Graziola et al., 2020; Schuller et al., 2021) in  
525 which a loss of striatal cholinergic interneurons has been reported (Kataoka et al., 2010).  
526 There is also strong evidence that striatal dopamine-depleted states are associated with  
527 disturbances in timing behavior in both animals and humans (Buhusi and Meck, 2005;  
528 Merchant et al., 2013), and it is conceivable that impaired local interactions between  
529 dopaminergic and cholinergic transmissions in the striatum are central to the expression of  
530 time processing abnormalities. Future work will be necessary to fully understand the  
531 integration of cholinergic TANs and dopaminergic input into striatal circuits underlying the  
532 encoding of time.

533 **Materials and methods**

534 *Animals and recording procedures*

535 Experiments were conducted on two adult male rhesus monkeys (*Macaca mulatta*), C and D,  
536 weighing 11 and 7 kg, respectively, kept in pairs in their home cage. This study was  
537 performed in accordance with the principles of the European Union Directive 2010/63/EU on  
538 the protection of animals used for scientific purposes and all procedures were approved by the  
539 Ethics Committee of the Institut de Neurosciences de la Timone (protocol #3057-  
540 2015120809435586). Prior to the experiments, each monkey was implanted with a head-  
541 holding device and recording chamber (25×35 mm) under general anesthesia (sevoflurane  
542 2.5%) and aseptic conditions. The center of the chamber was stereotaxically directed to the  
543 anterior commissure (AC) based on the atlas of Paxinos et al. (1988). The animals received  
544 antibiotics and analgesics for a period of 5 days after the surgery.

545 Recordings were obtained with glass-coated tungsten electrodes (impedance: 2–3 MΩ)  
546 passed inside a guide tube (0.6 mm outer diameter) and lowered to the striatum with a manual  
547 hydraulic microdrive (MO-95; Narishige, Tokyo, Japan). Electrode tracks were made  
548 vertically into regions of the striatum anterior and posterior to the AC, mostly in the putamen.  
549 Few penetrations were continued through the precommissural caudate nucleus and putamen to  
550 reach the ventral striatum.

551 Neuronal activity was amplified (x5000), bandpass-filtered (0.3–1.5 kHz), and spike  
552 sorting was performed on-line using a window discriminator (NeuroLog; Digitimer,  
553 Hertfordshire, UK). Continuous monitoring of the spike waveform on a digital oscilloscope  
554 allowed us to check the isolation quality of the recorded neurons. Recordings took place in  
555 the left striatum and all reach movements were made with the arm contralateral to the  
556 recorded hemisphere. In line with many previous single-neuron recording studies in the  
557 striatum of behaving monkeys, TANs were electrophysiologically identified by waveform  
558 shape, firing rate, and response to motivationally salient stimuli consisting of a transient  
559 decrease in activity (*pause*). A computer controlled the behavioral task and data acquisition  
560 using a custom-made software developed by E. Legallet under LabVIEW (National  
561 Instruments).

562

563 *Behavioral task*

564 Training and recording sessions took place in a setup similar to that described previously  
565 (Marche and Apicella, 2017). Monkeys were seated in a restraining box facing a vertical  
566 panel containing two metal knobs (diameter, 10 mm) serving as movement targets, positioned



567 18 cm apart at the animal's eye level, and two light-emitting diodes (LEDs), one above each  
568 target. A resting bar was mounted in the lower part of the panel and a tube was positioned  
569 directly in front of the monkey's mouth for dispensing drops of fruit juice (0.3 ml) as a  
570 reward. Animals were trained in a *time estimation task* (TET) in which one of the two LEDs  
571 was lit as a cue indicating both the target of movement and the minimum waiting period  
572 before initiating a reaching movement, the left and right locations being associated with *short*  
573 and *long* intervals, respectively. A schematic representation of the sequence of events in the  
574 TET is given in Figure 1A. The trial began when the monkey kept its hand on the bar. After 1  
575 s, a LED was illuminated for 0.5 s. This cue marked the beginning of the to-be-timed interval,  
576 referred to as the time threshold, and its left or right location indicated that the minimum  
577 waiting period before movement onset was short or long, respectively (monkey D: 1.0 and 2.0  
578 s; monkey C: 1.3 and 2.3 s from cue onset). The onset of movement after the minimum  
579 waiting period has elapsed was limited to 2 s. The cue location was pseudorandomly selected  
580 from trial to trial. Trials lasted 6 s, so that the overall temporal structure of the task remained  
581 the same. Because of this fixed trial duration, the monkey had no possibility to predict the  
582 timing of the cue accurately while keeping the hand on the bar.

583 Only monkeys' reaching movements made after the specified time interval had  
584 elapsed were rewarded. If the monkey released the bar before reaching the time criterion or  
585 touched the target in more than 1 s after bar release, it was not rewarded. In these cases, the  
586 trial continued until the end of its total duration, and the same trial was repeated until a  
587 rewarded movement was successfully completed. Trials on which animals did not react after  
588 cue onset or did not touch a target after bar release were excluded from the analyses. Both  
589 monkeys received training on the TET until they reached a stable level of performance  
590 corresponding to a criterion of at least 80% correctly performed trials.

591 We also tested the effects of intervals in the seconds range in a Pavlovian conditioning  
592 task (PCT) that excludes possible confounding factors that were linked to target reaching. In  
593 this condition, access to the working panel was prevented by closing the sliding door at the  
594 front of the restraining box and monkeys remained motionless with their arms relaxed in a  
595 natural position. Animals were then exposed to a visual stimulus (red light, 0.5 s duration),  
596 presented either on the left or right side, whose onset was initiated by the experimenter. This  
597 cue was followed by the delivery of reward after a fixed interval of 1.0 or 2.0 s, depending  
598 upon stimulus location. The PCT matched the TET in terms of location-interval combinations  
599 (left/short and right/long) and total trial duration (6 s). During PCT sessions, the licking  
600 movements of the monkeys were monitored using force transducers (strain gauges) attached

601 to the tube delivering liquid. Signals from the strain gauge device were digitized at 500 Hz  
602 and stored into an analog file. In addition, we sometimes reversed the cue-interval  
603 associations of the PCT. By comparing TAN activity before and after reversal, we assessed  
604 whether activity changes following the cue onset were related to the ensuing interval duration  
605 independent of the cue location.

#### 606 *Data analysis*

607 Performance in the TET was assessed by measuring the time between the onset of the cue and  
608 bar release (movement onset time, MOT), for each time-interval combination. We examined  
609 MOT distributions to determine whether the spread of these distributions is proportional to  
610 the length of the interval being estimated, as reflected by the ratio between the dispersion and  
611 the mean of MOT (coefficient of variation, CV).

612 Neurons were determined to be task-responsive according to methods similar to those  
613 described previously (Marche et al., 2017). Briefly, a “sliding window” analysis was used to  
614 quantify the time course of changes in TAN activity after a specific task event. A test window  
615 of 100 ms duration was advanced in 10 ms increments across the trial, starting at cue onset,  
616 movement onset, or target contact. The average spike counts in the test window was  
617 compared, at each step, with that calculated during the 500 ms immediately preceding the  
618 onset of the cue (control period). The onset of a modulation was taken to be the beginning of  
619 the first of at least five consecutive steps showing a significant difference (Dunn's test for  
620 multiple nonparametric comparisons,  $p < 0.01$ ) as against the activity in the control period.

621 Quantitative analysis of spike rates was also performed for windows with fixed  
622 durations that were specifically determined for each monkey on the basis of the analysis of  
623 latency and duration of statistically significant decreases in activity (i.e., pauses) detected  
624 with the sliding window procedure. We set these time windows such that they included most  
625 of the pause onset and offset times. According to this analysis, time windows after the cue  
626 onset were 93-309 ms for both short- and long-interval trials, for monkey C, and 127-313 ms  
627 and 211-418 ms for short- and long-interval trials respectively, for monkey D. The windows  
628 determined by the average onset and offset times of pause responses to reward were 139-369  
629 ms and 194–391 ms for both short- and long-interval trials, for monkey C and D, respectively.  
630 We then rated the magnitude of activity changes in each time window for each neuron and  
631 each time interval, irrespective of whether neurons were individually responsive or not. We  
632 set quantification windows in the same way for assessing the magnitude of TAN activity after  
633 the stimulus and reward in the PCT. The percentages of TANs showing pause responses in

634 relation to the total number of neurons tested were calculated for each condition and  
635 differences in proportions of responding neurons among conditions were statistically assessed  
636 with the  $\chi^2$  test. Differences in the magnitude of changes in activity were assessed with a  
637 Wilcoxon rank-sum test.

#### 638 *Recording sites*

639 The location of individual recorded neurons was confirmed histologically in both monkeys.  
640 Near the end of the experiments, small electrolytic lesions (20  $\mu$ A for 30 s, cathodal current)  
641 were made at several points along selected electrode tracks. The monkey was deeply  
642 anesthetized with pentobarbital and perfused through the heart with 0.9% saline followed by  
643 4% paraformaldehyde (pH 7.4 phosphate buffer). Frozen sections (40- $\mu$ m thick) were made in  
644 the frontal plane and stained with cresyl violet. We then reconstructed the location of each  
645 recorded neuron according to the depth and coordinates of electrode penetrations based on the  
646 retrieved sites of marking lesions. In line with previous work, we used the AC as a structural  
647 boundary separating the striatum into motor and associative parts. Based on the reconstruction  
648 of recording sites, TANs were sampled between 5 mm anterior and 4 mm posterior to the AC,  
649 mainly over the medio-lateral extent of the putamen. Only a few penetrations were made in  
650 the most ventral part of the precommissural striatum.

651

#### 652 *Acknowledgements*

653 We thank Dr. L. Renaud for assistance with surgery, Dr. K. Marche for assistance in  
654 MATLAB programming, and Dr. M. Esclapez for help with histology. We also thank animal  
655 care personnel of the Mediterranean Primate Research Center.

656

#### 657 *Data availability statement*

658 All datasets generated for this study will be made available upon publication. To make our  
659 data openly accessible, we will consider sharing source data files and codes by using a public  
660 open repository.

661

#### 662 *Competing interests*

663 The authors declare no competing interests.

664

665

666

667 *References*

668 Apaydin N, Üstün S, Kale EH, Çelikağ I, Özgüven HD, Baskak B, Çiçek M. 2018. Neural  
669 mechanisms underlying time perception and reward anticipation. *Frontiers in Human*  
670 *Neuroscience* **12**:115. DOI: 10.3389/fnhum.2018.00115

671 Aoki S, Liu AW, Zucca A, Zucca S, Wickens JR. 2015. Role of striatal cholinergic  
672 interneurons in set-shifting in the rat. *The Journal of Neuroscience* **35**:9424–9431. DOI:  
673 10.1523/JNEUROSCI.0490-15.2015

674  
675 Aosaki T, Tsubokawa H, Ishida A, Watanabe K, Graybiel AM, Kimura M. 1994. Responses  
676 of tonically active neurons in the primate's striatum undergo systematic changes during  
677 behavioral sensorimotor conditioning. *The Journal of Neuroscience* **14**:3969-3984. DOI:  
678 10.1523/JNEUROSCI.14-06-03969.1994

679  
680 Apicella P. 2007. Leading tonically active neurons of the striatum from reward detection to  
681 context recognition. *Trends in Neuroscience* **30**:299-306. DOI: 10.1016/j.tins.2007.03.011

682  
683 Apicella P. 2017. The role of the intrinsic cholinergic system of the striatum: What have we  
684 learned from TAN recordings in behaving animals? *Neuroscience* **360**:81–94. DOI:  
685 10.1016/j.neuroscience.2017.07.060

686  
687 Apicella P, Legallet E, Trouche E. 1997. Responses of tonically discharging neurons in the  
688 monkey striatum to primary rewards delivered during different behavioral states.  
689 *Experimental Brain Research* **116**:456-466. DOI: 10.1007/pl00005773

690  
691 Apicella P, Ravel S, Legallet E. 2006. A possible role for tonically active neurons of the  
692 primate striatum in learning about temporal relationships among salient stimuli. In: E. Bezdard  
693 (Ed) *Recent Breakthroughs in Basal Ganglia Research*. Nova Science Publishers, New York.  
694 pp. 55-63.

695  
696 Apicella P, Deffains M, Ravel S, Legallet E. 2009. Tonically active neurons in the striatum  
697 differentiate between delivery and omission of expected reward in a probabilistic task context.  
698 *The European Journal of Neuroscience* **30**:515–526. DOI: 10.1111/j.1460-9568.2009.06872

699

- 700 Apicella P, Ravel S, Deffains M, Legallet E. 2011. The role of striatal tonically active neurons  
701 in reward prediction error signaling during instrumental task performance. *The Journal of*  
702 *Neuroscience* **31**:1507–1515. DOI: 10.1523/JNEUROSCI.4880-10.2011  
703
- 704 Avlar B, Kahn JB, Jensen G, Kandel ER, Simpson EH, Balsam PD. 2015. Improving  
705 temporal cognition by enhancing motivation. *Behavioral Neuroscience* **129**:576-588. DOI:  
706 10.1037/bne0000083  
707
- 708 Bakhurin KI, Mac V, Golshani P, Masmanidis SC. 2016. Temporal correlations among  
709 functionally specialized striatal neural ensembles in reward conditioned mice. *Journal of*  
710 *Neurophysiology* **115**:1521–1532. DOI: 10.1152/jn.01037.2015  
711
- 712 Bakhurin KI, Goudar V, Shobe JL, Claar LD, Buonomano DV, Masmanidis SC. 2017.  
713 Differential encoding of time by prefrontal and striatal network dynamics. *The Journal of*  
714 *Neuroscience* **37**:854-870. DOI: 10.1523/JNEUROSCI.1789-16.2016  
715
- 716 Balci F. 2014. Interval timing, dopamine, and motivation. *Timing and time perception* **2**:379–  
717 410. DOI: 10.1163/22134468-00002035  
718
- 719 Bradfield LA, Bertran-Gonzalez J, Chieng B, Balleine BW. 2013. The thalamostriatal  
720 pathway and cholinergic control of goal-directed action: interlacing new with existing  
721 learning in the striatum. *Neuron* **79**:153–166. DOI: 10.1016/j.neuron.2013.04.039  
722
- 723 Brown HD, Baker PM, Ragozzino ME. 2010. The parafascicular thalamic nucleus  
724 concomitantly influences behavioral flexibility and dorsomedial striatal acetylcholine output  
725 in rats. *The Journal of Neuroscience* **30**:14390 –14398. DOI: 10.1523/JNEUROSCI.2167-  
726 10.2010  
727
- 728 Buhusi CV, Meck WH. 2005. What makes us tick? Functional and neural mechanisms of  
729 interval timing. *Nature Reviews Neuroscience* **6**:755. DOI: 10.1038/nrn1764  
730
- 731 Cachope R, Mateo Y, Mathur BN, Irving J, Wang H-L, Morales M, Lovinger DM, Cheer JF.  
732 2012. Selective activation of cholinergic interneurons enhances accumbal phasic dopamine

- 733 release: setting the tone for reward processing. *Cell Report* **2**:33–41. DOI:  
734 10.1016/j.celrep.2012.05.011  
735
- 736 Cai Y, Ford CP. 2018. Dopamine cells differentially regulate striatal cholinergic transmission  
737 across regions through corelease of dopamine and glutamate. *Cell Report* **25**:3148–3157.  
738 DOI: 10.1016/j.celrep.2018.11.053  
739
- 740 Chiba A, Oshio KI, Inase M. 2015. Neuronal representation of duration discrimination in the  
741 monkey striatum. *Physiological Reports* **3**:e12283. doi: 10.14814/phy2.12283  
742
- 743 Coull JT, Cheng R-K., Meck WH. 2011. Neuroanatomical and neurochemical substrates of  
744 timing. *Neuropsychopharmacology* **36**:3–25. DOI: 10.1038/npp.2010.113  
745
- 746 Daniels CW, Sanabria F. 2017. Interval timing under a behavioral microscope: Dissociating  
747 motivational and timing processes in fixed-interval performance. *Learning and Behavior*  
748 **45**:29-48. DOI: 10.3758/s13420-016-0234-1  
749
- 750 Daw ND, Courville AC, Tourtezky DS. 2006. Representation and timing in theories of the  
751 dopamine system. *Neural Computation* **18**:1637–1677. DOI: 10.1162/neco.2006.18.7.1637  
752
- 753 Ding JB, Guzman JN, Peterson JD, Goldberg JA, Surmeier DJ. 2010. Thalamic gating of  
754 corticostriatal signaling by cholinergic interneurons. *Neuron* **67**:294–307. DOI:  
755 10.1016/j.neuron.2010.06.017  
756
- 757 Fung BJ, Sutlief E, Shuler MGH. 2021. Dopamine and the interdependency of time  
758 perception and reward. *Neuroscience Biobehavioral Review* **125**:380-391. DOI:  
759 10.1016/j.neubiorev.2021.02.030  
760
- 761 Gable PA, Poole BD. 2012. Time flies when you're having approach-motivated fun: effects of  
762 motivational intensity on time perception. *Psychological Science* **23**:879-86. DOI:  
763 10.1177/0956797611435817.  
764
- 765 Galtress T, Marshall AT, Kirkpatrick K. 2012. Motivation and timing: Clues for modeling the  
766 reward system. *Behavioural Processes* **90**:142–153. DOI: 10.1016/j.beproc.2012.02.014

- 767 Gershman SJ, Moustafa AA, Ludvig EA. 2014. Time representation in reinforcement learning  
768 models of the basal ganglia. *Frontiers in Computational Neuroscience* 7:194. DOI:  
769 10.3389/fncom.2013.00194  
770
- 771 Gibbon J. 1977. Scalar expectancy theory and Weber's law in animal timing. *Psychological*  
772 *Review* 84 :279–325. DOI: 10.1037/0033-295X.84.3.279  
773
- 774 Gouvêa TS, Monteiro T, Motiwala A, Soares S, Machens C, Paton JJ. 2015. Striatal dynamics  
775 explain duration judgments. *eLife* 4:e11386. DOI: 10.7554/eLife.11386  
776
- 777 Graziola F, Pellorca C, Di Criscio L, Vigeveno F, Curatolo P, Capuano A. 2020. Impaired  
778 motor timing in Tourette syndrome: results from a case–control study in children. *Frontiers in*  
779 *Neurology* 11:552701. DOI: 10.3389/fneur.2020.552701  
780
- 781 Harrington DL, Boyd LA, Mayer AR, Sheltraw DM, Lee RR, Huang M, Rao SM. 2004.  
782 Neural representation of interval encoding and decision making. *Cognitive Brain Research*  
783 21:193–205. DOI: 10.1016/j.cogbrainres.2004.01.010  
784
- 785 Howe M, Ridouh I, Allegra Mascaro AL, Larios A, Azcorra M, Dombeck DA. 2019.  
786 Coordination of rapid cholinergic and dopaminergic signaling in striatum during spontaneous  
787 movement. *eLife* 8:e44903. DOI: 10.7554/eLife.44903  
788
- 789 Jin DZ, Fujii N, Graybiel AM. 2009. Neural representation of time in cortico-basal ganglia  
790 circuits. *PNAS* 106:19156-19161. DOI: 10.1073/pnas.0909881106  
791
- 792 Kataoka Y, Kalanithi PSA, Grantz H, Schwartz ML, Saper C, Leckman JF, Vaccarino FM.  
793 2010. Decreased number of parvalbumin and cholinergic interneurons in the striatum of  
794 individuals with Tourette syndrome. *The Journal of Comparative Neurology* 518:277–291.  
795 DOI: 10.1002/cne.22206  
796
- 797 Kimura M. 1992. Behavioral modulation of sensory responses of primate putamen neurons.  
798 *Brain Research* 578:204-214. DOI: 10.1016/0006-8993(92)90249-9  
799

- 800 Kimura M, Matsumoto N, Okahashi K, Ueda Y, Satoh T, Minamimoto T, Sakamoto M,  
801 Yamada H. 2003. Goal-directed, serial and synchronous activation of neurons in the primate  
802 striatum. *Neuroreport* **14**:799–802. DOI: 10.1097/00001756-200305060-00004  
803
- 804 Kirkpatrick K. 2014. Interactions of timing and prediction error learning. *Behavioural*  
805 *Processes* **101**:135–145. DOI: 10.1016/j.beproc.2013.08.005  
806
- 807 Lee IH, Seitz AR, Assad JA. 2006. Activity of tonically active neurons in the monkey  
808 putamen during initiation and withholding of movement. *Journal of Neurophysiology* **9**:2391-  
809 2403. DOI: 10.1152/jn.01053.2005  
810
- 811 Marche K, Martel AC, Apicella P. 2017. Differences between dorsal and ventral striatum in  
812 the sensitivity of tonically active neurons to rewarding events. *Frontiers in Systems*  
813 *Neuroscience* **11**:52. DOI: 10.3389/fnsys.2017.00052.  
814
- 815 Martel AC, Apicella P. 2021. Temporal processing in the striatum: Interplay between  
816 midbrain dopamine neurons and striatal cholinergic interneurons. *European Journal of*  
817 *Neuroscience* **53**:2090-2099. DOI: 10.1111/ejn.14741.  
818
- 819 Matell MS, Meck WH. 2000. Neuropsychological mechanisms of interval timing behavior.  
820 *BioEssays* **22**:94–103. DOI: 10.1002/(SICI)1521-1878(200001)22:1<94::AID-  
821 BIES14>3.0.CO;2-E  
822
- 823 Matell MS, Meck WH. 2004. Cortico-striatal circuits and interval timing: coincidence  
824 detection of oscillatory processes. *Cognitive Brain Research* **21**:139–170. DOI:  
825 10.1016/j.cogbrainres.2004.06.012  
826
- 827 Matell MS, Meck WH, Nicolelis MAL. 2003. Interval timing and the encoding of signal  
828 duration by ensembles of cortical and striatal neurons. *Behavioral Neuroscience* **117**:760-773.  
829 DOI: 10.1037/0735-7044.117.4.760  
830
- 831 Matsumoto N, Minamimoto T, Graybiel AM, Kimura M. 2001. Neurons in the thalamic CM-  
832 Pf complex supply striatal neurons with information about behaviorally significant sensory  
833 events. *Journal of Neurophysiology* **85**:60-976. DOI: 10.1152/jn.2001.85.2.960



- 834 Meck WH. 1996. Neuropharmacology of timing and time perception. *Cognitive Brain*  
835 *Research* **3**:227–242. DOI: 10.1016/0926-6410(96)00009-2  
836
- 837 Meck WH. 2006. Neuroanatomical localization of an internal clock: A functional link  
838 between mesolimbic, nigrostriatal, and mesocortical dopaminergic systems. *Brain Research*  
839 **1109**:93–107. DOI: 10.1016/j.brainres.2006.06.031  
840
- 841 Mello GB, Soares S, Paton JJ. 2015. A scalable population code for time in the striatum.  
842 *Current Biology* **25**:1113-2112. DOI: 10.1016/j.cub.2015.02.036  
843
- 844 Merchant H, Harrington DL, Meck WH. 2013. Neural basis of the perception and estimation  
845 of time. *Annual Review of Neuroscience* **36**:313–336. DOI: 10.1146/annurev-neuro-062012-  
846 170349  
847
- 848 Mikhael JG, Gershman SJ. 2019. Adapting the flow of time with dopamine. *Journal of*  
849 *Neurophysiology* **121**:1748–1760. DOI: 10.1152/jn.00817.2018  
850
- 851 Morris G, Arkadir D, Nevet A, Vaadia E, Bergman H. 2004. Coincident but distinct messages  
852 of midbrain dopamine and striatal tonically active neurons. *Neuron* **43**:133–143. DOI:  
853 10.1016/j.neuron.2004.06.012  
854
- 855 Noreika V, Falter CM, Rubia K. 2013. Timing deficits in attention-deficit/hyperactivity  
856 disorder (ADHD): evidence from neurocognitive and neuroimaging studies.  
857 *Neuropsychologia* **51**:235-266. DOI: 10.1016/j.neuropsychologia.2012.09.036.  
858
- 859 Parker KL, Lamichhane D, Caetano MS, Narayanan NS. 2013. Executive dysfunction in  
860 Parkinson’s disease and timing deficits. *Frontiers in Integrative Neuroscience* **7**. DOI:  
861 10.3389/fnint.2013.00075  
862
- 863 Pastor MA, Artieda J, Jahanshahi M, Obeso JA. 1992. Time estimation and reproduction is  
864 abnormal in Parkinson’s disease. *Brain* **115**:211–225. DOI: 10.1093/brain/115.1.211  
865
- 866 Paton JJ, Buonomano DV. 2018. The neural basis of timing: distributed mechanisms for  
867 diverse functions. *Neuron* **98**:687–705. DOI: 10.1016/j.neuron.2018.03.045

- 868 Paxinos G, Huang X, Petrides M, Toga A. 2008. *The Rhesus Monkey Brain in Stereotaxic*  
869 *Coordinates*. 2nd edn. San Diego: Academic Press.  
870
- 871 Petter EA, Gershman SJ, Meck WH. 2018. Integrating models of interval timing and  
872 reinforcement learning. *Trends in Cognitive Sciences* **22**:911–922. DOI:  
873 10.1016/j.tics.2018.08.004  
874
- 875 Ravel S, Sardo P, Legallet E, Apicella P. 2001. Reward unpredictability inside and outside of  
876 a task context as a determinant of the responses of tonically active neurons in the monkey  
877 striatum. *The Journal of Neuroscience* **21**:5730-5739. DOI: 10.1523/JNEUROSCI.21-15-  
878 05730.2001  
879
- 880 Ravel S, Sardo P, Legallet E, Apicella P. 2006. Influence of spatial information on responses  
881 of tonically active neurons in the monkey striatum. *Journal of Neurophysiology* **95**:2975-  
882 2986. DOI: 10.1152/jn.01113.2005  
883
- 884 Raz A, Feingold A, Zelanskaya V, Vaadia E, Bergman H. 1996. Neuronal synchronization of  
885 tonically active neurons in the striatum of normal and parkinsonian primates. *Journal of*  
886 *Neurophysiology* **76**:2083–2088. DOI: 10.1152/jn.1996.76.3.2083  
887
- 888 Rubia K, Halari R, Christakou A, Taylor E. 2009. Impulsiveness as a timing disturbance:  
889 neurocognitive abnormalities in attention-deficit hyperactivity disorder during temporal  
890 processes and normalization with methylphenidate. *Philosophical transactions of the royal*  
891 *society B* **364**:1919–1931. DOI: 10.1098/rstb.2009.0014  
892
- 893 Sardo P, Ravel S, Legallet E, Apicella P. 2000. Influence of the predicted time of stimuli  
894 eliciting movements on responses of tonically active neurons in the monkey striatum.  
895 *European Journal of Neuroscience* **12**:1801-1816. DOI:10.1046/j.1460-9568.2000.00068.x  
896
- 897 Schüller CB, Wagner BJ, Schüller T, Baldermann JC, Huys D, Kerner Auch Koerner J,  
898 Niessen E, Münchau A, Brandt V, Peters J, Kuhn J. 2021. Temporal discounting in  
899 adolescents and adults with Tourette syndrome. *PLoS* **16**:e0253620. DOI:  
900 10.1371/journal.pone.0253620  
901

- 902 Schulz JM, Reynolds JN. 2013. Pause and rebound: sensory control of cholinergic signaling  
903 in the striatum. *Trends in Neuroscience* **36**:41-50. DOI: 10.1016/j.tins.2012.09.006.  
904
- 905 Shimo Y, Hikosaka O. 2001. Role of tonically active neurons in primate caudate in reward-  
906 oriented saccadic eye movement. *The Journal of Neuroscience* **21**:7804-7814.  
907 DOI:10.1523/JNEUROSCI.21-19-07804.2001  
908
- 909 Soares S, Atallah BV, Paton JJ. 2016. Midbrain dopamine neurons control judgment of time.  
910 *Science* **354**:1273-1277. DOI: 10.1126/science.aah5234  
911
- 912 Stalnaker TA, Berg B, Aujla N, Schoenbaum G. 2016. Cholinergic interneurons use  
913 orbitofrontal input to track beliefs about current State. *The Journal of Neuroscience* **36**: 6242-  
914 6257. DOI: 10.1523/JNEUROSCI.0157-16.2016  
915
- 916 Stocco A. 2012. Acetylcholine-based entropy in response selection: a model of how striatal  
917 interneurons modulate exploration, exploitation, and response variability in decision-making.  
918 *Frontiers in Neuroscience* **6**:18. DOI: 10.3389/fnins.2012.00018  
919
- 920 Threlfell S, Cragg SJ. 2011. Dopamine signaling in dorsal versus ventral striatum: the  
921 dynamic role of cholinergic interneurons. *Frontiers in Systems Neuroscience* **5**. DOI:  
922 10.3389/fnsys.2011.00011  
923
- 924 Threlfell S, Lalic T, Platt NJ, Jennings KA, Deisseroth K, Cragg SJ. 2012. Striatal dopamine  
925 release is triggered by synchronized activity in cholinergic interneurons. *Neuron* **75**:58–64.  
926 DOI: 10.1016/j.neuron.2012.04.038  
927
- 928 Tomasi D, Wang GJ, Studentsova Y, Volkow ND. 2015. Dissecting neural responses to  
929 temporal prediction, attention, and memory: effects of reward learning and interoception on  
930 time perception. *Cerebral Cortex* **25**:3856–3867. DOI: 10.1093/cercor/bhu269  
931
- 932 Vicario CM, Martino D, Spata F, Defazio G, Giacchè R, Martino V, Rappo G, Pepi AM,  
933 Silvestri PR, Cardona F. 2010. Time processing in children with Tourette’s syndrome. *Brain*  
934 *and Cognition* **73**:28–34. DOI: 10.1016/j.bandc.2010.01.008  
935

936 Wang J, Narain D, Hosseini EA, Jazayeri M. 2018. Flexible timing by temporal scaling of  
937 cortical responses. *Nature Neuroscience* **21**:102-110. DOI: 10.1038/s41593-017-0028-6

938

939 Yamada H, Matsumoto N, Kimura M. 2004. Tonicly active neurons in the primate caudate  
940 nucleus and putamen differentially encode instructed motivational outcomes of action. *The*  
941 *Journal of Neuroscience* **24**:3500-3510. DOI: 10.1523/JNEUROSCI.0068-04.2004

942

943 Zhou F-M, Wilson CJ, Dani JA. 2002. Cholinergic interneuron characteristics and nicotinic  
944 properties in the striatum. *Journal of Neurobiology* **53**:590-605. DOI: 10.1002/neu.10150

945

946 Zhou S, Masmanidis SC, Buonomano DV. 2020. Neural sequences as an optimal dynamical  
947 regime for the readout of time. *Neuron* **108**:651-658.e5. DOI: 10.1016/j.neuron.2020.08.020.

948

949

950

951

952

953

954

955

956

957

958

959

960

961

962

963

964

965

966

967

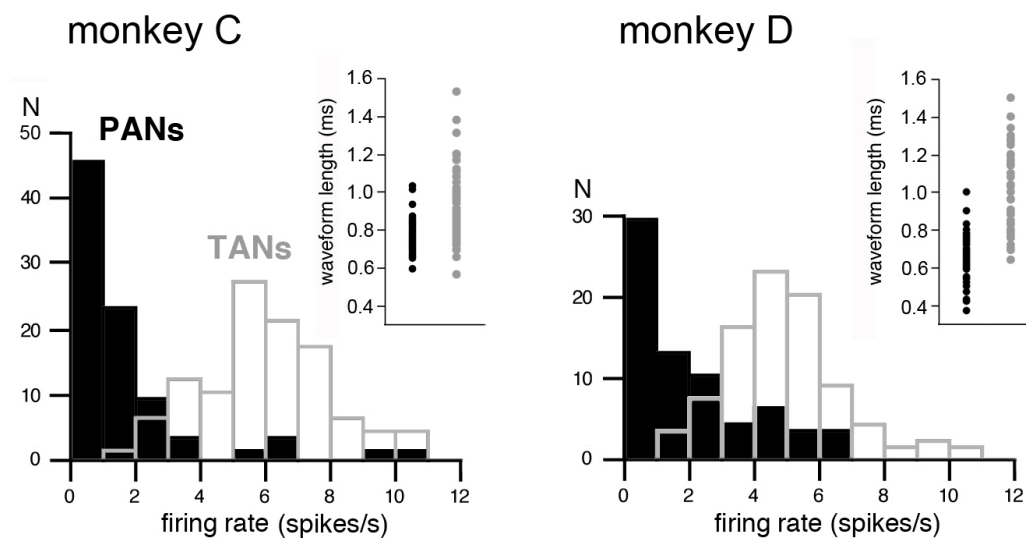
968

969

970

971

972 **Figure 2-figure supplement 1**  
973



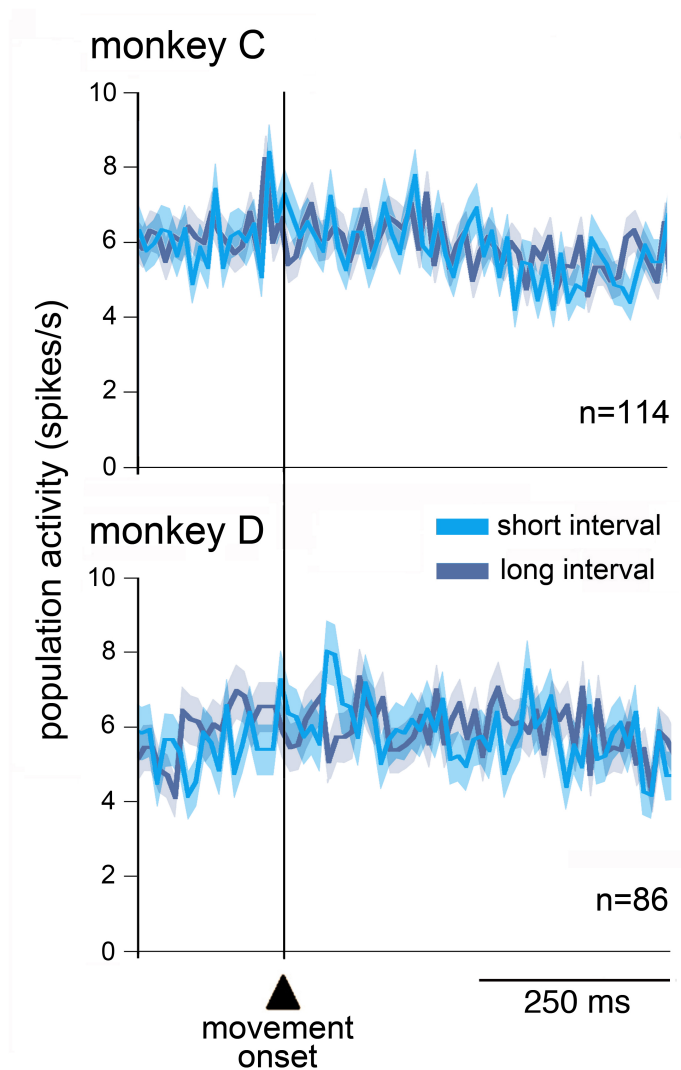
974  
975

### 976 **Electrophysiological characterization of TANs.**

977 Tonically active neurons (TANs), presumed cholinergic interneurons in the striatum, were  
978 identified based on previously established electrophysiological criteria, i.e., spontaneous  
979 firing rate and spike waveform width. Consistent with previous studies, the TANs had a mean  
980 baseline activity which is higher than that of the phasically active neurons (PANs), which  
981 correspond to striatal output neurons (Monkey C: TANs:  $n=103$ ,  $5.94 \pm 1.94$  spikes/s; PANs:  
982  $n=86$ ,  $1.47 \pm 1.91$  spikes/s; Monkey D: TANs:  $n=86$ ,  $4.82 \pm 1.72$  spikes/s; PANs:  $n=68$ ,  
983  $1.83 \pm 1.92$  spikes/s). Also, the mean spike waveform width of TANs was larger than that of the  
984 PANs (Monkey C: TANs:  $n=109$ ,  $0.90 \pm 0.17$  ms; PANs:  $n=104$ ,  $0.76 \pm 0.08$  ms; Monkey D:  
985 TANs:  $n=85$ ,  $1.08 \pm 0.22$  ms; PANs:  $n=68$ ,  $0.65 \pm 0.11$  ms). In addition, the stereotyped  
986 *pause* in activity in response to motivationally salient stimuli is a reliable indicator of TAN  
987 identity which clearly distinguishes them from PANs. We excluded from analysis a relatively  
988 small sample of the so-called fast spiking interneurons, presumed striatal GABAergic  
989 interneurons, characterized by having a higher firing frequency than TANs and a narrower  
990 spike waveform than PANs. Spontaneous activity was measured as the mean firing rate  
991 during the 500 ms before the onset of the timing cue. N indicates the number of neurons.  
992 Insets: distributions of average spike lengths. Each dot represents data from an individual  
993 neuron. The spike length of each neuron was defined as the time interval between the first  
994 negative and second positive peaks of the waveform.

995  
996  
997  
998  
999  
1000  
1001  
1002  
1003  
1004  
1005  
1006

1007 **Figure 4-figure supplement 1**  
1008



1009  
1010

1011 **Lack of changes in TAN activity around self-timed movements.**

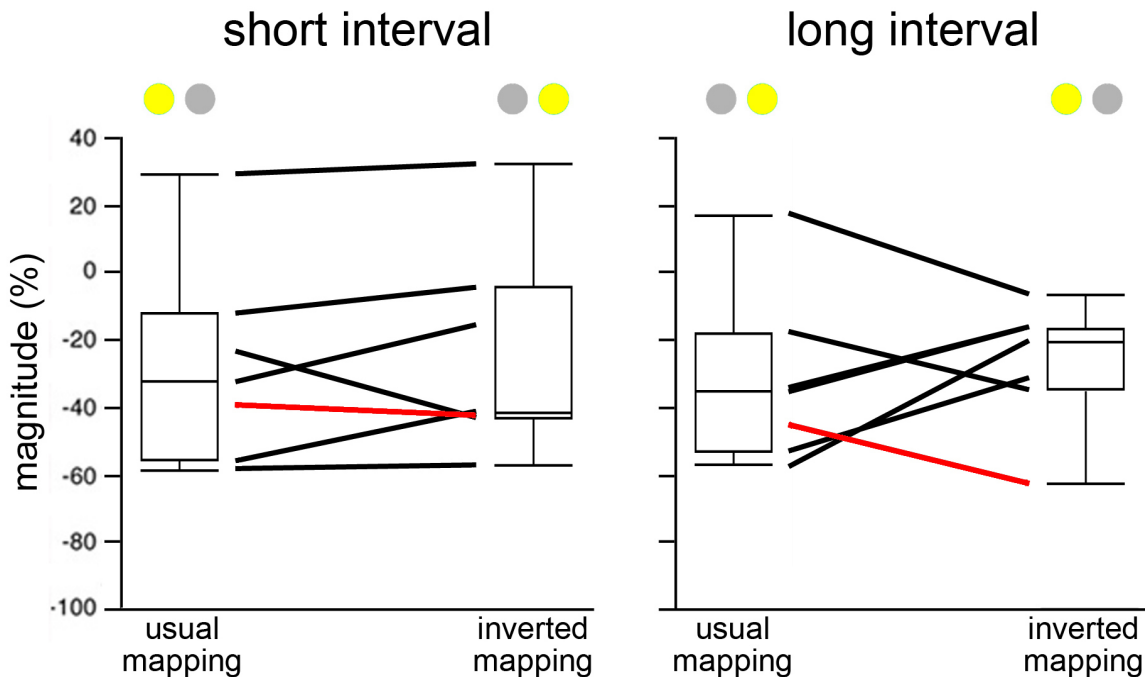
1012 Population activity of TANs aligned on the onset of movements marked by the vertical line.  
1013 At a population level, TANs did not exhibit clear activity modulation around the movement  
1014 onset, regardless of the time interval. Same conventions as in Fig. 2D.

1015  
1016  
1017  
1018  
1019  
1020  
1021  
1022  
1023  
1024  
1025  
1026  
1027  
1028

1029 **Figure 5-figure supplement 1**

1030

1031



1032

1033

1034 **Influence of changing the cue-interval mapping in the Pavlovian protocol.**

1035 Box plots of the magnitude of changes in TAN activity after the onset of the cue, separately  
1036 for short and long intervals. Boxes indicate 25–75 percentile ranges of the distributions, lines  
1037 through boxes indicate medians and vertical lines indicate tails of distributions. Red lines  
1038 correspond to the example TAN illustrated in Fig. 5B. We recorded a sample of seven TANs  
1039 in the PCT to test whether TAN responses to the cue are attributable to the time interval or the  
1040 location of the cue. After carrying out a block of trials with the usual assignment of cues for  
1041 the two time intervals (usual mapping), we reversed the assignments of the spatial cues for the  
1042 short and long interval (inverted mapping). The activity across this sample of TANs was not  
1043 altered when the cue-interval mapping was reversed, suggesting that it was not influenced by  
1044 the location of the cue.

1045