1 **Title: Concomitant processing of choice and outcome in frontal corticostriatal ensembles**

2 **correlates with performance of rats**

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19   **Summary**

20   The frontal cortex-basal ganglia network plays a pivotal role in adaptive goal-directed behaviors.

21   Medial frontal cortex (MFC) encodes information about choices and outcomes into sequential

22   activation of neural population, or neural trajectory. While MFC projects to the dorsal striatum (DS),

23   whether DS also displays temporally coordinated activity remains unknown. We studied this

24   question by simultaneously recording neural ensembles in the MFC and DS of rodents performing

25   an outcome-based alternative choice task. We found that the two regions exhibited highly parallel

26   evolution of neural trajectories, transforming choice information into outcome-related information.

27   When the two trajectories were highly correlated, spike synchrony was task-dependently modulated

28   in some MFC-DS neuron pairs. Our results suggest that neural trajectories concomitantly process

29   decision-relevant information in MFC and DS with increased spike synchrony between these regions.

30

31   **Keywords:**   win-stay lose-shift; frontal corticostriatal ensemble; multi-neuron recordings;

32   population coding; Linear Fisher's discriminant

33

Introduction

34

35   Deciding actions based on the outcome of past actions is crucial for the survival of animals. The

36   frontal cortex-basal ganglia network is engaged in various forms of decision making involved in

37   adaptive goal-directed behaviors [1-4]. These studies have shown how choice- and/or outcome-

38   related information is processed in the frontal cortex and the basal ganglia. However, how the

39   cortico-basal ganglia network encodes behavioral information at the neural ensemble level remains

40   largely unknown. How the related brain regions communicate such information during outcome-

41   based action selection is yet to be clarified [5]. In this study, we explore whether and how task-

42   related neural activity is coordinated between the medial frontal cortex (MFC) and dorsal striatum

43   (DS), which is a major input structure of the basal ganglia [6-8], of rats performing an outcome-based

44   alternative choice task.

45

46   The MFC is engaged in encoding reward and error signals [9,10] and hence is considered to

47   play a role in monitoring positive and negative outcomes from an action. In particular, the rostral

48   agranular medial cortex or the secondary motor cortex of rodent has been implicated in sensory-

49   cued [11-13] and outcome-based action selection [14,15]. Along the frontal cortico-basal ganglia

50   axis, excitatory MFC outputs project monosynaptically to DS [16,17], which also receives

51   glutamatergic inputs from some thalamic nuclei [7,18] and are modulated by midbrain dopaminergic

52   inputs conveying outcome information [19] and reward prediction error [20]. Accordingly, DS is

3

53   thought to associate a specific action with the resultant outcome [21-24]. Consistent with this,

54   lesions in MFC [14,15] and DS [25,26] impair outcome-based choice behaviors.

55

56   Evidence from MFC, or more specifically posterior parietal cortex (PPC) and orbitofrontal cortex

57   (OFC), shows that sequential activation of cortical neuron ensembles, which is termed neural

58   trajectory, underlies decision making [12,27,28]. We explore how this trajectory information is

59   conveyed to striatal neurons during decision making. If the role of the striatum is selection of cortical

60   inputs for action generation, as conventionally thought, such selection may occur sparsely in time,

61   making continuously evolving trajectories less relevant to striatal functions. We therefore clarify to

62   what extent cortical and striatal neuron ensembles are coherently activated during decision making.

63   We hypothesize that neural ensembles in the MFC and DS utilize synchronized spikes to

64   communicate with one another during adaptive behavior. Such spikes are ubiquitous in cortical

65   circuits [29,30] and were suggested to enhance information transmission and association between

66   directly or indirectly interconnected brain regions [31-32].

67

68   We trained head-restrained rats to lick either left or right spouts for earning reward (positive

69   outcome) and simultaneously recorded multi-neuron activity from the MFC and DS of these rats.

70   Because a rewarded spout was changed intermittently, the rats had to switch their choice responses

71   to maximize reward, exhibiting behavioral responses similar to a typical win-stay lose-away behavior.

72   Unexpectedly, we found that neural trajectories emerge and evolve highly coherently in both MFC

73   and DS during the entire period of the alternative choice task. In some MFC-DS neuron pairs, the

74   number of coincident spikes was modulated by task events when the trajectory evolution displayed

75   an enhanced coherence. Our results suggest that the adaptive control of outcome-based decision

76   making relies on the coevolution of neural trajectories in MFC and DS and that spike synchrony plays

77   a role in this temporal coordination.

78

79

80   **Results**

81   ***Adaptive outcome-based behavior under head-restrained condition***

82   Twenty head-restrained rats performed an outcome-based two choice task, in which the rats were

83   required to lick one of two spouts (Left and Right) and in each block of trials one spout delivered

84   reward, with a high reward acquisition probability (>75%, see Methods) (Fig.1a). The choice-reward

85   contingency was systematically reversed without any sensory feedback after the cumulative number

86   of rewarded trials reached 10 in each block. The rats figured out the reversal of choice-reward

87   contingency through the monitoring of no-reward events, time-out, and reward acquisition within

88   subsequent several trials (Fig. 1b). Rats tended to select the same spout as that chosen in the last

89   rewarded trial, but they switched the choice following one to several unrewarded trials (Fig. 1b, c).

90   This choice pattern is close to the so-called 'win-stay' and 'lose-shift' which is the optimal strategy
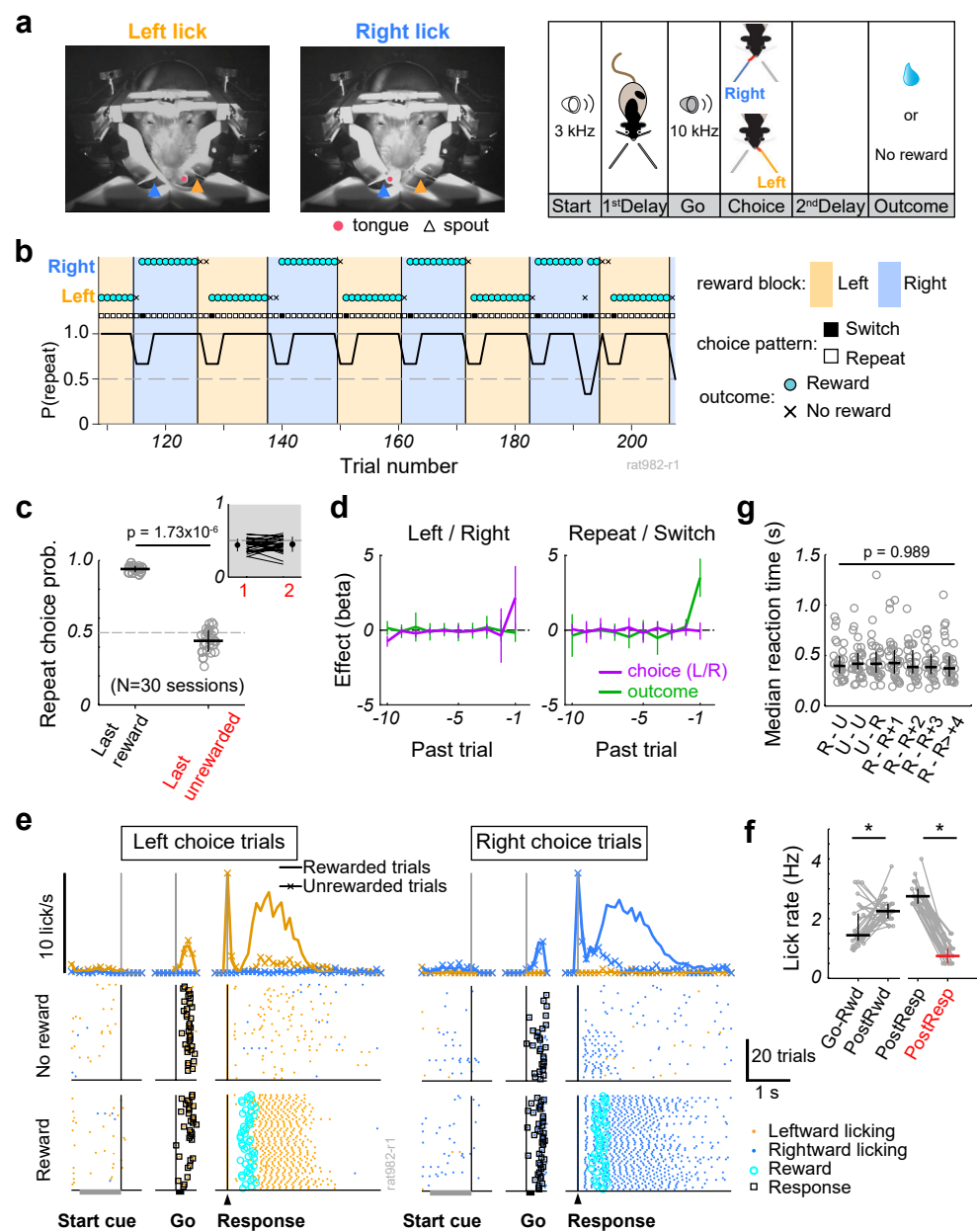
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Figure 1

a

Left lick   Right lick

● tongue △ spout

| 3 kHz | | 10 kHz | Right / Left | | or / No reward |
| Start | 1ˢᵗDelay | Go | Choice | 2ⁿᵈDelay | Outcome |

b

reward block: ▮ Left ▮ Right

choice pattern: ■ Switch □ Repeat

outcome: ⬤ Reward ✕ No reward

Right / Left / P(repeat) vs Trial number (120, 140, 160, 180, 200)

rat982-r1

c

p = 1.73×10⁻⁶

Repeat choice prob. — Last reward / Last Unrewarded

(N=30 sessions)

d

Left / Right   Repeat / Switch

Effect (beta) vs Past trial (-10, -5, -1)

— choice (L/R) — outcome

g

p = 0.989

Median reaction time (s)

R⁻ U⁻ / U⁻ U⁻ / U⁻ R⁻ / R⁻ R⁺¹ / R⁻ R⁺² / R⁻ R⁺³ / R⁻ R⁺⁴

e

Left choice trials   Right choice trials

— Rewarded trials ✕ Unrewarded trials

10 lick/s

No reward

Reward

rat982-r1

20 trials / 1 s

Start cue Go Response   Start cue Go Response

● Leftward licking
● Rightward licking
○ Reward
□ Response

f

Lick rate (Hz)

*   *

Go-Rwd / PostRwd / PostResp / PostResp

Figure 1. Adaptive choice patterns in response to change in choice-reward contingency.
(a) *Left and middle*: Snapshots of a head-restrained rat making lick responses toward Left (orange arrowhead) and Right (blue arrowhead) spouts. A circle indicates the tongue position. *Right*: A schematic illustration of an outcome-based two alternative choice task. Individual trial started with 'Start' tone (3 kHz) presentation. Head-fixed rats were required to wait for 'Go' tone (10 kHz) without a lick during '1st Delay' period, and then to make a choice response by licking either one of spouts within 5 s after 'Go' tone onset. Reward was given after '2nd Delay' of various durations (0.3-0.7 s) if the chosen position corresponded to current reward location. Otherwise no reward, no sensory feedback, and timeout of 5 s were given. (b) Representative flexible choice behavior by a rat across 9 block-reversals of reward position. Vertical lines and colored areas denote the block-reversal and reward position in each block, respectively. Circle and cross indicate the outcome, reward and no-reward respectively, after choice response (Left or Right). Square shows choice pattern (Switch or Repeat). (c) Probability of repeating choice after rewarded and unrewarded outcomes (30 sessions, 20 rats). Circle indicates individual sessions. Horizontal lines and error bars represent mean and SD, respectively. The statistical significance was confirmed by Wilcoxon signed-rank test. Inset: Probability of repeating choice following one or two consecutive unrewarded trials. (d) Effects of past choice (purple) and outcome (green) on (*left*) current choice position and (*right*) current choice type (repeat and switch). The ordinate shows averaged coefficients of a logistic regression model (N = 30 sessions). Error bars show SD. (e) Licking patterns during rewarded and unrewarded trials in the session shown in b. Rastergrams denote licking times and locations (Left: orange, Right: blue). Histograms show averaged Left and Right licking rates in rewarded (solid line) and unrewarded (line with cross) trials. (f) Median licking rate in an epoch between Go tone onset and reward delivery (Go-Rwd), during outcome period of 4-s after reward delivery (PostRwd), and for 4-s after response in rewarded (PostResp in black) and unrewarded (PostResp in red) trials. Asterisks indicate statistical significance (Wilcoxon signed-rank test, $p < 0.05$). (g) Comparison of reaction times among different choice patterns. Circles denote median reaction times in individual trials. Horizontal and vertical lines indicate median and 1st-3rd quartiles across trials, respectively. Trials were classified by last and current outcomes at each session (e.g., U–R denotes unrewarded last and rewarded current choices, and R+1, R+2, … denote the number of continuous rewarded outcomes).

91   to maximize reward in the present task. To examine if the choice pattern (repeating post rewarded

92   trial and switching post no-reward trial) was biased by past outcome (reward or no-reward) or by

93   past choice position (Left and Right), we estimated the effect of past outcomes and past choices by

94   a logistic regression analysis (Methods). The last outcome provided the greatest effect on the current

95   choice type (repeat or switch) regardless of past choice position (t-test with Bonferroni correction,

96    $p = 3.73 \times 10^{-15}$). In contrast, the current choice position was the most affected by the last choice

97   position regardless of outcome (t-test with Bonferroni correction, $p = 5.17 \times 10^{-6}$) (Fig. 1d).

98   Trial-by-trial licking behavior was strongly influenced by outcome events. Rats licked either

99   one of the two spouts immediately after the appearance of Go tone, and then showed fast and

100   rhythmic licking at around 6 Hz just after reward delivery (Fig. 1e). In contrast, rats showed sparse

101   licking at around 1 Hz during outcome period in unrewarded trials. The lick rate of population data

102   was significantly different before and after reward delivery in rewarded trials (Fig. 1f, Wilcoxon

103   signed-rank test, $p = 0.00295$) and between rewarded and unrewarded outcome periods (Wilcoxon

104   signed-rank test, $p = 1.50 \times 10^{-6}$). In behavioral studies on primates and rodents, prolonged response

105   times, called post-error slowing, are observed after no reward outcome in sensory-cued choice tasks

106   [33,34]. At 13 sessions, we observed significantly differential reaction times among different choice

107   patterns classified by recent outcome history (Kruskal-Wallis test, $p < 0.05$). Although the reaction

108   times were indeed longer after no or first reward outcome than after consecutive reward outcomes

6

109   in some sessions, we did not find any tendency toward post-error slowing at the population level (p

110   = 0.989, Kruskal-Wallis test, n = 30 sessions) (Fig. 1g).

111

112   ***Simultaneous multi-neuron recordings from MFC and DS***

113   To test whether and how neuronal population activities of the MFC-DS circuit are collectively

114   coordinated during the outcome-based action selection, we recorded multi-neuron activity in both

115   MFC and DS using two silicon probes during task performance. In addition, we simultaneously

116   performed juxtacellular recordings of Neurobiotin-loaded neurons near the silicon probe for the

117   MFC recording (Fig. 2a). We confirmed the projection from the recording site in the MFC to that in

118   the DS by using a retrograde tracer Fluoro-Gold (FG) injected into the DS (Methods). FG-labelled

119   corticostriatal neurons were primarily observed in certain restricted regions of the MFC (Fig. 2b and

120   c), particularly in the rostral agranular medial cortex as previously reported [6,7]. Observing the track

121   of the silicon probe insertion, Neurobiotin-loaded neurons (in some cases), FG-labelled neurons in

122   layers 3 and 5 of MFC (Fig. 2b-c and Fig. S1) and the tracks of another probe for the DS recording at

123   or near FG injection site in DS (Fig. 2b), we confirmed that multi-neuron activity was presumably

124   recorded from a subset of MFC-DS circuit.

125

126   ***Single MFC and DS cells encode both positive and negative outcomes***
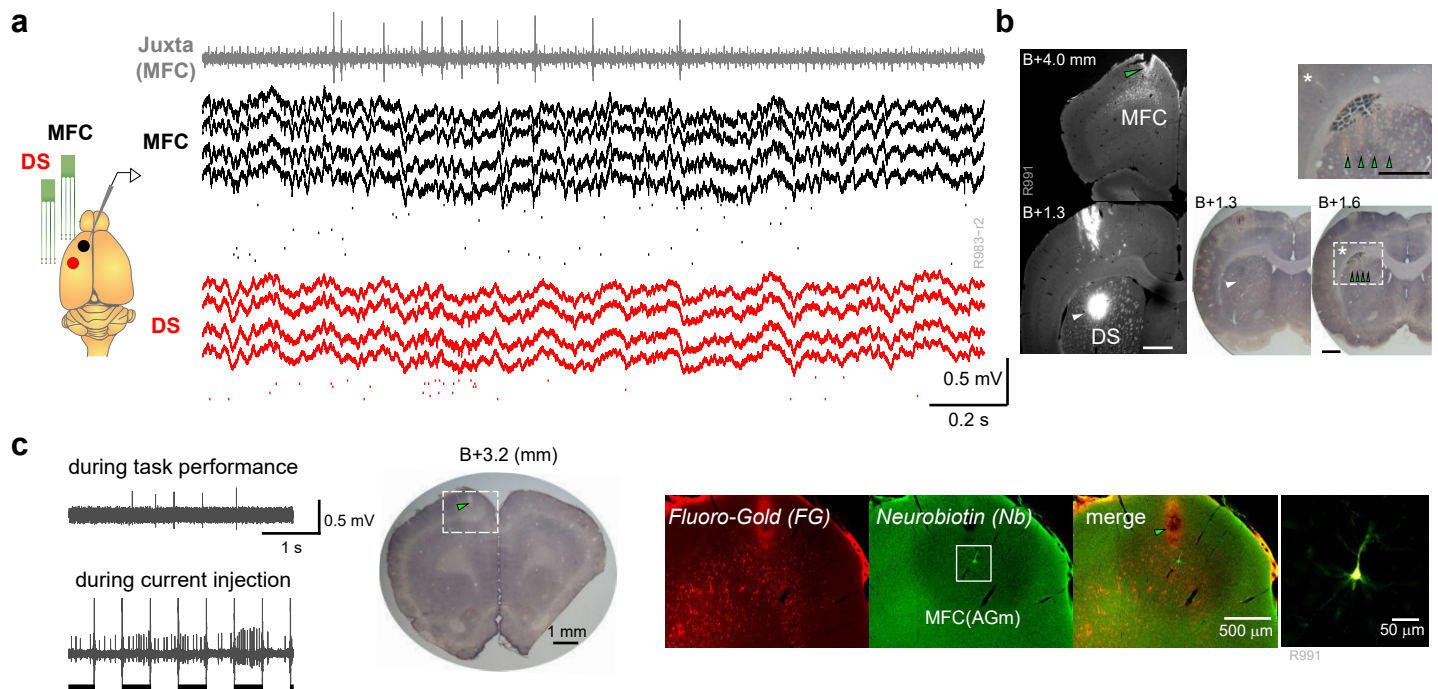
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Figure 2

Figure 2. Simultaneous recording from MFC and DS. (a) Left: A schematic illustration of recordings. One silicon probe (black) and a glass pipette electrode (gray) were inserted into MFC and another silicon probe into DS (red). Right: Multiunit activity recorded with tetrodes in MFC and DS and juxtacellularly recorded activity (gray). Spikes of isolated units are depicted below multiunit signals. (b) Left: Fluorescent images show corticostriatal projection neurons labelled with Fluoro-Gold (FG) in MFC (top) and the injection site (white arrowhead) of FG in DS (bottom). Right: Nissl stained brain sections specified by the AP coordinates from Bregma. One shown at bottom left is identical to the lower fluorescent image and one at bottom right indicates silicon probe tracks (scars, green arrowhead) in DS. The area surrounded by a dash-lined box was magnified (top). Scale bars show 1 mm. (c) Left: Juxtacellularly recorded activity of a neuron during task performance (top) and current injection (bottom). Horizontal bars indicate epochs of periodic current injection whereas long vertical lines are artifacts by the on/off cycles of injection. Middle: A Nissl stained brain section including probe track (scar, green arrowhead) in MFC (agranular medial cortex). Right: Fluorescent images reveal FG labelled corticostriatal neurons and a Neurobiotin (Nb)-labeled neuron in MFC. The rightmost image enlarges the inside of the box area.
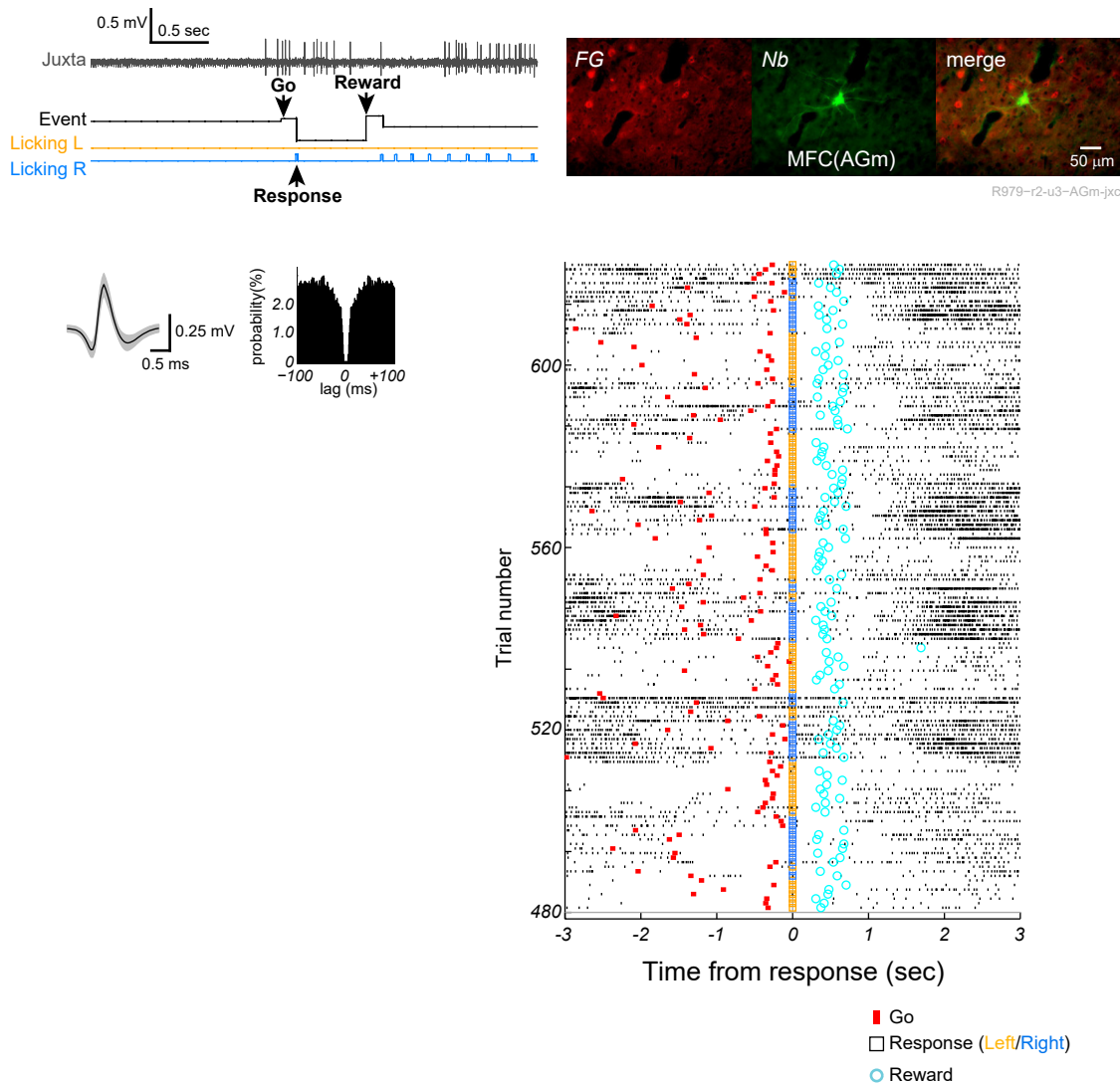
Figure S1

Figure S1. **Juxtacellular recording during task performance and labelling the recorded neuron.** Top left: Juxtacellular activity (gray) during task performance. Black, orange, and blue lines indicate event signal, left licking signal, and right licking signal, respectively. Top right: Post hoc visualization of the recorded neuron and corticostriatal projection neurons. Bottom left: Auto-correlogram and spike waveform of the identified neuron. Bottom right: Firing patterns of the labelled neuron aligned to response execution.

127   We analyzed event-related firing patterns of well-isolated single neurons recorded simultaneously

128   from MFC (n = 468, mean ±SD = 39 ±18 cells per session) and DS (n = 489, mean ±SD = 40 ±14 cells

129   per session) in 10 rats. The recordings were performed in the left hemisphere while these rats were

130   performing the learned task through 12 recording sessions (Methods). The firing rates of both MFC

131   and DS cells were correlated with choices (Left vs. Right) and outcomes (Reward vs. No-reward) (two-

132   way ANOVA, p <0.05, Methods).

133

134   Many outcome-modulated neurons decreased firing rate during the period of positive

135   outcome but not during that of negative outcome (Fig. 3a, b). However, some neurons increased

136   firing rate most strongly for negative outcomes (Fig. 3c), and others raised firing rate for positive

137   outcomes (Fig. 3d). We then calculated peri-event time histograms (PETHs), which revealed similar

138   sequential dynamics of MFC and DS cells (Fig. 3e and Fig. S2a). The PETHs also indicated a prominent

139   decrease in firing rate in the majority of MFC and DS cells after reward delivery. The fractions of

140   choice- and outcome-modulated neurons evolved similarly in MFC and DS, but these fractions

141   differed significantly between MFC and DS in large portions of task period (Fig. 3f). We quantified

142   the degree and preference of selectivity across different sessions and neurons by performing

143   receiver operating characteristic (ROC) analysis of neurons' firing rates and calculating the area

144   under ROC curve (AROC) (Methods). In both MFC and DS, choice-selective neurons exhibited a bias

145   towards right (i.e., contralateral) choice responses whereas outcome-selective neurons towards
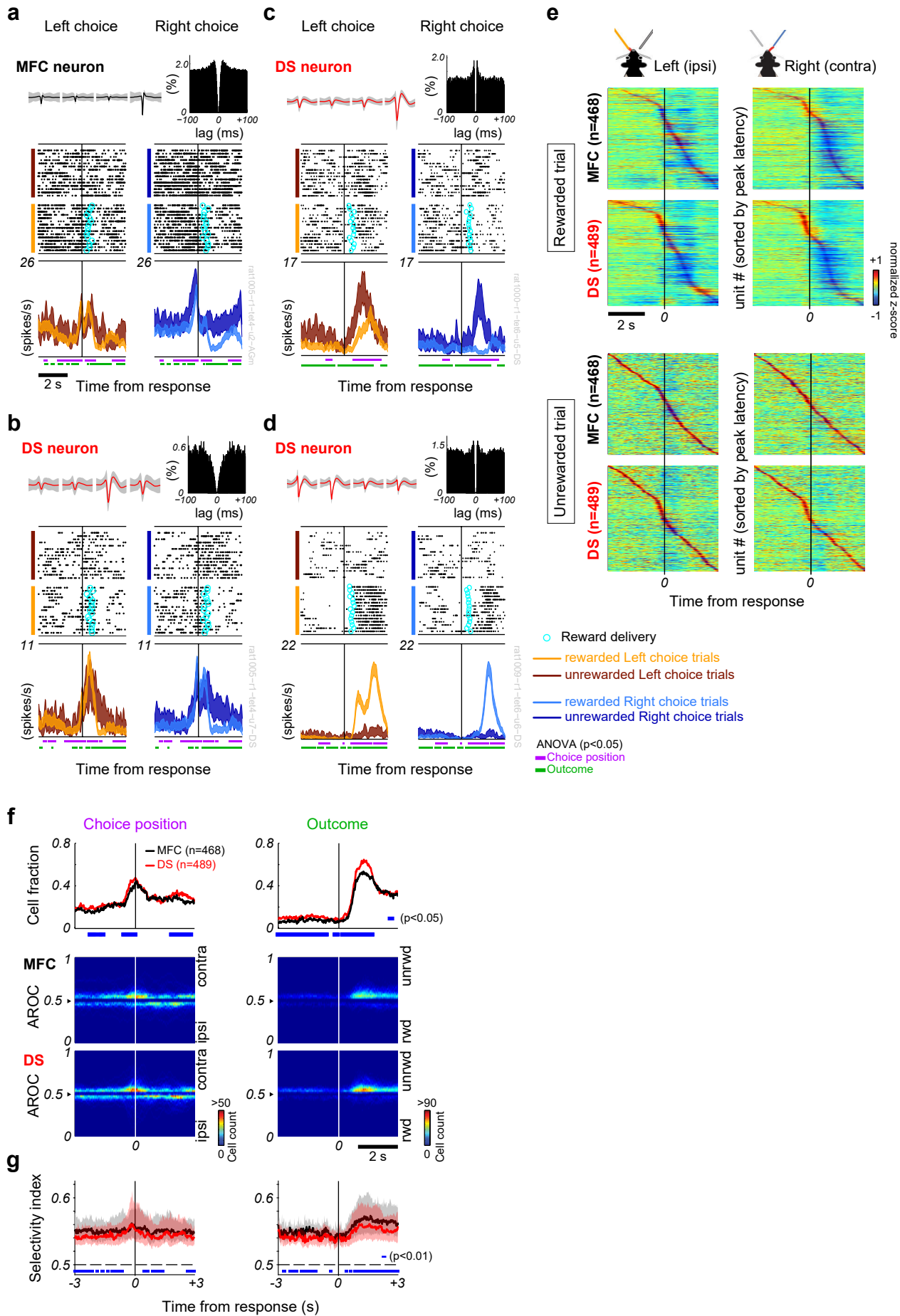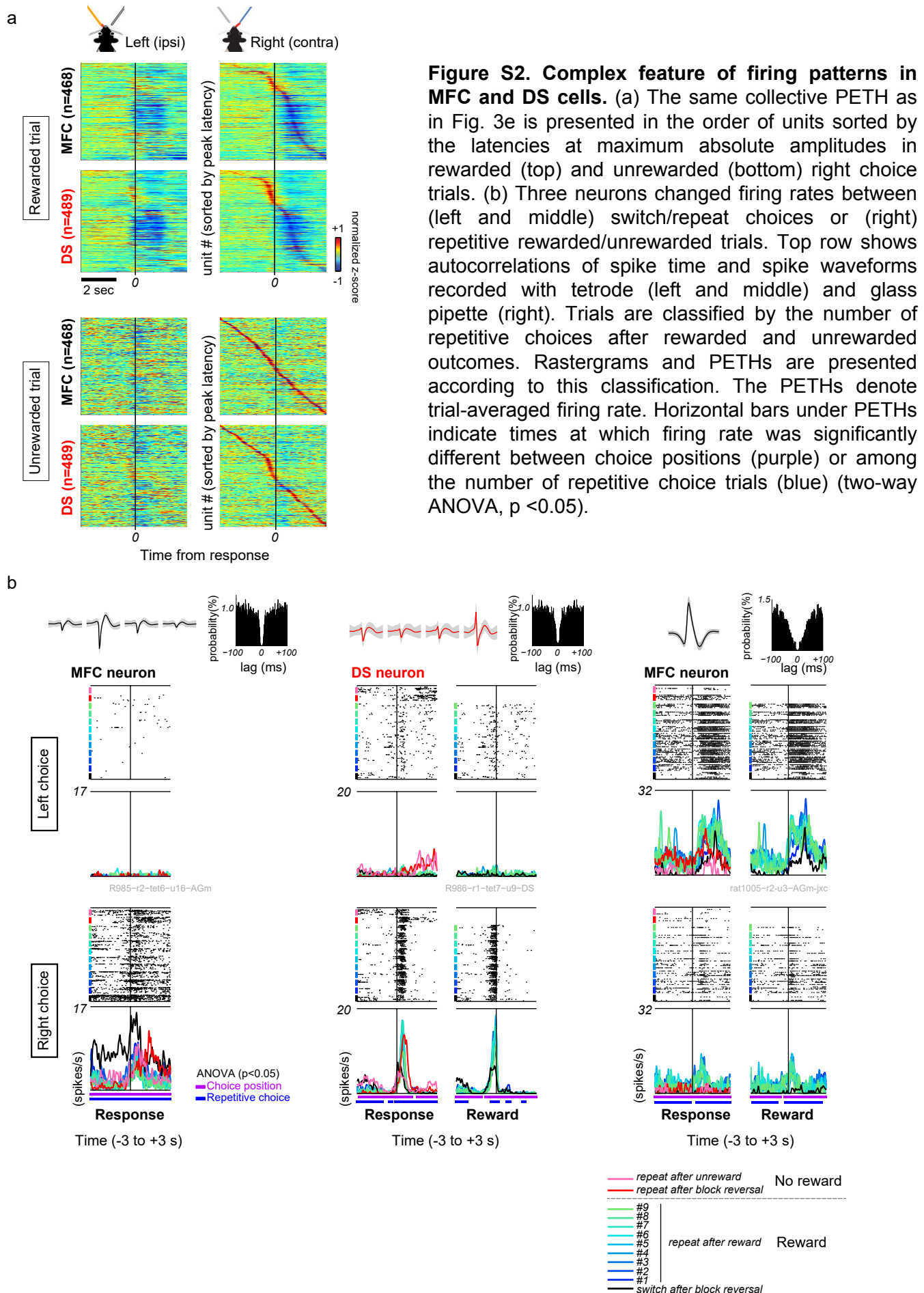
Figure 3

a
Left choice   Right choice
MFC neuron
26   26
(spikes/s)
2 s   Time from response
rat1005-r1-tet4-u2-AGm

b
DS neuron
11   11
(spikes/s)
Time from response
rat1005-r1-tet4-u7-DS

c
Left choice   Right choice
DS neuron
17   17
(spikes/s)
Time from response
rat1000-r1-tet6-u5-DS

d
DS neuron
22   22
(spikes/s)
Time from response
rat1009-r1-tet6-u6-DS

e
Left (ipsi)   Right (contra)
Rewarded trial
MFC (n=468)
DS (n=489)
Unrewarded trial
MFC (n=468)
DS (n=489)
unit # (sorted by peak latency)
normalized z-score   +1 / -1
2 s
Time from response

○ Reward delivery
rewarded Left choice trials
unrewarded Left choice trials
rewarded Right choice trials
unrewarded Right choice trials

ANOVA (p<0.05)
— Choice position
— Outcome

f
Choice position   Outcome
Cell fraction
MFC (n=468)
DS (n=489)
— (p<0.05)
MFC   AROC   contra / ipsi   contra / unrwd
DS   AROC   contra / ipsi   unrwd / rwd
Cell count >50 / 0   Cell count >90 / 0
2 s

g
Selectivity index
— (p<0.01)
-3   0   +3
Time from response (s)

Figure 3. Choice and outcome coding in single neurons of MFC and DS. Below, neuronal activities are aligned to choice response (vertical line). (a-d) Representative firing patterns modulated by choices and outcomes on MFC and DS single neurons. Simultaneously recorded Right-choice selective responses of (a) MFC and (b) DS cells. These cells decreased firing rate immediately after reward delivery (cyan circle). Another DS cell increased firing rate during post-outcome periods in (c) unrewarded and (d) rewarded trials. Top row shows spike waveforms (mean ± SD) in four channels of a tetrode and auto-correlogram. Peri-event time histogram (PETH) shows mean ± SEM of firing rate (bin = 200-ms, sliding 20 ms), and horizontal bars indicate the times at which firing rate was significantly different (two-way ANOVA, $p < 0.05$) with respect to choice (purple) or outcome (green) factor. (e) Normalized PETHs of MFC (n = 468) and DS (n = 489) neurons are shown in rewarded and unrewarded trials. In each condition, units are sorted by the latency of maximum absolute peaks. (f) *Top*: Proportion of MFC (black) and DS (red) cells showing (*left*) choice or (*right*) outcome-selective activity (two-way ANOVA, $p < 0.05$). Blue bars represent the times at which the cell proportions were significantly different between MFC and DS ($\chi 2$ test, $p < 0.05$). *Middle and bottom*: The number of choice- and outcome-selective neurons are shown together with AROC values revealing preference between contralateral/ipsilateral choices or unrewarded/rewarded outcomes, respectively. (g) Comparison of selectivity index for choice (*left*) and outcome (*right*) between MFC (black) and DS (red). Thick solid lines and shaded area represent median and 1st/3rd quartiles, respectively. Blue bars represent the times at which the selectivity indices were significantly different between MFC and DS (Mann-Whitney U test, $p < 0.01$).

Figure S2. Complex feature of firing patterns in MFC and DS cells. (a) The same collective PETH as in Fig. 3e is presented in the order of units sorted by the latencies at maximum absolute amplitudes in rewarded (top) and unrewarded (bottom) right choice trials. (b) Three neurons changed firing rates between (left and middle) switch/repeat choices or (right) repetitive rewarded/unrewarded trials. Top row shows autocorrelations of spike time and spike waveforms recorded with tetrode (left and middle) and glass pipette (right). Trials are classified by the number of repetitive choices after rewarded and unrewarded outcomes. Rastergrams and PETHs are presented according to this classification. The PETHs denote trial-averaged firing rate. Horizontal bars under PETHs indicate times at which firing rate was significantly different between choice positions (purple) or among the number of repetitive choice trials (blue) (two-way ANOVA, $p < 0.05$).

146   unrewarded outcomes (Fig. 3f). At the group level, selectivity indices were significantly larger in MFC

147   than in DS for both choice- and outcome-selective neurons in large portions of task period (Fig. 3g,

148   Mann-Whitney U test, $p < 0.01$). A small number of MFC and DS cells dynamically changed their firing

149   patterns according to the histories of outcome and choice (Supplementary information and Fig. S2b).

150

151   Thus, choice- and outcome-modulated neurons show similar dynamical evolution in MFC and

152   DS, but their fractions and degrees of selectivity were significantly different between the two brain

153   regions. These results suggest parallelism between MFC and DS in the neural population coding of

154   task-relevant information in this outcome-based choice task.

155

156   ***Functional correlation of neural trajectories between MFC and DS***

157   The above similarity in neural dynamics raises a question about the way neural population activities

158   are coordinated in MFC and DS to encode information about decision making. To clarify this, we

159   explored how the neural trajectories encoding Left and Right decisions were separated in each

160   session (12 sessions in total) by using Fisher's linear discriminant (Methods). This method yields a

161   hyperplane that optimally separates datasets belonging to two categories, such as Left/Right choices

162   or positive/negative outcomes, in the high dimensional space of the instantaneous population rate

163   vectors [12,13,35]. We calculated discriminability index *d'*, which is the distance between the

164   categorized rate vectors measured orthogonally to such a hyperplane. To keep a sufficient number

9

165   of cells (n ≧ 25 in both regions per session, Supplementary information), below we omitted three

166   data sets (R986-r1, R1000-r1, R1004-r1 in Fig. S3a) and only used 9 datasets (R982-r1, R983-r1, R983-

167   r2, R985-r1, R985-r2, R991-r1, R1005-r1, R1009-r1, R1012-r1).

168

169   For choice-selective or outcome-selective trajectories, the index d' peaked around the time of

170   choice response or outcome, respectively (Figs. 4a and S3c). However, the peak times were not

171   significantly different between MFC and DS (Fig. 4a). Then, we studied how neural trajectories

172   evolved in each session by projecting the instantaneous rate vectors onto the axes orthogonal to

173   choice (Ch) and outcome (Ot) hyperplanes obtained by the linear discriminant analysis (Fig. 4b).

174   Hereafter, these trajectories are termed "Ch-projected" and "Ot-projected" trajectories. The

175   temporal patterns of these trajectories varied from session to session in both MFC and DS (Fig. S4).

176   However, their trial-averages exhibited transiently or gradually correlated increases between MFC

177   and DS around choice (r = 0.62) and outcome (r = 0.50) events, respectively (Fig. 4b and 4e). The

178   inter-trajectory correlations were particularly strong (Pearson correlation coefficient was about 0.5

179   or greater) in 4 sessions (R983-r2 in Fig. 4, R983-r1, R985-r1, and R985-r2 in Fig. S4). The neural

180   trajectories diverged along with choice responses (Left and Right) around the response time (t1: 0.2

181   s before choice response), and afterwards diverged along with outcomes (reward and no-reward)

182   during outcome period (t2: 1 s after choice response), as exemplified in Fig. 4c. Figure 4d shows the

183   trial-by-trial values of Ch-projected and Ot-projected trajectories in MFC and DS at times t1 and t2,
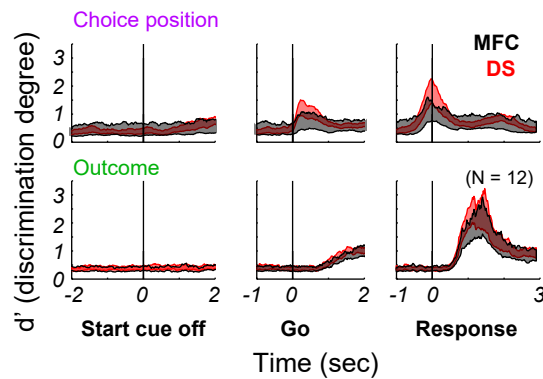
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Figure S3

a
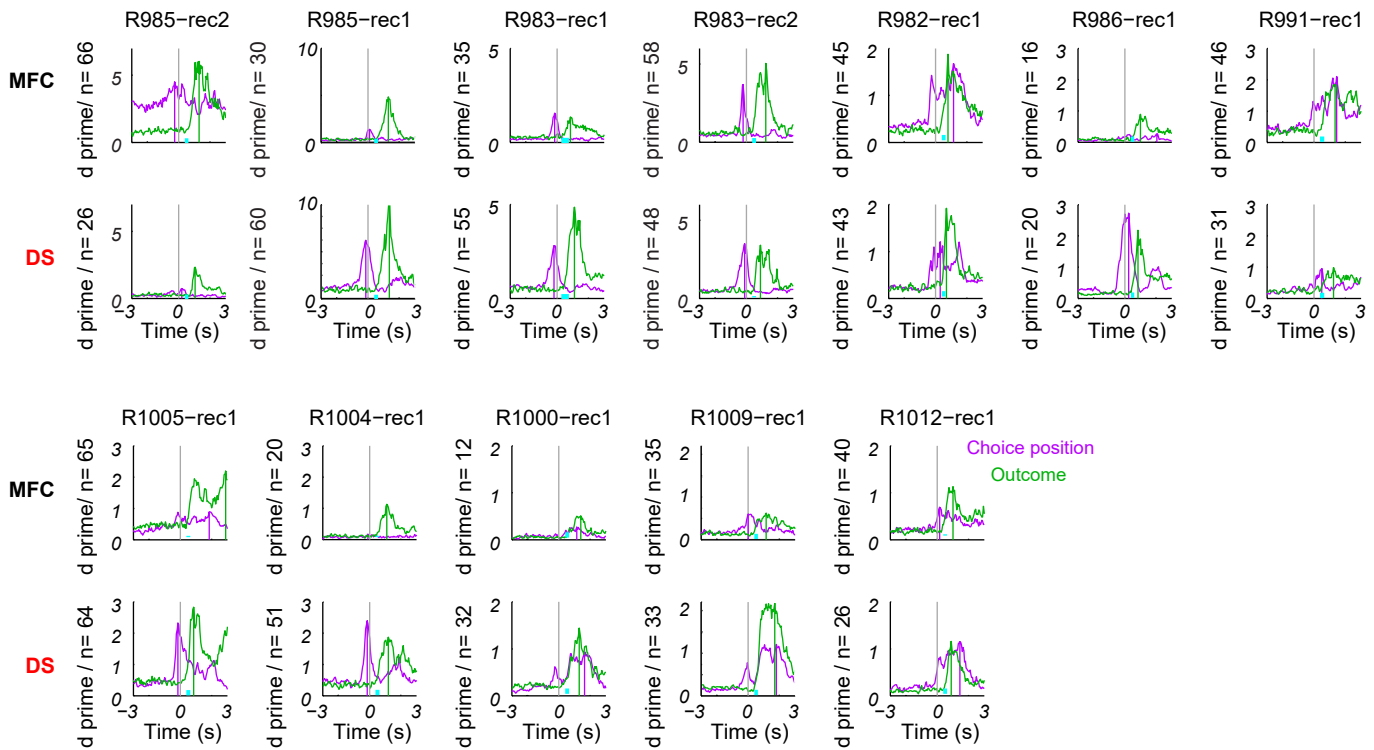
b
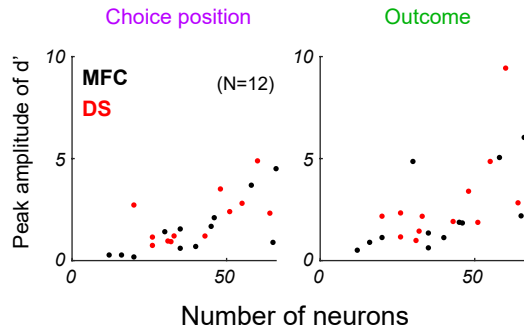
c

Figure S3. Degree of discrimination by population activity at individual sessions. (a) Time evolution of discrimination degree (d') on choice (purple) and outcome (green) axes around choice responses. Vertical line indicates the peak time of d' and cyan bar the interval of reward delivery. (b) Correlations between peak d' values and the number of neurons in population data. (c) Group data of d' on choice and outcome axes in MFC (black) and DS (red). Data were aligned at start cue end, go cue end, and response execution.

Figure 4

a

MFC (n=58)

— Choice
— Outcome

DS (n=48)

Time from response (s)

session: R983-r2

Peak time of *d'* from Response (s)

p = 0.900

MFC   DS

p = 0.355

(N=9)

MFC   DS

b

Ch-projected trajectory   Ot-projected trajectory

MFC   MFC

DS   DS

Time from response (s)   Time from response (s)

— rewarded Left choice trials   — rewarded Right choice trials
— unrewarded Left choice trials   — unrewarded Right choice trials

c

t1 = −0.2 s   t2 = +1.0 s

MFC

○: t1   ○: t2

DS

Ot-projected trajectory

d

MFC   t1 = −0.2 s

DS

MFC   t2 = 1.0 s

DS

Trial number

e

Corr. coeff.

Time from response (s)

— Ch-projected trajectory
— Ot-projected trajectory

f

Ch-projected trajectory   Ot-projected trajectory

Mutual info (%)

MFC
DS

(N=9)

Time from response (s)

g

Reward acquisition prob.

p = 0.0092

Lower corr. (5 sessions)   Higher corr. (4 sessions)

Figure 4. Temporal correlations of neural trajectories between MFC and DS. Unless otherwise stated, all results are shown for a typical session (R983-r2). (a) *Left*: Time series of discrimination degree (*d'*) for neural populations simultaneously recorded in MFC (n = 58, *Top*) and DS (n = 48, *Bottom*). Vertical lines indicate the peak times of *d'* measured between different choices (purple) and outcomes (green). *Right*: Comparison of the peak times of d' between MFC and DS (N = 9 sessions) at Ch- and Ot- hyperplanes. Squares and error bars denote mean and SD. P-value was assessed by paired t-test. (b) Trial-averaged (*left*) choice-projected and (*right*) outcome-projected trajectories of population vectors in MFC (*top*) and DS (*bottom*). Gray lines show standard errors. Vertical lines indicate times t1 (-0.2 s) and t2 (+1.0 s) at which the trajectories were near maximally separated on choice and outcome axes, respectively. (c) Evolution of trial-averaged population vectors from -3 s to t1 (*left*) or t2 (*right*) in the space spanned by choice and outcome axes. Circles indicate population activities at t1 (*left*) and t2 (*right*). (d) The distances of choice- and outcome-projected trajectories from the separating hyperplanes at t1 (*top*) and t2 (*bottom*) are shown in MFC (*1st row*) and DS (*2nd* row). (e) Time evolution of Pearson's correlation coefficients was calculated between MFC and DS projected trajectories. Purple and green lines represent the mean correlation coefficients, and gray lines show individual correlation coefficient calculated from randomly sampled 431 trials. Circles indicate the times of the maximum correlation. (f) *Top*: Mutual information about choice (*left*) and outcome (*right*) obtained from the trajectories in MFC (black) and DS (red) shown in b. *Bottom*: Mutual information from 9 sessions (thin) and the average (thick). Asterisk indicates statistical significance (Mann-Whitney U test, p <0.05). (g) Reward acquisition probability was significantly different between sessions with lower (< 0.5) and higher (> 0.5) trajectory correlations (t test, P <0.0092).
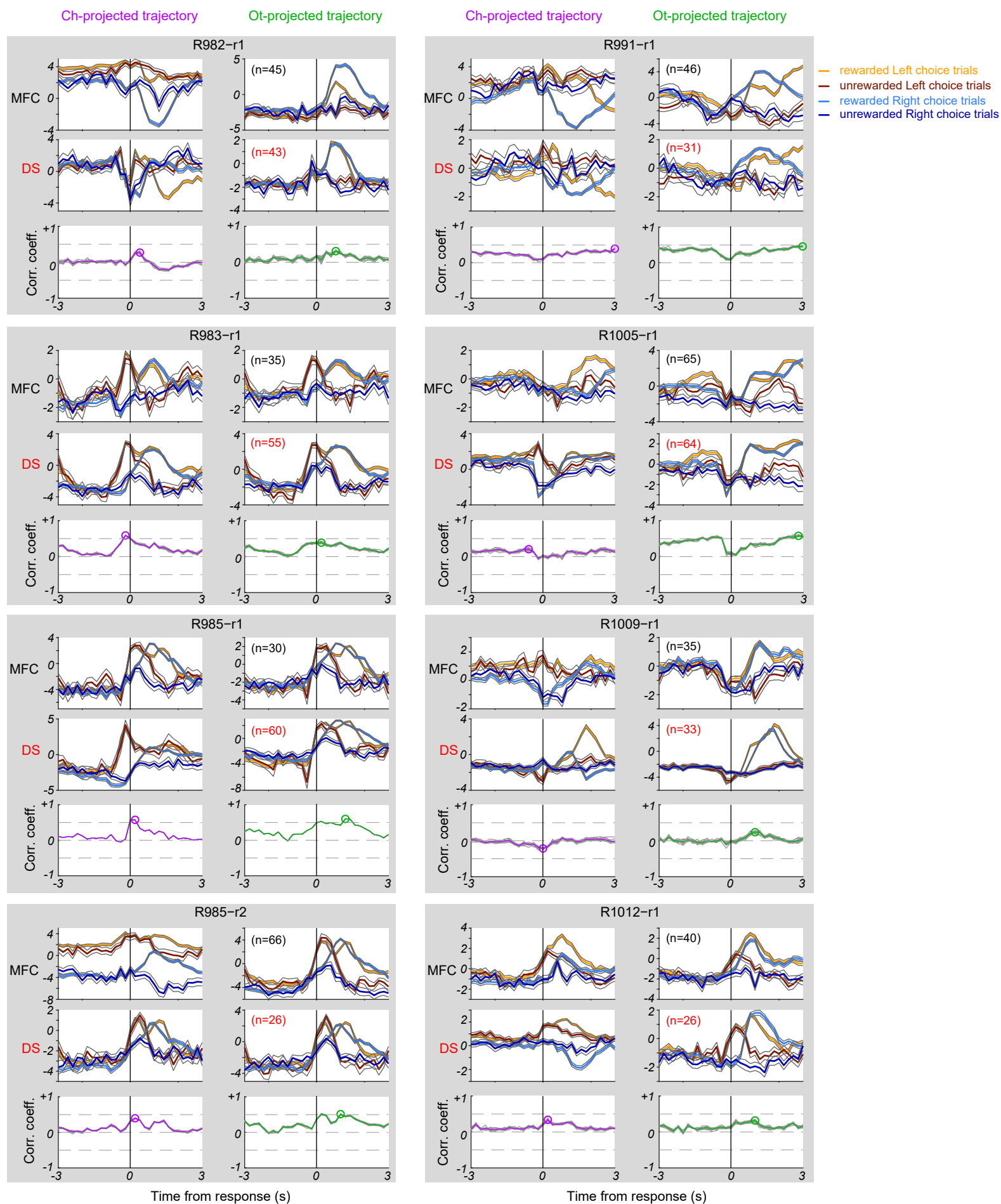
Figure S4

Figure S4. MFC and DS trajectories and their session-by-session correlations. Each gray box shows results from one session. Within a box, neural trajectory in MFC (*top*) and DS (*middle*) on choice (*left*) and outcome (*right*) axes are shown. Color codes indicate Left rewarded trials (orange), Left unrewarded trials (brown), Right rewarded trials (light blue) and Right unrewarded trials (dark blue). Thin lines represent S.E.M. (*Bottom*) Time evolution of correlation coefficient between MFC and DS trajectories. Gray lines show the correlation coefficients calculated with randomly sampled 431 trials in session R985-r1, which contains the minimum trial numbers among the 9 sessions. The averaged correlation coefficients between MFC and DS were displayed for Ch-projected (purple) or Ot-projected (green) trajectories. Circles indicate the times of maximum correlation.

184 respectively. These trajectories were highly correlated between the two areas around the

185 corresponding time points (Fig. 4e). In the other 5 sessions, while the Ot-projected trajectories were

186 similar, the Ch-projected ones behaved differently in MFC and DS and the inter-trajectory

187 correlations were also weaker between the two areas (Fig. S4).

188

189   The enhanced correlations of Ch-projected and Ot-projected trajectories likely reflect choice

190 responses and the monitoring of reward, respectively. As the degree of correlations varied during

191 the task in all sessions, we addressed the behavioral variables correlated to the high MFC-DS

192 correlation of neural trajectories. We investigated the temporal relationships among licking behavior,

193 choice responses, reward delivery and the times of peak inter-trajectory correlations. As shown in

194 Fig. 1e, the licking rate quickly reached a peak when the choice response was made and then

195 decayed slowly during the outcome period ('reward licking period') in rewarded trials. In

196 unrewarded trials, the decay was much faster. The peak correlation times of Ch-projected

197 trajectories tended to cluster around choice response (t-test, $p = 0.366$) and reward delivery (t-test,

198 $p = 0.620$), while those of Ot-projected trajectories were significantly later than choice response (t-

199 test, $p = 2.65 \times 10^{-3}$) and reward delivery (t-test, $p = 0.0297$) (Fig. S5a), and were retained high during

200 the following licking behavior (Fig. S5b). These results suggest that the epochs of highly correlated

201 Ch- and Ot-projected trajectories are related to licking behavior or the intake of reward, respectively.

202 The trajectory correlations exhibited different temporal profiles in rewarded and unrewarded trials
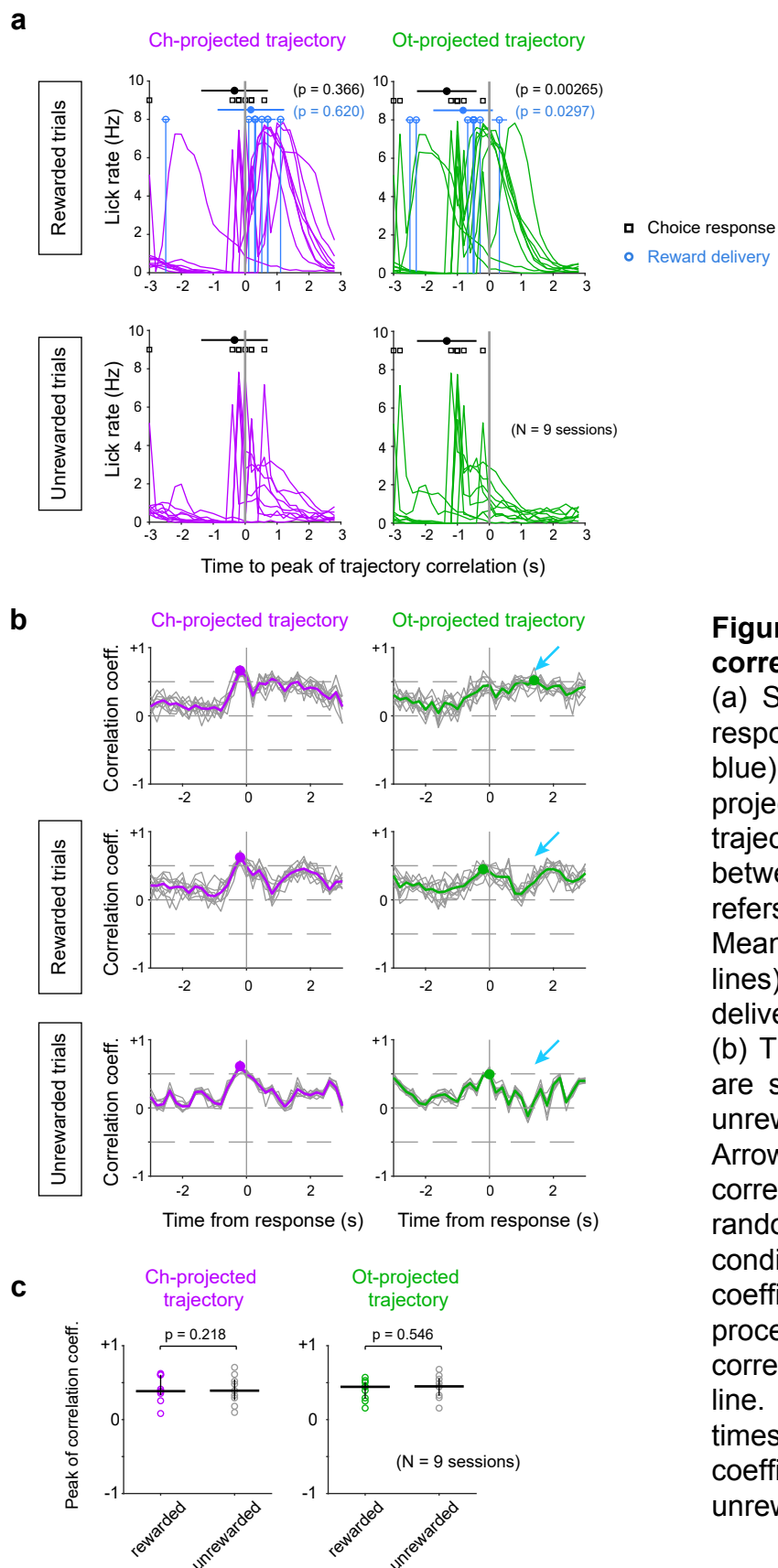
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Figure S5. Behavioral variable when the correlation of trajectories reached peak.

(a) Session-by-session licking rate, choice response (black) and reward delivery (light blue) are aligned at the time when Ch-projected (purple) and Ot-projected (green) trajectories showed peak correlations between MFC and DS. The origin of time refers to the time of peak correlations. Means (filled symbols) and SDs (horizontal lines) of choice response and reward delivery time are shown (N = 9 sessions). (b) Time evolution of trajectory correlations are shown for a rat in rewarded (middle), unrewarded (bottom), and mixed (top) trials. Arrows indicate the times of peak correlation in the mixed trial condition. We randomly selected 63 trials in each condition and calculated correlation coefficient (gray line). We repeated this procedure by 10 times. Averaged correlation is represented by colored thick line. Circles indicate the peak values and times. (c) Comparison of peak correlation coefficients between rewarded and unrewarded trials.

203   (Fig. S5b), but the peak correlations of Ch- (Wilcoxon signed-rank test, p = 0.218) and Ot-projected

204   (p = 0.546) trajectories were not significantly different between the two trial types, suggesting that

205   the enhanced correlations reflect the monitoring of reward rather than actual reward delivery.

206

207   It is intriguing to examine whether neural trajectory switched in either or both of MFC and DS

208   when the rats switched their choices after unrewarded trials. In the present task, rats switched their

209   choices after a reversal of reward block within a couple of unrewarded trials (Fig. 1b and c). We

210   hypothesized that during the unrewarded trials neural trajectory should cross the hyperplane

211   (Unless otherwise stated, hyperplane refers to Ch-hyperplane) from one side to the opposite side

212   (i.e. 'Right to Left' or 'Left to Right') before a choice response in the next trial. Out of 9 sessions, in

213   3 sessions we observed a gradual crossing of MFC trajectory during ITIs, while a similar crossing of

214   DS trajectory was observed only in one session (Fig. S6). Although the crossing was somewhat more

215   prominent in MFC than in DS, it was difficult to determine which region dominated switching choice

216   responses.

217

218   We further verified the analogous encoding of choice and outcome information in MFC and DS

219   by calculating the time evolution of the information carried by the neural trajectories (Fig. 4f). The

220   amount of information varied from session to session and was slightly greater in MFC than in DS.

221   However, the mutual information averaged over nine sessions was not statistically different between
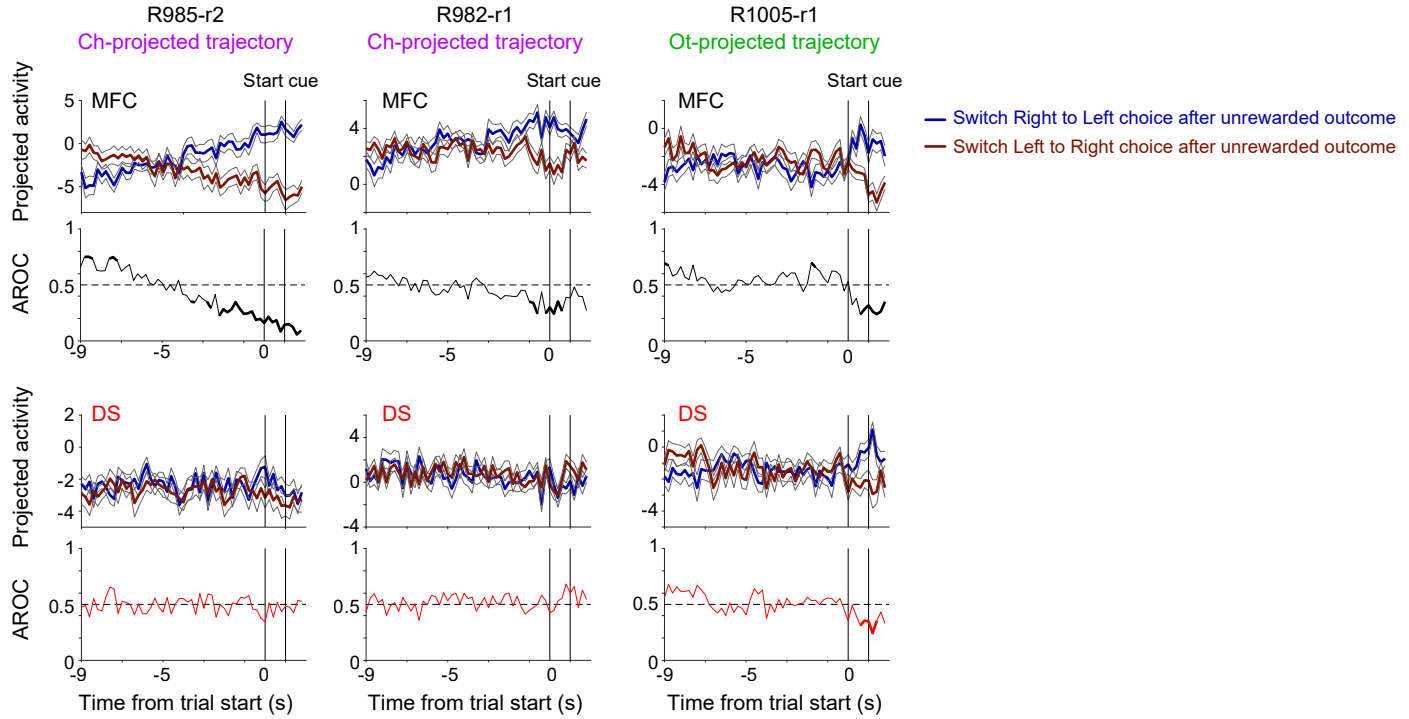
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Figure S6

Figure S6. Crossing trajectories between two switching choice trials during inter-trial interval (ITI) period. Neural trajectory during ITI period post unrewarded episode in 3 sessions (left: R985-r2, Ch-projected trajectory; middle: R982-r1, Ch-projected trajectory; right: R1005-r1, Ot-projected trajectory) in MFC (1st row) and DS (3rd row). Dark blue and brown denote the neural trajectories when rats switched choices from Right to Left and from Left to Right, respectively. Black thin lines denote standard errors. Differences in the two trajectories were quantified by AROC values in MFC (2nd row, black) and DS (4th row, red). A gradual shift from high values (> 0.5) to low values (< 0.5), suggests that the trajectories evolved to the opposite side of the corresponding hyperplane. Statistical significance of AROC values is estimated by Mann-Whitney U test (p <0.05) and represented by thick lines. Two vertical lines show the duration of start cue presentation.

222   MFC and DS at most of the times around choice response (Mann-Whitney U test, p >0.05). These

223   results indicate that neural trajectories evolve in parallel in MFC and DS and transiently increase the

224   mutual correlation to transfer choice and outcome information without a large loss. Reward

225   acquisition probability was significantly larger (p = 0.0092, two-sample t test) in sessions with high

226   inter-trajectory correlations (> 0.5) during choice and outcome periods than in sessions with low

227   correlations (Fig. 4g). In this analysis, the session R1005-r1 was included in the low-correlation group

228   because the correlation was low during choice and outcome periods. Omission of this session did

229   not change the essential result of comparison (p = 0.0193, two-sample t test).

230

231   ***Synchronous spiking when neural trajectories were highly correlated between MFC and DS***

232   As shown above, neural trajectories in MFC and DS exhibit task event-dependent modulations of

233   coherence. This result raises questions about whether neuronal firing is also temporally correlated

234   between these regions and whether correlated spikes, if any, are related to neural trajectories. To

235   study these questions, we calculated cross-correlograms (CCGs) of MFC-DS cell pairs in the time

236   window in which MFC-DS correlations of trajectories were maximized (see Fig.4e and Fig. S4)

237   (Methods). Then, we compared task-event relevant and irrelevant CCGs in three trial categories (i.e.,

238   Left-choice, Right-choice and rewarded trials).

239

13

240   Our analysis revealed MFC-DS cell pairs displaying statistically significant spike synchrony in

241   a task-event-dependent manner (Fig. 5a). Shuffling spike trains across trials within the individual

242   pairs eliminated the peaks in CCGs (Fig. S7), implying that these cell pairs fired with a close temporal

243   relationship in each trial. The peak values of CCGs showed significant correlations between MFC and

244   DS (> 4 SD of the base line) in task-event related windows, but these values were greatly decreased

245   if CCGs were calculated in task-event-irrelevant windows (i.e., at random time points) (Fig. 5a and b).

246   These results suggest that the emergence of spike synchrony between MFC and DS cells is correlated

247   with task events.

248

249   Spike synchrony tended to occur when neural trajectories were highly correlated between

250   MFC and DS, which further supports the role of spike synchrony in coordinating neural population

251   activities in the two regions. The spike count of MFC cells exhibited statistically significant peaks at

252   near zero time lags from spikes of DS cells in 4 sessions (R983-r1, R983-r2, R985-r1 and R985-r2 in

253   Fig. 5c). At this epoch, the maximum correlation coefficients between MFC and DS trajectories were

254   near or over 0.5 (Fig. 4e and Fig. S4). By contrast, the CCG distributions showed no significant peaks

255   in the other 5 sessions (R982-r1, R991-r1, R1005-r1, R1009-r1, and R1012-r1). However, in these 5

256   sessions the correlations of neural trajectories were also weaker compared to the former 4 sessions

257   (Fig. S4). Interestingly, in rewarded trials the correlation coefficient between Ot-projected

258   trajectories was correlated with the fraction of cell pairs showing CCG peaks within ±2 ms (Pearson
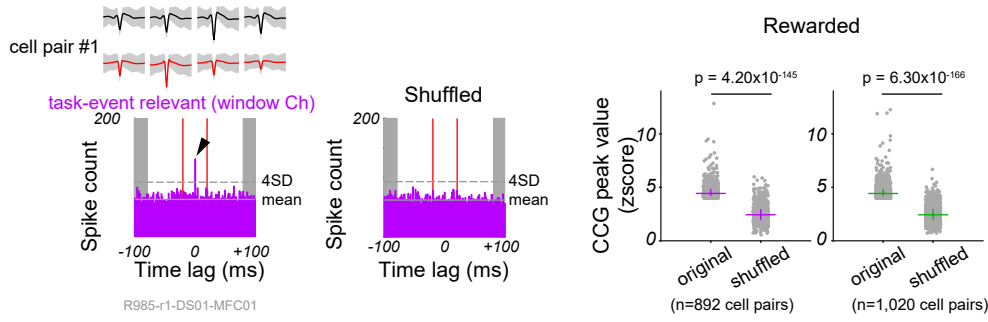
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Figure S7. Significant cross correlations between MFC and DS cell were dependent on the spike timing in the ongoing trial but not on the number of spikes. Left: CCGs of the cell pair #1 in Fig. 5a were calculated in the original (left) and shuffled (right) trial orders in rewarded trials. Right: Normalized CCG peak amplitudes were calculated for all cell pairs showing significant CCG peaks in windows Ch (purple) and Ot (green). The peak amplitude are compared with those obtained in shuffled trial orders. Horizontal lines and error bars show the median and 1st/3rd quartiles, respectively. P-values were calculated by Wilcoxon signed-rank test.

Figure 5
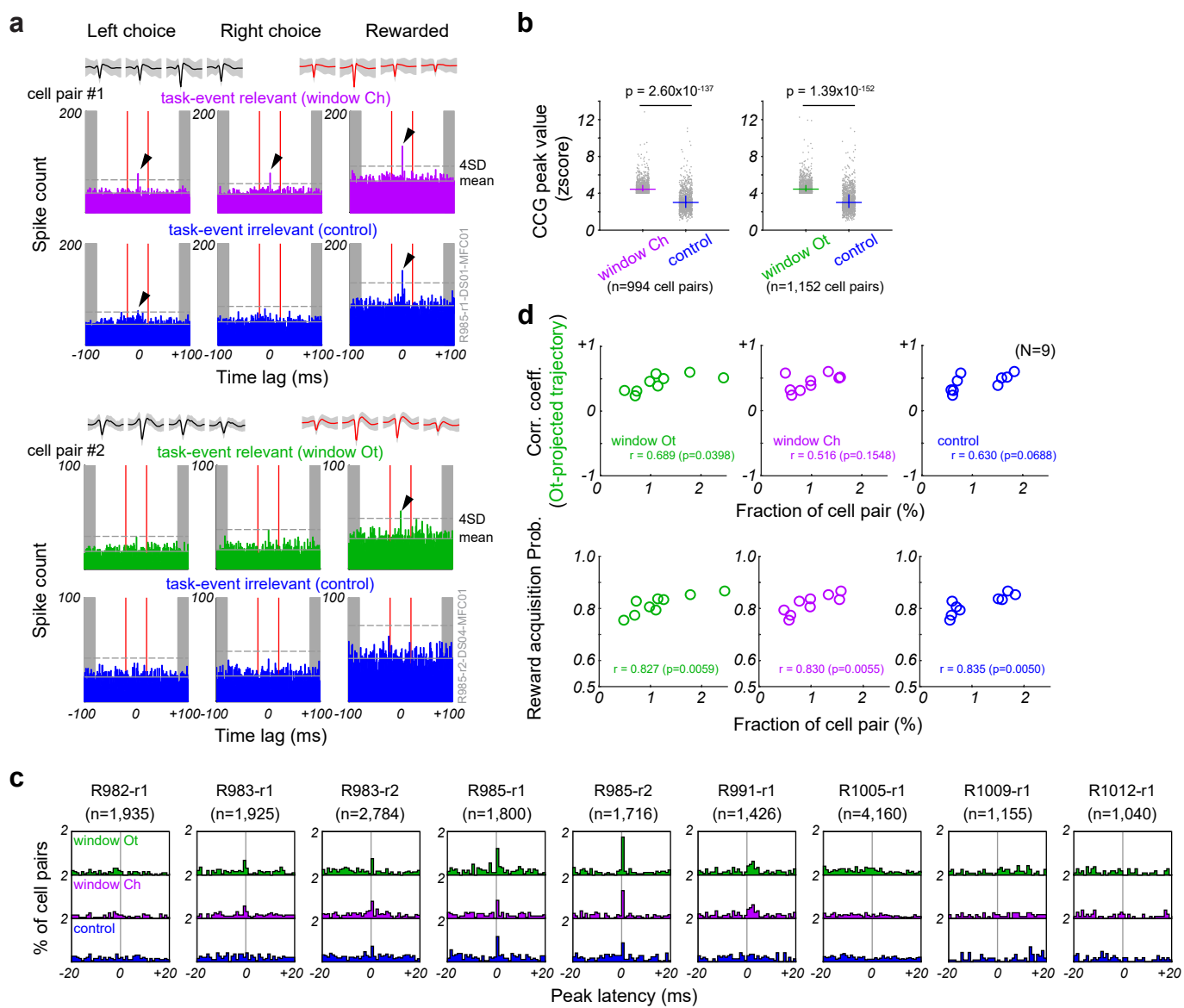
Figure 5. Relationships among MFC-DS spike synchrony, trajectory correlation and task performance. (a) Spike CCGs of two MFC-DS cell pairs. For each cell pair, time lags of MFC cell spike firing relative to DS cell spike firing were calculated in task-event relevant (*middle row*) and irrelevant (*bottom row*) time windows. Average and SD (gray shade) of spike waveforms recorded with tetrodes in MFC (black) and DS (red) are also shown (*top row*). *Left and middle columns*: Left or Right choice trials with arbitrary outcomes, respectively. *Right column*: rewarded trials with arbitrary choice positions. Windows Ch (purple) and Ot (green) refer to the analyzed time windows centered around the peak MFC-DS correlations of Ch-projected and Ot-projected trajectories, respectively. Task-event irrelevant CCG gives a control and was calculated at a randomly selected time point. Arrowhead indicates the peak of CCGs above the mean + 4SD of the baseline defined by spike counts from -100 to –80 ms and from +80 to +100 ms (gray shaded ranges). (b) CCG peak values of cell pairs in windows Ch (purple) and Ot (green) are compared with those in task-event irrelevant windows (black). Each dot represents a cell pair with a significant CCG peak (peak > 4SD). Horizontal lines and error bars show the median and $1^{st}/3^{rd}$ quartiles, respectively. P-values were calculated by Wilcoxon signed-rank test. (c) Proportion of MFC-DS cell pairs that displayed an excess amount of spike coincidences in rewarded trials was plotted for the 9 sessions against CCG peak time lags. The numbers of such cell pairs are given in parentheses. (d) (*Top*) Correlation coefficients between MFC and DS Ot-projected trajectories are plotted against the fraction of MFC-DS cell pairs showing a CCG peak within ±2 ms for different time windows. (*Bottom*) Relationship between such MFC-DS cell pairs and animal's reward acquisition probability. Symbols represent individual sessions, and Pearson correlation coefficient and P-value are denoted for each diagram.

259   correlation test, window Ot, r = 0.689, p = 0.0398) (Fig. 5d, top). Further, the fraction of such cell

260   pairs was correlated with the probability of animal's reward acquisition (Fig. 5d, bottom). These

261   results suggest that precisely correlated firing of MFC and DS cells underlies the coherent evolution

262   of neural trajectories between these areas and boost animal's task performance.

263

264   ***Inactivation of MFC attenuated neural representation of choice and outcome in DS cells***

265   To confirm the contributions of inter-trajectory correlations between MFC and DS to adaptive control,

266   we examined the effect of bilateral MFC inhibition on the choice behavior and DS-cell activity in 5

267   rats (muscimol group) (Fig. 6a, Methods). It is known that MFC is one of the main regions projecting

268   excitatory inputs to DS [6-8]. Another 10 rats were examined the choice behavior without bilateral

269   MFC inhibition as a control group. The muscimol injection induced variable choice patterns in five

270   muscimol-treated rats (F test, F = 16.95, p = 0.0179). After the injection (Day 2), the reward

271   acquisition probabilities of four rats were significantly different from those prior to the injection (Day

272   1) (χ2 test, p<0.05). Among these four, the reward acquisition behavior was impaired in three while

273   another rat rather improved the behavioral performance (Fig. 6b). Figure 6c shows an example of

274   impaired task performance. This rat reduced "stay" behavior after preceding rewarded outcomes

275   but increased such behavior after preceding unrewarded outcomes (Fig. 6c-d). Indeed, the fractions

276   of "stay" and "switch" choice patterns were significantly different between Day1 and Day2 in the

277   muscimol group (two-way ANOVA, $F_{1,4}$ = 5.774, p = 0.021) but not in the control group ($F_{1,9}$ = 0.248,
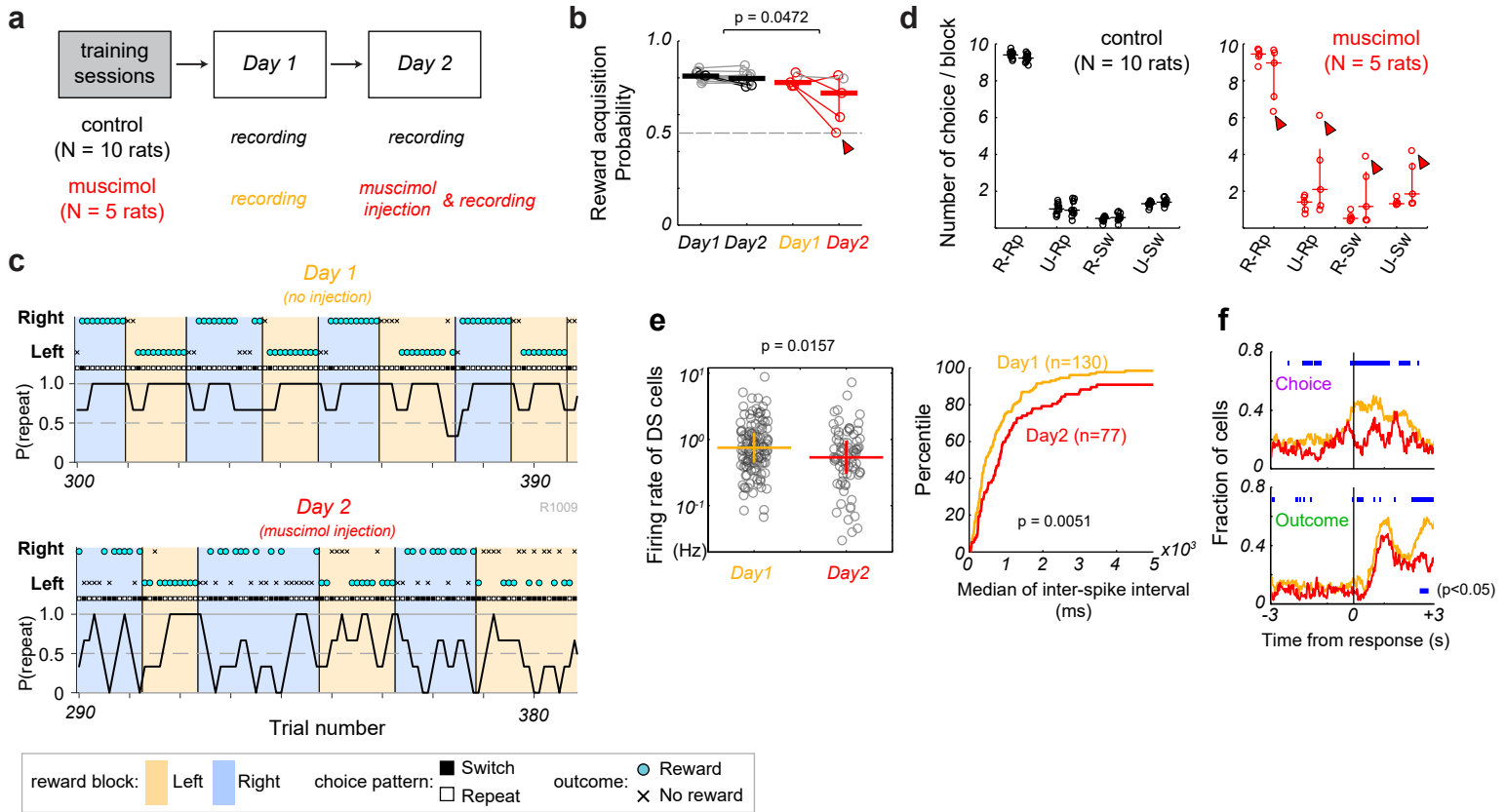
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Figure 6

Figure 6. Inactivation of MFC attenuated neuronal representation of choice and outcome in DS. In statistical comparisons, horizontal and vertical lines indicate the median and 1st/3rd quartiles, respectively. (a) Experimental schedules for control (N = 10 rats) and muscimol (N = 5 rats) groups. At the first session (Day1), all 15 rats underwent the recordings of multi-neuron activity from MFC and DS without any pharmacological treatments. At the second session (Day2), we injected muscimol into the MFC of five rats (muscimol group) and recorded multi-neuron activity from DS. For the remaining 10 rats (control group), neural activities were recorded from MFC and DS without any injection. (b) Reward acquisition probabilities in Day1 and Day2 are shown in control (black) and muscimol (red) groups. Each symbol shows data from a rat. Colored (black or red) symbols indicate the individual rats that showed significantly different probabilities (χ2 test, p <0.05) between Day1 and Day2. Arrowhead indicates the rat shown in c. The differences in reward acquisition probabilities between Day1 and Day2 were compared between groups by two-sample t-test. (c) An example of impaired adaptive choice behavior of a muscimol-injected rat is shown on Day1 (top, no injection) and Day2 (bottom, muscimol injection). (d) Mean number of choice patterns per block on Day1 (left) and Day2 (right). Left: control group, Right: muscimol group. R: rewarded in last trial, U: unrewarded in last trial, Rp: repeat in current trial, Sw: switch in current trial. (e) Firing patterns of DS cells were compared (Mann-Whitney U test) in the muscimol group in terms of firing rate (Left) and inter-spike interval (Right) between Day1 (n = 130, orange) and Day2 (n = 77, red). Circles indicate the firing rates of individual DS neurons. (f) Time evolution of the fraction of (top) choice-modulated and (bottom) outcome-modulated DS cells (two-way ANOVA, 200-ms-long sliding-by-20-ms bins, p <0.05) on Day1 (orange) and Day2 (red). Blue bars show the epochs of significantly different fractions between Day1 and Day2 (χ2 test, p <0.05).

278   p = 0.620). Accordingly, the control and muscimol groups showed significantly different reward

279   acquisition probabilities between Day 1 and Day2 (two-sample t-test, p = 0.0472). The behavioral

280   variability between the two groups was unlikely attributed to the attenuation of their motivations

281   for the task because the number of trials was not significantly different between Day1 (mean ±SD =

282   731 ±86 trials) and Day2 (740 ±96 trials) in the muscimol group (unequal variance t-test, p = 0.877)

283   and these numbers were not significantly different from those of the control group. Those results

284   suggest that MFC is not a sole contributor to the present adaptive choice behavior.

285

286   The inhibition of MFC diminished neuronal activities and their selective modulations in DS.

287   The firing rates of DS cells (n = 77) were significantly lower (Mann-Whitney U-test, p = 0.0157) and

288   the median of inter-spike intervals was significantly longer (Mann-Whitney U-test, p = 0.0051) on

289   Day2 than on Day1 (n = 130) (Fig. 6e), suggesting an attenuated excitatory drive or an enhanced

290   inhibition on DS cells. The MFC inhibition also altered the task-related activities of DS cells, especially

291   those encoding choices and outcomes (two-way ANOVA, p <0.05). The proportion of choice-

292   modulated cells around and after choice responses was significantly smaller on Day2 than on Day1

293   ($\chi 2$ test, p <0.05) (Fig. 6f, top), and so was the proportion of outcome-modulated cells during the

294   latter part of outcome period (more than 2 s after a choice response). However, this proportion was

295   not significantly different during the former part of outcome period (Fig. 6f, bottom). Taken together,

296   our results suggest that neuronal activation in MFC is engaged in DS neural coding both choice-

16

297 related information around response time and outcome-related information during reward licking

298 behavior.

299

300

301 **Discussion**

302 This study revealed a highly parallel evolution of MFC and DS neural trajectories regulating outcome-

303 based choice behavior. These trajectories initially encoded a current choice and later reflected the

304 outcome of the choice response. Despite variation in temporal patterns of the trajectories across

305 sessions and individuals, the amount of information carried by the trajectories was not significantly

306 different between MFC and DS. Furthermore, cross-area spike coincidences in the millisecond range

307 were enhanced in rewarded trials when the trajectories were highly correlated between the two

308 regions, suggesting that the parallel trajectory evolution in MFC and DS actively participates in

309 coordinating the behavioral task.

310

311   Along the frontal cortex-basal ganglia axis, the MFC-DS channel is thought to be the stage

312 to process action selection based on past outcomes [1,3]. A widely hypothesized mechanism for this

313 process is that neural ensemble in DS selectively gates the necessary information (or suppresses the

314 unnecessary information) received from MFC. However, our results provide no support for this

315 hypothesis because neural populations in MFC and DS preserve similar amounts of information

17

316   about choices as well as outcomes and their trajectories evolve quite similarly in the two regions

317   (Fig. 4).

318

319   Our data suggest in contrast to the gating hypothesis that the neural trajectory of DS

320   ensembles reflects that of MFC ensembles, which is consistent with previous work demonstrating a

321   simultaneous and correlated activation of time-dependent ramping activity in MFC and DS during a

322   temporal judgment task [36]. An alternative interpretation, given that MFC innervates DS

323   unidirectionally, is that MFC cells exhibit a choice-related signal prior to DS cells and then convey the

324   signal to DS in a sequence, as previously suggested [14,37]. However, our data does not support this

325   interpretation because choice-related signals concomitantly emerge in MFC and DS. This discrepancy

326   may be attributed to several differences in experimental conditions. For instance, unlike other

327   studies, we recorded neuronal activities in a head-restrained condition, did not change the

328   probability of reward delivery during the task, and directly compared simultaneous neuronal

329   activations between MFC and DS.

330

331   A possible role of the corticostriatal circuit suggested by our findings is that construction of

332   temporal correlation of neural activation between MFC and DS could lead to consolidating efficient

333   transmission of information from frontal cortex to downstream of basal-ganglia or to Inhibiting

334   inappropriate action. A previous study demonstrated the synchronization of oscillatory local field

18

335 potentials at 5-13 Hz across motor cortex, DS and substantia nigra pars reticulata, which is the output

336 terminal of the basal ganglia, and the propagation of spindle-like spike-and-wave oscillations along

337 this cortico-basal ganglia pathway in freely moving rats. Spikes of individual neurons were phase-

338 locked to the oscillatory local field potential and synchronized within the cortical-basal ganglia

339 network [38]. Since synchronized neuronal discharges may enable a reliable information

340 transmission [29,30], the temporal correlation between neural ensembles in the frontal cortex-basal

341 ganglia network could be a consequence of learning the decision behavior. In accordance with this

342 view, during skill learning, neural ensembles in both motor cortex and DS are known to develop spike

343 correlations [39,40] and precise temporal spiking patterns [31,41]. In the present study, synchronous

344 spiking was coordinated between MFC and DS cells when neural trajectories in these areas were

345 strongly correlated in the well learned rats, supporting the hypothesis (Fig. 5).

346

347   These results raise the question about how such a precise spike synchrony, which is

348 indicated by the short latency of CCG peak between MFC and DS cells (Fig. 5), was generated

349 between the cortical and subcortical regions. There are at least two possible explanations. One

350 possibility is that corticostriatal neurons in MFC make monosynaptic connections to the postsynaptic

351 DS cells. In this case, presynaptic MFC cells should discharge prior to the firing of postsynaptic DS

352 cells, but this was not the case at all in the CCGs between these cells (Fig. 5). Another possibility is

353 that the firing of MFC and DS cells was driven by a common input from other corticostriatal neurons

354   in the MFC [42,43] or neurons in other cortical areas projecting to both MFC and DS. Medium spiny

355   DS cells receive excitatory inputs from ipsilateral and contralateral MFC [16], while MFC cells' output

356   projects to both ipsilateral and contralateral cortical areas and DS through corpus callosum, the so-

357   called crossed-corticostriatal (or intratelencephalic) cells [6,44]. Since excitatory connections are

358   abundant between MFC corticostriatal pyramidal cells [45], synchronous discharges of these

359   neurons generate large EPSPs in their postsynaptic target cells in MFC and DS, which increases the

360   probability of synchronous firing between MFC and DS cells. Alternatively, both MFC [46] and DS

361   [7,8] receive afferent connections from higher-order cortical areas such as the ventrolateral OFC and

362   PPC. Since the OFC and PPC are implicated in goal-directed decision-making [47,48], excitatory

363   inputs from a common subpopulation of OFC and/or PPC neurons may evoke synchronous spiking

364   of MFC and DS cells during decision-making. In the present study, bilateral MFC inactivation

365   attenuated, but did not completely eliminate, choice and outcome representations in DS cells (Fig.

366   6), suggesting that brain regions other than MFC also drive DS cells in outcome-based action

367   selection. For instance, optogenetic inhibition of PPC corticostriatal projection neurons altered the

368   history dependent choice bias during decision making [49]. To distinguish between the different

369   causes of spike synchrony between MFC and DS cells further recordings from multiple regions

370   involving MFC, OFC, PPC, and DS are required.

371

372   It was recently shown in the three-layered cortex of the reptile that just a few spikes of

20

373   single pyramidal neurons can trigger a cascade of firing sequences of neuron ensembles [50], as was

374   suggested by theoretical studies [29,51]. Our results indicate the possibility that a similar cascade of

375   firing sequences is triggered in the cortico-basal ganglia pathway during the parallel evolution of

376   frontal cortical and striatal neural trajectories. Such spike sequences may not only enhance reliable

377   information transmission (i.e., spikes) from the cortex to the basal ganglia, but also may contribute

378   to regulating the gain of synaptic plasticity together with modulations by dopaminergic afferents

379   [52,53]. Further experiments are required to clarify these points.

380

381   In sum, we have shown that neural trajectories evolve in DS during goal-directed decision

382   making in parallel with a similar trajectory evolution in MFC. When the parallel trajectory evolution

383   in DS and MFC exhibits a strong coherence, spike synchrony is also enhanced between the two

384   regions and behavioral performance tends to be improved. Our results suggest that the parallel

385   evolution of cortical and striatal neural trajectories is a hallmark of successful decision making.

386

387

388   **Acknowledgments**

395   **Author contributions**

396   TH and TF designed the project. TH and RH conducted experiments. TH analyzed data. TH and TF

397   wrote the manuscript.

398

399   **Declaration of Interests**

400   The authors declare no competing interests.

401

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31

563   **Methods**

564   ***Animal preparation.***   All experiments were approved and carried out in accordance with the

565   Animal Experiment Plan by the Animal Experiment Committee of RIKEN. Male Long-Evans rats (N =

566   34, 6 weeks, 200-220 g, Japan SLC, Inc.) were used. Home cages were set in a temperature and

567   humidity controlled environment with lights maintained on a 12-h light/dark cycle. Prior to a primary

568   surgery, rats were handled briefly and habituated to a stainless-steel cylinder in the cages. All surgical

569   procedures were operated under sterile circumstances. Animals underwent three surgical

570   procedures. Rats were anaesthetized with 2% isoflurane. Their body temperature was monitored

571   with a rectal probe and maintained at ~37°C on a heating pad during the surgery.

572   At a primary surgery, a sliding head-attachment (Narishige) was implanted on the skull with

573   dual-curing resin cement (Panavia, Kuraray Noritake Dental) and dental resin (Unifast II, GC) as done

574   before in our laboratory [12,13,23,54]. Reference and grounding electrodes (teflon coated silver

575   wire) were put on dura mater above the cerebellum. After recovery from the surgery, the rats were

576   deprived water intake in the home cages in order to utilize water as a reward for the execution of a

577   task, although food was available *ad libitum*. Water (10 ml) was supplemented at every weekend. At

578   a second surgery 3 days prior to a first recording session, we injected a retrograde tracer Fluoro-Gold

579   (FG) into the DS in order to confirm if our recording sites corresponded to the MFC sending

580   corticostriatal projection to the DS. A glass micropipette filled with 2% FG (Fluorochrome) dissolved

581   in 0.1 M cacodylic acid was installed on a micromanipulator angled medially by 27 deg. The pipette

32

582   was inserted through a tiny burr hole drilled in the skull over left hemisphere (+1.5 mm to Bregma,

583   1.0 mm lateral to midline, 4.3 mm traveling distance) so that the pipette tip reached the dorsocentral

584   part of striatum (+1.5 mm anterior to Bregma, approximately 3.0 mm lateral to midline,

585   approximately 3.8 mm ventral to pia mater), which rostral agranular medial cortex (AGm) pyramidal

586   neurons directly innervate (Figs. 2 and S1). FG was iontophoretically loaded through 7 s pulse of +5.0

587   µA by 7 s interval for 30 or 60 minutes with an iontophoresis pump (BAB-501, Kation Scientific). Then,

588   the tiny hole was covered with sterilized spongel and dental silicone sealants (Matsukaze). At a third

589   surgery after the training sessions were completed, two cranial windows (1.2 mm diameter) were

590   made above the DS and MFC of left hemisphere (+1.0 and +3.0 mm to Bregma, 3.0 and 1.0 mm to

591   midline for DS and rostral MFC, respectively) and then its dura maters were removed. For bilateral

592   muscimol injection session, another tiny burr hole was additionally drilled in the skull above MFC of

593   right hemisphere (+3.0 mm to Bregma, 1.0 mm to midline). The cranial windows were covered with

594   the silicone sealants.

595

596   ***Behavioral training apparatus.***   After recovery from the first surgery, the rats were trained to

597   perform a behavioral task controlled under a customized multiple-rats training system (O'hara),

598   which enabled training several rats to learn a task paradigm in parallel [23,54]. The behavioral task

599   was controlled by a custom-written software with LabVIEW (National Instruments). In an isolated

600   training chamber, each rat was placed at a body-supporting cylinder, and its head was fixed rigidly

33

601   and painlessly by screwing a sliding head-holder on a stereotaxic frame. Auditory stimuli were

602   presented at 60-dB SPL via a speaker placed in front of the rat. The timing and direction of licking

603   movement were detected when its tongue interrupted an infrared beam placed below the left and

604   right spouts. The detection sensitivity was much enough to detect tongue movements during licking

605   spout, but not sensitive enough to detect other potential movements such as whisking and

606   mastication. White plastic plates were located at both besides of the face in order to prohibit rats

607   from approaching the beam detectable spaces by forelimb reaching (Fig. 1a). Spouts were connected

608   to a syringe set on a single-syringe pump (AL-1000, World Precision Instruments) via silicon tubing.

609   Water delivery from each spout was regulated by an audible pinch valve triggered by TTL signal

610   which also triggered the syringe pumping.

611

612   ***Behavioral task.***   A trial began with a pure tone presentation (3 kHz, 1 s) (Start in Fig. 1a).

613   Rats were required not to lick any spouts from 0.3 s after Start cue offset until an appearance of

614   another auditory cue (10 kHz, 0.2 s) (Go). If the rats licked any spouts during the delay period, the

615   trial was immediately aborted. This pseudorandom delay period ranged between 0.7-2.3 s. After the

616   Go signal onset, the rats were allowed to lick one of either left or right spout within a response

617   window (5 s). A first lick was judged as a choice response (Choice). When the chosen spout location

618   corresponded to ongoing reward location, 0.1% saccharin water (15 μl) was delivered as a reward

619   after a pseudorandom delay period ranging between 0.3 and 0.7 s (2nd Delay). After an outcome

34

620   period (4 s, Outcome), a next trial began. On the other hand, when the rats chose no-reward spout,

621   they did not receive any sensory feedback but had an additional time-out of 5 s after the outcome

622   period. After the time-out, a next trial began.

623   At a first training session, the rats learned to associate licking a single spout located at left

624   (or right) side with a reward delivery. Once the rats voluntarily licked the spout to acquire a reward,

625   they learned to associate licking another spout set at the opposite side with a reward acquisition

626   through another spout. At this stage, the second delay period after choice was fixed as zero (no

627   delay). At a second training session, the reward-associated spout location was switched after a

628   bunch of cumulative rewarded trials were observed at each side. As rats reliably showed repetitive

629   rewarded choices rather than random choices, the number of reversal of reward-location increased.

630   Eventually, once the cumulative rewarded trial number reached 10 within each block, the reward-

631   associated spout position was reversed without any feedback like sensory or physical differences in

632   the task. Therefore, rats could not know the block reversal *a priori* without experiencing forthcoming

633   trials. From 13th and 17th sessions, the first and second delay periods were prolonged by extending

634   to 0.7-2.3 s and to 0.3-0.7 s, respectively. After each rat was trained at a training chamber for 19

635   days, they were afterwards habituated to another task chamber, where electrophysiological

636   recording experiments were conducted, through two more training sessions.

637

638   ***Electrophysiological recordings.*** Two daily recording experiments were conducted. Multi-neuron

35

639   activity was simultaneously recorded from MFC and DS of left hemisphere with two 32-channels

640   silicon probes consisting of four shanks (0.4 mm shank separation), on which tetrode-like electrode

641   sites were spaced vertically by 0.5 mm (A4x2-tet-7/5mm-500-400-312, NeuroNexus Technologies).

642   Each probe was connected to a custom-made headstage installed on either one of two fine

643   micromanipulators (1760-61; David Kopf Instruments) mounted on a stereotaxic frame (SR-8N,

644   Narishige). A silicon probe was penetrated vertically (depth from pia mater: 1.2 mm) into MFC (+2.4-

645   4.2 mm to Bregma, 1.0-1.4 mm to midline), and the shanks were aligned along midline. Another

646   silicon probe angled posteriorly by 6 deg was inserted into DS through a cranial window (+0.6-1.0

647   mm to Bregma, 2.1-3.7 mm to midline, 4.0 mm traveling distance), and the shanks were aligned

648   along coronal suture. Multiunit signals were amplified by the headstages before being fed into main

649   amplifiers (Nihon Koden, 2000 gain) with a band-pass filter (0.5 Hz to 10 kHz). At the second

650   recording session, juxtacellular activity was recorded from the MFC of left hemisphere. A glass

651   microelectrode was prepared by a laser puller (P-2000; Sutter Instrument) and filled with 2-3%

652   Neurobiotin (Vector Laboratories) dissolved in 0.5 M potassium chloride (9-19 MΩ). The electrode

653   was inserted into MFC through the cranial hole for MFC silicon probe with a stereotaxic hydraulic

654   micromanipulator (SM-25C, Narishige). Juxtacellular activity was amplified (final gain 1000) with two

655   amplifiers (IR-283, Cygnus Technologies; EX4-400, Dagan) and filtered (0.3-10 kHz). All neural data

656   was sampled at 20 kHz with two hard-disc recorders (LX-120, TEAC), together with time of task

657   events and licking each spout (left and right). After juxtacellular recording, we tried to electroporate

36

658   Neurobiotin into the recorded cell with positive current injection (2-14 nA, duration of 0.5s at 1 Hz

659   interval, for 5-15 mins) in order to visualize the recorded cell *post hoc* and to verify the recording

660   location.

661

662   ***Inactivation of the MFC.*** Of 34 rats, 15 reached a performance criterion (>75%) at the first recording

663   session. Of the 15 rats, 10 and 5 were tested without and with injection of GABAa agonist muscimol

664   into bilateral MFC at the second recording session, respectively. For the muscimol group, the rats

665   were placed in the body restrainer and their heads were fixed on the stereotaxic frame. A

666   microinjection syringe (Hamilton) filled with 0.1% muscimol (muscimol hydrobromide, Sigma-

667   Aldrich) dissolved in 0.1M phosphate-buffered saline [55] was set on a microsyringe pump (Legato

668   130, KD Scientific) which was installed on the micromanipulator (1760-61; David Kopf Instruments).

669   An injection needle (25 or 31 gauge) was vertically inserted into the MFC (+3.0 mm to Bregma, 1.0

670   mm to midline, -1.5 mm from pia mater). Muscimol solution was injected via the microsyringe pump

671   at 0.2 µl/min by totally 0.5 ul in each hemisphere. After injections were completed, the needle was

672   left for 5 minutes to allow for diffusion and then slowly retracted. Then, a silicon probe was inserted

673   into DS of left hemisphere as described above. Then, the rats were tested the behavioral task 1 hour

674   after end of muscimol infusion. For control group, multi-neuron and juxtacellular recordings were

675   conducted.

676

677   ***Histology.***   Animals were deeply anaesthetized with Urethane (2-3 g/kg, i.p.) and then perfused

678   intracardially with 0.9% chilled saline followed by 4% paraformaldehyde (PFA) dissolved in 0.1 M

679   phosphate buffer (PB). The fixed brain was stored in 4% PFA overnight and then stored in 30%

680   sucrose solution in 0.1 M PB over 2 weeks. Postfixed brains were frozen and coronally sliced into 50-

681   μm thick serial sections with a microtome Cryostat (HM500OM, Microm). The brain sections were

682   stored in 0.1 M PB at 4°C overnight. The brain sections were subject for immunostaining to detect

683   FG and Neurobiotin (Nb). For fluorescent visualizations of FG labelled neurons and Nb-loaded

684   neuron, the brain sections were incubated with a rabbit antibody of FG (AB153, 1:3000 dilution,

685   Millipore) at 4°C overnight, followed by incubation with a goat anti-rabbit antibody of FG conjugated

686   with Alexa-594 (A11012, 1:500 dilution, Invitrogen) together with antibody Strept Avidine Alexa-488

687   (A11012, 1:250 dilution, Invitrogen) of Nb over 2 hours. The slices were washed with PB and mount

688   on a slide glass and dried in a shaded box overnight. Fluorescent images were imaged with a

689   fluorescence microscopy (Olympus, AX70) in order to check if FG labeled neurons were observed

690   around the silicon probe recording locations in MFC and near the FG injection site in DS as well as if

691   the Nb-loaded neurons were observed in MFC. After the fluorescent imaging, the brain sections

692   were re-stained the Nb-loaded neuron using the avidin-biotin-horseradish peroxidase complex

693   (Vectastain Elite ABC; Vector Laboratories, 1:200 dilution) with diaminobenzidine and nickel as

694   demonstrated previously [23,54]. Finally, the slices were counterstained with Neural Red Nissl, and

695   observed with a microscopy to check the tracks (scars) due to probe insertion. We judged the

38

696   recording locations in MFC and DS and the AP coordinate of brain sections according to the rat brain

697   atlas [56].

698

699   ***Data analysis.***   All of behavioral and neuronal data were analyzed by custom-written MATLAB

700   scripts (The MathWorks, Inc.). We analyzed behavioral data on 20 rats performing the task at high

701   reward acquisition probability (>0.75) through 30 recording sessions.

702

703   *Logistic regression analysis.* We estimated effects of past outcome and past choice on current choice

704   (repeat or switch) by the following regression model [14].

705
$$\log\left(\frac{Pr}{Ps}\right) = \sum_{k=1}^{10}\beta_{Rk}R(i-k) + \sum_{k=1}^{10}\beta_{Ck}\left(C_L(i-k) - C_R(i-k)\right) + \beta_0$$

706   where p_r (or p_s) is the probability of repeat choice (or switch choice) in the ith trial. The variables R(i)

707   and C_L(i) (or C_R(i)) indicate presence of reward delivery (0 or 1) and left (or right) choice (0 or 1) in

708   the ith trial, respectively. The coefficient β_{Rk} and β_{Ck} represent the effect of past rewards and choices,

709   respectively. β_0 is an intercept. These coefficients were calculated at each session. We checked if the

710   coefficient was significantly different from zero by t-test with Bonferroni correction (Fig. 1d).

711

712   *Spike sorting, clustering, and refining.* Spike event from multiunit (or juxtacellular) activity was

713   isolated by a custom-made semi-automatic spike-sorting program EToS [12 (or 5) feature dimensions

714   for 4 (or 1) channels; high-pass filter, 300 Hz; time-resolution, 20 kHz; spike-detection interval, >0.5

715   ms) [57,58]. The sorted spike clusters were combined, divided, and discarded manually to refine

716   single-neuron clusters by Klusters [59]. To avoid overlapping of detection of same units recorded

717   among spatially distinct tetrodes, we checked cross-correlations of spike times among isolated units

718   across all of tetrodes. If there was a high correlation peak only at zero time between a pair of units,

719   one of the units was excluded from further analyses because those spikes which originated from the

720   same neuron were presumably recorded through different tetrodes.

721   For comparison of neural activity between MFC and DS, we analyzed behavioral and

722   electrophysiological data (isolated cells from multi-neuron activity) in 12 sessions from 10 rats:

723   experiment-id (reward acquisition probability, the number of well isolated cells), R982-r1 (p = 0.828,

724   MFC/DS = 45/43), R983-r1 (p = 0.837, MFC/DS = 35/55), R983-r2 (p = 0.834, MFC/DS = 58/48), R985-

725   r1 (p = 0.853, MFC/DS = 30/60), R985-r2 (p = 0.867, MFC/DS = 66/26), R986-r1 (p = 0.813, MFC/DS

726   = 16/20), R991-r1 (p = 0.806, MFC/DS = 46/31), R1000-r1 (p = 0.784, MFC/DS = 12/32), R1004-r1 (p

727   = 0.792, MFC/DS = 20/51), R1005-r1 (p = 0.794, MFC/DS = 65/64), R1009-r1 (p = 0.774, MFC/DS =

728   35/33), R1012-r1 (p = 0.755, MFC/DS = 40/26). To examine effects of muscimol injection in MFC on

729   neuronal activation of DS cells, we analyzed data from 130 and 77 DS cells of the 3 rats in muscimol

730   group at the first and second recording sessions, respectively.

731

732   *Event-related neuronal activity*. Task-event-related neuronal activity was examined on well isolated

733   units. To make peri-event time histograms (PETHs), we calculated mean and SEM of instantaneous

40

734 firing rate (FR) in a 20-ms bin around task events (Start cue onset, Go tone onset, Choice response,

735 and Reward delivery) and the PETHs were smoothed with a Gauss filter (SD = 40 ms). To compare

736 firing pattern across neurons and recording sessions, the PETHs were transformed the FR into z-

737 score by using the mean and SD of control activity, which were FRs in 200-ms at pseudorandom

738 times (1000 time points) in each recording session. Then, the z-scores were normalized by the

739 absolute peak value (Fig. 3e).

740

741 *Neuronal selectivity for choice and outcome*. Statistical significance of neuronal modulation by

742 choice and outcome was analyzed by a two-way ANOVA with choice (left and right) and outcome

743 (reward and no-reward) factors ($p < 0.05$) for each FR in 0.2-s wide sliding window by 20 ms

744 increment around choice response (±3 s) (Fig. 3f). To quantify the preference of selectivity for choice

745 and outcome, we used a receiver-operating characteristic (ROC) analysis for neurons showing the

746 statistical significance by two-way ANOVA at each window [60]. The proportion of trials for a

747 condition (left choice or rewarded trials) was plotted against that of trials for another condition,

748 (right choice or unrewarded) while changing a criterion for the FR. If the area under the plotted ROC

749 curve (AROC) was 0.5 (0 or 1), the two conditions are indistinguishable (or completely different,

750 respectively). If AROC was larger (smaller) than 0.5, FR is larger in right choice (left choice) or

751 unrewarded outcome (rewarded outcome) than under the opposite condition. To compare the

752 selectivity for choice and outcome modulated activity between MFC and DS, the AROC was

41

753 transformed as a selectivity index. Selectivity index = |AROC − 0.5| + 0.5 (Fig. 3g).

754

755 *Analysis of neural trajectory separation.* All analyses for population activity were separately

756 performed on data sampled at individual recording session. Fisher's linear discriminant [35] was

757 used to find the degree of discrimination between neural trajectories classified into two conditions

758 (e.g., Left and Right choices). To define a Ch-hyperplane, we constructed the distributions of the

759 corresponding *r*(*t*), which is *N*-dimensional population FR vector (N corresponds to the number of

760 population of neurons), separately for Left and Right choice trials in each 0.2-s sliding window by 50

761 ms increment time around choice response (±3 s). Our task is to find a hyperplane, or equivalently

762 the normal vector **w** of this hyperplane, that best divides the two distributions projected onto the

763 direction of **w**. If the two distributions have the means and covariance matrices $\mathbf{m}_1$, Σ_1 and $\mathbf{m}_2$, Σ_2,

764 respectively, we can obtain **w** by maximizing the ratio of the between-class variance to the within-

765 class variance,

766 $$S = (\mathbf{w}^T\mathbf{m}_1 - \mathbf{w}^T\mathbf{m}_2)^2 / \mathbf{w}^T(\Sigma_1 + \Sigma_2)\mathbf{w},$$

767 where $\mathbf{w}^T\mathbf{m}_1$ and $\mathbf{w}^T\mathbf{m}_2$ are the means of the two projected distributions and $\mathbf{w}^T\Sigma_1\mathbf{w}$ and $\mathbf{w}^T\Sigma_2\mathbf{w}$ are

768 their variances. We can show that *S* is maximized if $\mathbf{w} \propto (\Sigma_1 + \Sigma_2)^{-1}(\mathbf{m}_2 - \mathbf{m}_1)$. The coefficient of

769 proportion can be determined by the normalization condition: $|\mathbf{w}| = 1$.

770 Thus, we can calculate the discrimination function between Left and Right choice trials as

771 $S = (r_L - r_R)^2 / (\sigma_L^2 + \sigma_R^2)$ from the means (r_L, r_R) and standard deviations (σ_L, σ_R) of the trial-by-

42

772   trial firing rates in these trials. The discrimination degree between the two clusters is defined as d'

773    $= S$.

774   The $r(t)$ in each 0.2-s wide window (0.2 s increment time) around choice response (±3 s) was

775   projected onto the axis orthogonal to the hyperplane by calculating inner product of $r(t)$ and the

776   normal vector $\mathbf{w}_{choice}$, which yielded maximal degree of discrimination (d'). For instance, population

777   rate at time t vectors was projected onto the axis orthogonal to "Ch-hyperplane", $v_t = < r(t), \mathbf{w}_{choice}>$.

778   In this case, we termed the projected rate vector "Ch-projected trajectory". We performed the same

779   procedure to find "Ot-hyperplane" ($\mathbf{w}_{outcome}$) by classifying trials into rewarded and unrewarded

780   conditions, and to obtain the projected rate vector "Ot-projected trajectory".

781

782   *Mutual information.* To quantify the difference in Ch-projected trajectory (or Ot-projected

783   trajectory), we calculated the mutual information [61] between choices (or outcomes) and the

784   projected population rate vectors v_t:

785    $$I(C;V_t) = H(C) - H(C|V_t) = -\sum_c p(c)\log p(c) + \left\langle \sum_c p(c|v_t)\log_2 p(c|v_t) \right\rangle_v,$$

786   where C and V_t denote the set of choices (or outcome) c and the set of projected population activity

787   v_t, respectively, and $p(c)$ is the choice probability (or outcome probability), $p(c|v_t)$ is the conditional

788   probability of choice (or outcome) c when v_t is observed at time t, and the parenthesis means an

789   averaging over the values of v_t. The mutual information was calculated at each time window (bin =

790   0.2 s) around choice response (±3 s), and was normalized by the total entropy of choice $H(C)$ (or total

43

791   entropy of outcome) to provide the percentage of information as demonstrated before [12].

792

793   *Pairwise correlation analysis of population activity*.   A Pearson correlation was performed

794   for Ch-projected trajectory and Ot-projected trajectory between MFC and DS at each time window

795   (bin = 0.2 s) around choice response (±3 s). Because the correlations are sensitive to the number of

796   trials, we randomly selected 431 trials, which was the minimum total number of trials among 9

797   sessions, and calculated correlation coefficient so that the correlation coefficient was calculated with

798   the same total number of trials sampled. We repeated this procedure 10 times to obtain mean

799   correlation coefficients (Fig. 4e and Fig. S4). To compare the correlations of trajectories between

800   rewarded and unrewarded trials, we calculated correlation coefficient by randomly selected 63 trials,

801   which was the minimum total number of unrewarded trials among 9 sessions, for each outcome

802   condition (only rewarded trials, only unrewarded trials, trials without distinction). We repeated this

803   procedure 10 times to obtain mean correlation coefficients (Fig. S5b).

804

805   *Cross-correlation analysis of spike times*. We calculated cross-correlograms (CCGs) under 3

806   conditions (left choice trials, right choice trials, and rewarded trials) for all MFC-DS cell-pairs. We

807   used spike times in a 5-s wide window centered on a specific time at which a maximum absolute

808   correlation of neural trajectories was yielded at Ch-hyperplane (or Ot-hyperplane) referred as

809   window Ch (or window Ot) (Fig. 4e and Fig. S4). As a control, we calculated task-event irrelevant

44

810   CCGs by randomly sampling X time points within the same recording session for the same cell-pair.

811   X corresponds to the number of trials used for calculation of task-event relevant CCGs under each

812   condition. For example, the CCG between MFC and DS cells was calculated by counting spikes of

813   MFC cell in a window ranging from -0.1 to +0.1 s relative to each spike time of DS cell (bin = 1 ms).

814   Spike synchrony was detected by comparing the peak value in CCGs within ±20 ms with the

815   mean+4SD of values in a baseline window (-100 to -80 ms and +80 to +100 ms) under each condition.

816   If the peak value was larger than this criterion, we judged that the neuron pair showed spike

817   synchrony. We did not assess the spike synchrony if the mean of baseline values was less than 1 or

818   the SD was 0 due to scarce spiking. We did not look into negative spike correlation. For each cell-pair

819   showing significant CCG peak values, we also calculated CCGs by shuffling trials between MFC and

820   DS cells (Fig. S6). To compare peak value of CCGs, each CCG was z-scored by the mean and SD of the

821   baseline values (Fig. S6 and 5b).