1   **Title: Distinct Oscillation Dynamics Selectively Coordinate Excitatory and Inhibitory Neurons in**

2   **Prefrontal Cortex during Sensory Discrimination**

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9   **Abstract**

10   The prefrontal cortex (PFC) is crucial for many cognitive functions. PFC individual neuron activity and

11   ensemble oscillation dynamics have been linked to unique aspects of behavior. However, it remains

12   largely unclear how different neuron types relate to oscillation features. To understand how excitatory

13   and inhibitory neurons in PFC are coordinated by distinct oscillation signatures, we designed a 3-choice

14   auditory discrimination task and used tetrode devices to examine individual PFC neuron activity and

15   ensemble LFP oscillations in task performing mice. We found that PFC neurons and ensemble LFP

16   oscillations exhibited complex sensory evoked responses that are context and task-progression

17   dependent. While both excitatory and inhibitory neurons were transiently modulated at different

18   phases of the task, inhibitory neurons were increasingly recruited as trial progressed compared to

19   excitatory neurons. Inhibitory neurons in general showed higher spike-field coherence with LFP

20   oscillations than excitatory neurons throughout the task period, first at higher frequencies at the

21   beginning of the task, and then transitioned to lower frequencies in the middle of the task that

22   sustained beyond task completion. Together, our results demonstrate that excitatory and inhibitory

23   neurons selectively engage distinct oscillation dynamics during sensory discrimination in mice.

24

Introduction

26   The prefrontal cortex (PFC) is known to be critically involved in decision making, and damage to the PFC

27   leads to deficits in various cognitive performance (Miller, 2000, Miller and Cohen, 2001, Dalley et al.,

28   2004, Rossi et al., 2007, Gregoriou et al., 2014, Duan et al., 2015, Hanks and Summerfield, 2017). Goal-

29   orientated decision making involves detecting sensory stimuli, applying learned rules, and executing an

30   outcome. PFC activities—both individual neurons' spiking patterns and population local field potential

31   (LFP) oscillation dynamics—have been correlated with many aspects of decision making process, such as

32   attention, sensory processing, rule utilization, working memory, task progression tracking, and result

33   anticipation.

34   Recent studies using optogenetics to manipulate the activity of genetically defined cell types have

35   showed that different PFC cell types are associated with distinct aspects of cognitive tasks (Hussar and

36   Pasternak, 2009, Kvitsiani et al., 2013, Sparta et al., 2014, Murray et al., 2015, Kim et al., 2016a). In

37   general, excitatory neurons participate in various aspects of a task, whereas different subtypes of

38   inhibitory neurons seem to be preferentially recruited during different stages of a task. Calcium imaging

39   of different PFC neuron subtypes in a go/no-go task further revealed that excitatory neurons exhibit

40   heterogeneous responses, while inhibitory neuron activity tends to be more correlated within their

41   subtypes (Pinto and Dan, 2015, Kim et al., 2016a, Kamigaki and Dan, 2017) , presumably due to gap

42   junction coupling (Gibson et al., 1999). Parvalbumin-expressing (PV) neurons were shown to respond to

43   various aspect of a task (Pinto and Dan, 2015, Lagler et al., 2016), especially reward (Kvitsiani et al.,

44   2013, Sparta et al., 2014), whereas somatostatin-expressing (SST) neurons tend to be more selective and

45   respond primarily to sensory stimuli and motor activity (Kvitsiani et al., 2013, Pinto and Dan, 2015).

46   LFP oscillations in the PFC have also been associated with distinct cognitive functions, and are

47   coordinated with LFP oscillations in other cortical and subcortical areas. For example, theta oscillations

48   (~5-10Hz), closely related to working memory (Liebe et al., 2012), can coordinate long range connections

49   between the PFC and the hippocampus (Dejean et al., 2016). Beta oscillations (~15-30Hz), largely

50   associated with status-quo and rule application (Buschman et al., 2012), are often synchronized over

51   shorter distances, within the PFC, or between the PFC and other cortical areas. Higher frequency

52   oscillations, such as gamma oscillations (~35-100Hz) are found to be involved in encoding/decoding of

53   working memory (Lundqvist et al., 2016) and attention (Gregoriou et al., 2009).

54   It has been suggested that LFP oscillations may organize neurons into functional ensembles (Watrous et

55   al., 2015, Helfrich and Knight, 2016). For example, spike-field coherence between the PFC and the

56   sensory cortex was increased during covert attention (Gregoriou et al., 2009), and spike-field coherence

57   within the sensory cortex was found to be correlated with behavioral performance (Womelsdorf et al.,

58   2006). Recent optogenetic experiments showed that abnormal activity of inhibitory neurons can disrupt

59   gamma oscillations and lead to cognitive deficiency, highlighting the power of transgenic mouse models

60   in cell type-specific investigation of PFC neural networks (Cho et al., 2015). While much of our

61   knowledge of the PFC has been obtained in humans, monkeys, and rats, much less is known about the

62   PFC in mice, a model organism with advanced genetic tools that allow detailed examination of the

63   functional significance of distinct neuron subtypes. Here we recorded both spikes and LFPs

64   simultaneously in the PFC while mice were freely moving and performing a 3-choice auditory

65   discrimination task. With tetrode devices, we were able to identify well-isolated individual PFC neurons

66   and distinguish excitatory neurons from inhibitory neurons using waveform features. We found that

67   mouse PFC neurons and LFP oscillations were modulated during the sensory discrimination task, as

68   observed in other animal models and humans. In addition, spike-field coherence was modulated across

69   multiple frequency bands, in a behavioral stage- and cell type-dependent manner. Inhibitory neurons

70   overall showed higher coherence with LFPs compared to excitatory neurons, which were significant first

71   at gamma frequencies at the beginning of the task, then at theta and alpha frequency as trial

72   progressed, and subsequently transitioned back to gamma frequencies towards trial completion.

73

Results

A large fraction of Mouse PFC neurons exhibited task related spiking activities

76   To understand how distinct cell types are recruited during sensory discrimination, we designed a 3-

77   choice auditory discrimination task, which required freely moving mice to associate a specific auditory

78   stimulus with a predefined "reward" location (Figure 1A). During the task, mice self-initiated each trial

79   by stepping into the "initiation" location to trigger one of the three auditory cues (10 kHz sine wave, 25

80   click/second and 100 click/second), which was presented to the mice throughout the trial. After

81   initiation, mice were given 5 seconds to reach the "reward" location on the other end of the arena to

82   receive a reward (correct trial). If mice reached the other two incorrect "reward" locations (incorrect

83   trial) or failed to reach any "reward" location within 5 seconds (incomplete trial, excluded in this study),

84   they were presented with a 5-second timeout, with bright light illuminating all "reward" locations.

85   Throughout the recording period, all mice regained and maintained a performance of >60% correct rate

86   over the recording period (Figure 1B). Most mice were able to complete each trial within 3 seconds, with

87   an averaged reaction time of 1.31±0.57 seconds across all completed trials in 6 mice (Figure 1C).

88   We performed a total of 251 recording sessions in 6 mice, in PFC bilaterally, and identified 711 single

89   neurons based on simultaneously recorded waveforms from four closely positioned tetrode wires (Fig.

90   1D). Among these 711 neurons, 552 (78%) showed significant changes in their firing rates during the task

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91   when compared to the inter-trial interval (ITI) ($p<0.05$, Wilcoxon rank sum test). We excluded 159 (22%)

92   neurons that were not modulated by the task from further analysis. Of the 552 task-relevant neurons,

93   the width of their spike waveforms followed a bi-normal distribution (Fig. 1E) with one peak centered at

94   0.45 ms, consistent with the general observation of putative excitatory neurons (Figure 1 D1), and

95   another peak centered at 0.25 ms, consistent with the general observation of putative inhibitory

96   neurons (Figure 1 D2) (Cardin et al., 2007, Mitchell et al., 2007, Han et al., 2009, Lee et al., 2017). Thus,

97   we further divided these neurons into putative excitatory neurons and putative inhibitory neurons

98   based on their spiking width, using 0.4 ms as a threshold. We identified 441 excitatory neurons (80%)

99   and 111 inhibitory neurons (20%). The recorded excitatory neurons exhibited a lower firing rate

100   comparing to the inhibitory neurons (excitatory: 1.66±1.84 Hz, inhibitory: 2.62±2.68 Hz; $p<0.05$,

101   Wilcoxon Ranksum test).

102

103   **Excitatory neurons and inhibitory neurons were preferentially modulated at different phases of the**

104   **task**

105   We first examined the timing of PFC spiking relative to the phase of a trial. When aligned to trial start,

106   some neurons showed an immediate increase in firing rate centered at trial start upon sound onset

107   (Figure 2A), some exhibited a delayed increase in firing in the middle of the trial (Figure 2B), and some

108   increased firing at the end of the trial (Figure 2C). Given the variation in the time to complete each trial,

109   we normalized the firing rate to task progression to track neural responses throughout the task. Indeed,

110   firing rate were task progression-dependent, with increase in firing rates at different stages of the task

111   (Figures 2A-C).

112   To compare responses of excitatory and inhibitory neurons, we first calculated the firing rates of each

113   individual neuron, and then sorted them according to the timing when their firing rates peaked. As a

114   population, we found that both excitatory and inhibitory neurons were transiently modulated at

115   different phases of the task (Figure 2D). Excitatory neurons tended to be modulated at the early stage of

116   the task, whereas most inhibitory neurons were modulated towards the end of the task (Figure 3E;

117     $p<0.01$, χ^2 test). These results suggest that discrete PFC neuron subgroups are related to different

118   aspects of sensory discrimination, and that excitatory and inhibitory neurons are recruited at different

119   phases of the task.

120   The diverse temporal profiles, and the existence of delayed and transient activity throughout the task

121   suggested that PFC responses were relevant to the discrimination task; however, an alternative

122   explanation could be that they were driven primarily bottom up by auditory stimuli presented

123   throughout the trial. To rule out the possibility that the PFC was solely driven by bottom up auditory

124   stimuli, we designed a "passive listening" block, during which mice received the same auditory stimuli

125   but without performing the discrimination task. Mice were placed in the same arena with a floor that

126   covered the sensors at the "initiation" and "reward" locations. The "passive listening" block contained

127   200-250 trials. During each trial, one of the same three auditory stimuli was randomly presented for 1.5-

128   3 seconds, followed by a 1.5-3 seconds long inter-trial interval (ITI). To compare the activity of the same

129   neuron during discrimination task versus during passive listening, the "passive listening" block was

130   performed either before or after the "discrimination task" in the same recording session.

131   Of the 552 task-modulated neurons, 422 neurons were also tested with the "passive listening"

132   condition. Of these 422 neurons, only 12 (3%) neurons showed significant differences in firing rate when

133   auditory stimuli were presented during passive listening ($p<0.05$, Wilcoxon rank sum test). Neurons that

134   were modulated during the task showed largely no response to auditory stimuli during passive listening

135   (representative neurons shown in Figure 2). As a population, PFC neurons failed to produce any

136   responses during the "passive listening" condition, confirming that PFC neurons exhibit task-specific

137   modulation, rather than responding to bottom-up auditory stimuli alone (Figure 2D). While PFC neurons

138   are known to respond to auditory stimuli during passive conditions (Romanski and Goldman-Rakic, 2002,

139   Miller et al., 2015), in our study, using the three auditory stimuli at ~70dB over an ambient environment

140   of ~60dB, was not sufficient to evoke significant passive responses in the PFC.

141   We further examined whether PFC neurons responded differently according to task outcomes. We

142   calculated the firing rates during correct and incorrect trials, and sorted the excitatory and inhibitory

143   neurons based on their peak firing rates during correct trials. We found that both excitatory and

144   inhibitory neurons showed clear sequential activity during the correct trials (Figure S1A), but not during

145   the incorrect trials (Figure S1B), confirming that both excitatory and inhibitory neurons are modulated

146   by task outcomes.

147

148   **PFC neurons exhibited complex activation patterns during three stimuli discrimination, and inhibitory**

149   **neurons were increasingly recruited as trial progressed.**

150   While PFC neurons are broadly tuned to auditory stimuli, they are known to discriminate sensory

151   stimuli, such as categorizing sensory inputs (Freedman et al., 2001, Miller and Cohen, 2001, Lee et al.,

152   2009). To examine whether mouse PFC neurons are selectively modulated by different auditory cues or

153   can discriminate different cues, we compared their responses to different auditory cues. We observed

154   that PFC neurons exhibited highly heterogeneous responses when different cues were presented (Figure

155   3A and B). Some PFC neurons responded to only one auditory cue but not the other two (Figure 3A),

156   whereas some responded to all cues but with distinct time course, i.e. small and transient response to

157   one cue and robust and long lasting response to the other two (Figure 3B). This demonstrates that while

158   some PFC neurons responded only to one specific cue, others may show complex and distinct response

159   to different cues. It further suggests that when animals were required to distinguish more than two

160   cues, they were able to utilize the combination of neurons with various response amplitude and

161   temporal kinetics, which may effectively expand their coding capacity.

162   To quantify the selectivity of each PFC neuron between different auditory cues at different phase of the

163   task, we calculated the p-value (One-way ANOVA) between the firing rates during different auditory

164   cues throughout trial progression. We found that many PFC neurons exhibited differential responses to

165   the presentation of different auditory cues (Figure 3C). As a population, we found that the fraction of

166   neurons that discriminated sound identity increased as the trial progressed (Figure 3D).

167   While both excitatory and inhibitory neurons showed stronger discrimination between sounds as trials

168   progressed, a larger fraction of the inhibitory population were recruited toward the end of the task

169   compared to the excitatory neuron population (Figure 3D, excitatory: dark blue, inhibitory: dark red,

170   χ^2 test, $p<0.01$). In contrast, during passive listening, these same PFC neurons failed to discriminate

171   sound identity (Figure 3C), and the percentage of modulated neurons stayed low and constant

172   throughout the auditory stimuli (Figure 3D). Together, these results demonstrate that PFC firing rates

173   were modulated by auditory stimuli in a context-dependent manner. While both excitatory and

174   inhibitory neurons showed greater discrimination ability as trials progressed, inhibitory neurons tended

175   to be significantly more engaged towards trial completion.

176

177   **PFC exhibits context- and outcome-dependent changes in LFP oscillation power**

178   LFP oscillations have been broadly associated with many cognitive functions. To examine changes of LFP

179   oscillations in the mouse PFC during the task, we analyzed LFP oscillations during both the "auditory

180   discrimination" block and the "passive listening" block. We observed that LFP power was modulated at

181   trial start and trial end during the discrimination task, but not during passive listening (Figures 4A1 and

182   4A2). More specifically, during the discrimination task, oscillation power first decreased across multiple

8

183   frequency bands upon trial initiation when compared to the powers before trial onset, then recovered in

184   the middle of the trial, which subsequently decreased again at the trial end (Figures 4A1, before: blue,

185   after: red, window size: 500 ms). Conversely, during passive listening, LFP powers remained constant

186   (Figure 4A2, before: blue, after: red, window size: 500 ms).

187   To further examine the changes in power across mice, we calculated the z-score of LFP powers for each

188   trial (2 second window centered at either trial start or trial end), and averaged across all trials for each

189   animal. We then calculated the power for five frequency bands for each animal, alpha (5-8 Hz), theta (9-

190   14 Hz), beta (15-30 Hz), low-gamma (30-50 Hz), and high-gamma (70-100 Hz), and averaged across all

191   animals. Upon trial initiation, oscillation powers transiently decreased across all frequency bands from

192   alpha to high-gamma (Figure 4C and E). Interestingly, for beta and low-gamma frequencies, we observed

193   a brief rise in power before trial-start, which was not present in theta, alpha, or high-gamma

194   frequencies. After the initial decrease, oscillation powers increased in the middle of the trial, and then

195   decreased again towards the end of the trial at all frequencies (Figure 4D and F). In contrast, during the

196   passive listening block, oscillation power largely stayed constant throughout the trial and progressed

197   into the ITI. Closer examination revealed a small but significant increase across all frequencies upon cue

198   presentation (Figure 4C and E), which is in sharp contrast to the observed decrease in oscillation powers

199   during the discrimination task. At the end of the cue presentation period during passive listening,

200   oscillation power decreased upon the termination of the auditory cue (Figure 4D and F). Together, these

201   results demonstrate that PFC oscillatory activity is context and task progression dependent. The same

202   auditory cues can result in broad reduction in oscillation powers during sensory discrimination, or slight

203   increase during passive listening. Such observations highlight the correlation of dynamic changes in PFC

204   oscillation patterns during the sensory discrimination task described here, consistent with that generally

205   observed in other studies in other animal models.

206   We then examined whether LFP oscillations were differently modulated by task outcome. LFP oscillation

207   power was modulated strongly when aligned to trial start, for both correct and incorrect trials (Figure 5

208   A1). However, when aligned to trial end, correct trials showed more dynamics than incorrect trials

209   (Figure 5 A2). We further examined the changes in various frequency bands across all mice. At the start

210   of a trial, oscillation powers decreased across all frequencies compared to that before trial-start during

211   both the correct and the incorrect trials (Figure 5B). The fact that oscillations at higher frequencies (beta

212   and gamma) were more suppressed in correct trials highlight the relevance of beta and gamma

213   oscillations in mediating PFC related behaviors. The suppression of LFP powers, along with the selective

214   increase in many individual PFC neurons' firing rate, suggests that changes in LFPs are mainly due to the

215   synaptic inputs to the PFC, rather than PFC spiking alone.

216   To capture the difference in the oscillation dynamics between correct and incorrect trials, we calculated

217   the average power for 500ms before versus after trial-start, and found that the drop in LFP power was

218   larger in correct trials than in incorrect trials, except at alpha frequency (Figure 5D, N=5 mice, paired t-

219   test, *: $p<0.05$). When aligned to trial-end, oscillation powers diverged between the correct and the

220   incorrect trials (Figure 5C). For correct trials, oscillation powers continued to decrease and remained low

221   over a prolonged period into the ITI, across all frequencies (Figure 5C, black line). For incorrect trials,

222   oscillation powers first decreased for a couple hundred milliseconds and then rose across all frequency

223   bands (Figure 5C, gray line). As a result, the power changes at the trial end were significantly different

224   between the correct and the incorrect trials (Figure 5D, N=5 mice, paired t-test, *: $p<0.05$). The

225   divergence of LFP powers at trial-end between correct and incorrect trials within a couple hundred

226   milliseconds is interesting. However, we cannot rule out the possibility that this change reflects a

227   difference in animal behavior, since in correct trials, animals retrieved a water reward, whereas in

228   incorrect trials, animals experienced a 5-second timeout. Together, these results demonstrate that LFP

229   powers were in general decreased when animals started the task regardless of trial outcome, but the

230   changes were great at trial-start during correct trials.

231

232   **Inhibitory neurons exhibit higher spike-field coherence than excitatory neurons during distinct phases**

233   **of auditory discrimination**

234   To investigate the relationship between PFC spiking patterns and LFPs, we calculated spike-field

235   coherence (SFC) between individual neurons and the LFPs recorded from the adjacent electrode within

236   the ipsilateral hemisphere. We first examined whether the SFCs were selectively modulated by contexts.

237   Among the 552 recorded neurons, 422 were from the recording sessions containing both discrimination

238   blocks and the passive listening blocks. When aligned to trial start, SFCs at theta frequencies during the

239   discrimination task exhibited a transient increase only at the beginning of the trial when compared to

240   those during passive listening, which returned to approximately the same level in the middle of the trial

241   (Figure 6A, left). SFCs then increased again toward trial end (Figure 6A, right). After the end of the trial,

242   SFC showed no difference between the discrimination blocks and the passive listening blocks. Similar

243   trends were observed for SFCs at alpha frequencies (Figure 6B). Interestingly, for beta frequencies, we

244   observed a lower SFC during discrimination, but only in the middle of the trial (Figure 8C). For both low

245   and high gamma frequencies, SFCs were lower during discrimination compared to passive listening at

246   the trial start, which then reversed towards the trial end (Figure 6D and E).

247   Given that the pattern of LFP power changes across multiple frequencies was similar at trial-start (Figure

248   4A), the change in SFC at theta frequencies reflect stronger coupling of PFC spiking and theta

249   oscillations, likely coordinated by inputs to the PFC. As trials progressed, SFCs became weaker at higher

250   gamma frequencies, but stronger at lower theta to beta frequencies, and then at the end of the trial,

251   SFCs rose across all frequencies except beta. Together, these results demonstrate wide spread changes

252   in SFC across all frequencies from alpha to high gamma. Similar to spike activity, the modulation of SFC

253   was also context-dependent, which further suggest the difference in network states when animals were

254   performing the task. Our results in general are in agreement with the idea that theta oscillations provide

255   long-range connections between different brain regions, and gamma oscillations predominately parallel

256   local spiking activities.

257   Although many studies have demonstrated that spikes and LFP oscillations are related to behavior, the

258   details about how excitatory versus inhibitory neurons interact with oscillations are largely unknown.

259   We compared SFCs between excitatory and inhibitory neurons. At trial start, inhibitory neurons

260   exhibited higher coherence with higher frequency oscillations (both low- and high-gamma) (Figure 7D

261   and E) than excitatory neurons, but not at lower frequencies (theta, alpha, and beta) (Figure 7A-C). As

262   trials progressed, SFCs increased in both neuron types, but inhibitory neurons showed increasingly

263   stronger coherence at lower frequencies (theta and alpha) that sustained beyond trial completion

264   (Figure 7A and B). At higher frequencies (beta and low-gamma), the divergence of SFC between

265   excitatory and inhibitory neurons only occurred after the trial end, where inhibitory neurons showed

266   stronger coherence across almost all frequencies than excitatory neurons (Figure 7C and D).

267   Interestingly, excitatory neurons showed higher coherence only at high gamma frequency bands, and

268   only right before the end of the trial (Figure 7E). In summary, these results demonstrate that inhibitory

269   neurons showed a stronger coherence with LFPs than excitatory neurons, across multiple frequencies,

270   towards the end of the task. In contrast excitatory neurons only exhibit stronger coherence with the

271   higher frequency gamma oscillations at trial-end. These results provide support for the idea that

272   inhibitory neurons are critical in supporting higher beta and gamma frequency oscillations, whereas,

273   lower frequency theta oscillations may provide long range coordination between the PFC and other

274   areas.

275

276 **Discussion**

277 To understand the functional significance of excitatory versus inhibitory neurons and their relationships

278 with LFP oscillation patterns in sensory discrimination in mice PFC, we performed tetrode recordings in

279 mice performing a 3-choice auditory discrimination task. Using tetrodes, we distinguished excitatory

280 neurons and inhibitory neurons based on spike waveforms, and found that inhibitory neurons were

281 preferentially recruited at later stages, whereas excitatory neurons were most active at early stages of

282 the trial. Inhibitory neurons also showed stronger spike-field coherence than excitatory neurons with

283 LFP oscillations at high frequencies at task start, which switched to lower frequencies for the remaining

284 period of the task. While oscillation power increased before both the start and the end of the task, the

285 coherences between spiking activity and oscillations only rose at the end of the trial. Together, our

286 results demonstrate that excitatory neurons and inhibitory neurons were recruited in a context-

287 dependent manner at different phases of the task in mouse PFC, similar to the characteristic activity

288 patterns in other species. The fact that inhibitory neurons exhibited a higher degree of coherence across

289 all frequency bands is suggestive of inhibitory neural networks in supporting PFC oscillation dynamics,

290 which may play a crucial role in organizing cell assemblies within the PFC in a context-dependent

291 manner.

292 PFC neurons exhibit diverse response profiles during cognitive tasks, from task progression (Ma et al.,

293 2014), to different aspects or stages of a task (Asaad et al., 1998). PFC neurons increase their firing rates

294 when anticipating task-relevant sensory stimulation (Rodgers and DeWeese, 2014), responding to

295 sensory stimuli presentation (Ninokura et al., 2004, Russ et al., 2008a), maintaining working memory

296 (Rainer et al., 1998, Romo et al., 1999, Constantinidis et al., 2001, Kopec et al., 2015), predicting and/or

297 anticipating reward (Matsumoto et al., 2003, Toda et al., 2012), or outcome (Amiez et al., 2006, Russ et

298 al., 2008b, Hayden et al., 2011, Del Arco et al., 2017), which can linger into the inter-trial interval

13

299   (Marcos et al., 2016). We here show that mouse PFC neurons exhibit transient increases at specific

300   stages of the task during a 3-choice discrimination task, suggesting that different subgroups of PFC

301   neurons are recruited to process task-relevant information sequentially during our task.

302   PFC neurons have been shown to respond to task relevant auditory cues in many studies with a 2-choice

303   auditory discrimination task. In this study, using a 3-choice auditory discrimination task allows us to

304   examine more complex features of the PFC encoding ability. We found that, while a number of neurons

305   increased their firing rate to only one auditory cue, many responded to two sounds. To a certain extent,

306   these results suggest that PFC neurons can negatively respond to auditory cues of a certain identity. The

307   combination of both positive and negative responses could expand the PFC's coding capacity; while an

308   individual neuron could respond to multiple stimuli, ensemble of neurons could collectively represent or

309   code a specific stimulus. It is interesting to speculate how such complex identification coding arises in

310   the PFC, and how such coding scheme contributes to the discrimination process. Further experiments

311   are needed to examine the detailed network mechanisms.

312   Different types of PFC neurons have been shown to exhibit distinct task-related responses (Hussar and

313   Pasternak, 2009, 2012, Murray et al., 2015). Excitatory neurons increase their firing rates with less

314   variability upon sensory stimulation (Hussar and Pasternak, 2010), whereas inhibitory neurons are more

315   correlated with the later stage of a task, such as reward (Kvitsiani et al., 2013). Most recently, using

316   optogenetics, Sparta et al. demonstrated that selective activation of PV neurons facilitated the

317   extinction of cue-reward association (Sparta et al., 2014). We found that an increasing proportion of

318   neurons, both excitatory and inhibitory, were modulated by auditory cues as the task progressed,

319   suggesting gradual recruitment of the PFC neurons during sensory discrimination, consistent with the

320   idea that the PFC accumulates information about sensory identity and outcome decision (Freedman et

321   al., 2001). It is interesting that a higher fraction of inhibitory neurons was recruited towards the end of

322   the task. It is possible that inhibitory neurons were likely involved in associating task information with

323   task outcome.

324   Although mice were presented with the same auditory cues in both the "discrimination task" and

325   "passive-listening" blocks, the PFC was only modulated during the "discrimination task" block, indicating

326   that PFC neurons' activities are context-dependent. Such context-dependent modulation has been

327   shown when animals were exposed to different environments (Hyman et al., 2012), or were performing

328   tasks requiring different rules (White and Wise, 1999, Wallis et al., 2001, Stokes et al., 2013, Ma et al.,

329   2016, Siniscalchi et al., 2016). In our study, almost all modulated neurons lost their modulation during

330   the "passive-listening" blocks, suggesting a state difference when the animals were actively engaged in

331   the task utilizing sensory information versus when they were passively presented with sensory

332   information. Such state difference may involve many neuromodulators, such as acetylcholine and

333   dopamine that have been shown to mediate attentional states (Del Arco et al., 2017).

334   Over the years, LFP oscillations at specific frequencies have been related to different aspects of

335   behavioral tasks and states (Helfrich and Knight, 2016, Wimmer et al., 2016, Kamigaki and Dan, 2017),

336   and LFP synchrony within the PFC and between the PFC and other areas has been observed in many

337   tasks (Antzoulatos and Miller, 2016). In addition to single neuron responses in the PFC, we also observed

338   wide spread changes in LFP oscillation patterns across all frequencies from alpha to high gamma, in

339   general agreement with other studies. While beta oscillations have been associated with status quo and

340   movement, in our current task, we cannot dissociate movement and task outcome. Interestedly, we

341   observed that the power of oscillations reacted differently in response to task outcomes. We found that

342   beta power first increased before trial onset, and then decreased upon sensory stimulation followed

343   with a rebound, similar to previous reports where no movement was involved (Panagiotaropoulos et al.,

344   2013). The oscillation power showed much stronger dynamics in correct trials than in incorrect trials,

345   indicating the enhancement of oscillations may be crucial in successfully performing the task.

346   Spike-phase coherence at theta frequencies within the PFC and between the PFC and sensory areas

347   increases during the delay period of working memory tasks (Liebe et al., 2012), whereas spike-field

348   coherence at beta frequencies rose in monkey PFCs when monkeys were performing visual attention

349   tasks (Buschman et al., 2012), and between PFCs and parietal cortex when monkeys performing

350   category tasks (Antzoulatos and Miller, 2016). Moreover, phase-locking of pyramidal neurons to gamma

351   oscillations in the PFC occurs during attentional processes (Kim et al., 2016b) and spiking activity is

352   modulated by the phase of gamma oscillations in the visual cortex (Ni et al., 2016). Inhibitory neurons

353   have been implicated in supporting LFP oscillations in the PFC (Lodge et al., 2009, Kim et al., 2016b) and

354   other brain areas (Cardin et al., 2009, Miller et al., 2015). In our study, although the oscillation powers

355   increased prior to both trial-initiation and trial completion, only the latter is associated with an increase

356   in spike-field coherence, suggesting that the same oscillation frequencies may serve different functions

357   depending on the ongoing task. More interestingly, while changes in oscillations patterns are similar

358   across multiple frequency bands during the task, their interactions with spikes differ depending on the

359   LFP frequency bands and cell types. In general, inhibitory neurons showed stronger coherence than

360   excitatory neurons in a trial progression-dependent manner. These results further support the rationale

361   that inhibitory neurons play an important role in generating or supporting beta and gamma frequency

362   oscillations.

363

364   **Materials and methods**

365   **Three--choice auditory discrimination task**

366   <u>Behavior Arena</u>

367   The behavior arena (12 inch x 12 inch) was constructed with black plastic walls and a mesh floor. The

368   start location was located at one side of the arena (1 inch away from the wall), and three reward

16

369   locations were at the opposite side (1 inch away from the wall) (Figure 1). Each location was equipped

370   with an IR beam sensor and a white LED light on the floor. A speaker was located outside of the box, at

371   the reward side, and delivered a 70 db auditory cue when the animal initiated the task. The room had a

372   consistent background noise of approximately 60 db. LabVIEW (2012, National Instruments, Austin, TX)

373   scripts were used to control the sensors and LED lights in the behavior arena via a NiDAQ board (NI USB-

374   6259, National Instruments, Austin, TX) and recorded behavior events.

375   <u>Behavioral training</u>

376   All animal procedures were approved by the Boston University Institutional Animal Care and Use

377   Committee. Female C57BL/6 mice (Taconic, Hudson, NY), were water-restricted during behavioral

378   testing, and were closely monitored to ensure that they maintained at least 85% of their pre-experiment

379   body weight. Adult female mice (2-3 months old at the start of the experiments) were trained to

380   perform a 3-choice auditory discrimination task in following steps:

381   Step 1: Obtain water from reward port. At this step, only the middle reward port was accessible to the

382   animal. It delivered a water reward whenever an animal reached the reward location and triggered the

383   IR beam sensor in front of the water port.

384   Step 2: Detection of the first auditory cue. A white LED at the start location was illuminated to indicate

385   trial initiation. Mice learned through trial and error to initiate a trial by reaching the start location, which

386   triggered the IR beam sensor and the first auditory cue was presented. Mice were allowed to reach the

387   reward location to obtain the reward without any time limit.

388   Step 3: Detection of the second auditory cue. After the animal learned initiating the task and responding

389   to the first cue, we made the reward location associated with the second cue accessible, while blocking

390   the reward locations for the other two cues. At this step, when animals initiated a trial, only the second

17

391   cue was presented, and animals were required to reach the corresponding location for reward with no

392   time limit.

393   Step 4: Two-choice auditory discrimination task. At this step, the reward locations for both the first and

394   the second cues were accessible. When animals initiated the trial, one of two auditory cues would be

395   presented randomly, and mice were required to reach the corresponding reward location for reward

396   with no time limit. When animals reached the incorrect reward location, a 5 second timeout occurred,

397   indicated by white LED lights around the reward locations.

398   Step 5: Detection of the third auditory cue: After the animal learned the 2-choice discrimination task, we

399   repeated Step 3 to introduce the third auditory cue. At this step, only the third reward location was

400   accessible and the other two were blocked. When an animal initiated the trial, only the third auditory

401   cue was played, and the animal was required to reach the third reward location to complete the trial

402   and receive the reward with no time limit.

403   Step 6: Three-choice auditory discrimination task. All three reward ports were accessible. When animals

404   initiated the trial, one of the three auditory cues was presented, and mice were required to reach the

405   corresponding reward location to obtain reward. At this stage, a reaction time limit of 5 seconds was

406   introduced. Failure of reaching the reward location within 5 seconds would cause a timeout, indicated

407   by white LED lights around the reward locations. To obtain a balanced number of trials with each cue,

408   auditory cues were presented in a group of three, and within each group, each auditory cue was

409   presented once in a random order.

410   During training, each mouse was trained 20 minutes per day. Once well trained, defined as performing

411   over 60% correct rate per day over 3 consecutive training days and capable of completing a minimum of

412   100 trials per day, animals were provided free water access in their home cage for a week and then

413   underwent tetrode implantation. After tetrode implantation and recovery from surgery, animals were

18

414   briefly re-trained using procedures described in Steps 2-6 until their performance reached 60%, and

415   then recordings were performed.

416   **Electrophysiology**

417   Custom tetrode devices (16 channels) were assembled in house, which contained four tetrode bundles,

418   two bundles targeting each hemisphere. The four tetrode bundles were designed to target the PFC

419   bilaterally (AP: +1.8, ML: +0.2; AP: +2.2; ML +0.2; AP: 2.2; ML: -0.2; AP 2.2, ML: +0.2). A tetrode bundle

420   was made by twisting together 4 tetrode wires (Sandvik-Kanthal, Ahmerst, NY), and adjusting the

421   impedance to 0.5-1 MOhm with gold plating (SIFCO 5355, SIFCO ASC, Independence, Ohio). Tetrodes

422   were implanted with the center positioned at the midline (AP: 2, ML: 0), so that the tetrode bundles

423   targeted the PFC (AP: 2+/-0.2, ML: +/- 0.2). We advanced tetrode bundles gradually during the re-

424   training period, so that the tip of the tetrode bundles reached the PFC at the recording stage (AP: 1.0 –

425   1.9). All recordings were performed in freely-moving mice. During recording, the tetrode device was

426   connected to a commutator (ACO32, Tucker-Davis Technologies, Alachua, FL) to ensure free movement

427   in the behavior arena. Data was acquired with a Plexon OmniPlex system (Plexon Inc, Dallas, TX). Spike

428   waveforms and local field potentials were sampled at 40 kHz and at 1 kHz respectively. The Plexon

429   OmniPlex system also received time stamps from the NiDAQ board to record timing of behavioral

430   events.

431   Mice underwent one recording session per day. Each recording session constituted one "discrimination

432   task" block and one "passive listening" block in random order. Animals could move freely in the

433   behavioral arena during the entire recording session. The "discrimination task" block lasted about 20

434   minutes, and animals were allowed to initiate the task as many times as they desired. In general, mice

435   performed 100-200 trials within 20 minutes. During the "passive listening" block, mice were placed in

436   the same behavioral arena with a plastic floor positioned above all IR beam sensors and LEDs, so that

19

437   mice had no access to any sensors or water ports. The same three auditory cues (~70db) used in the

438   "discrimination task" were played in pseudo-random order for 200-250 trials. The durations of the

439   auditory cues were randomized from 1.5 to 3 seconds with random 1.5 - 3 seconds inter-trial intervals.

440   **Data analysis**

441   Spike: Spikes were sorted with Offline Sorter (Plexon Inc, Dallas, TX) and then imported into Matlab

442   (2014, MathWorks, Natick, MA) for further analysis. Spike width was defined as the duration between

443   the valley and the peak of a spike waveform. Due to the difference in lengths of each trial, to calculate

444   the firing rate throughout trial progression, we first normalized each trial based on its duration, so that

445   trial start and trial end were aligned at 0% and at 100% of trial progression, respectively. We then

446   calculated the firing rate from -20% to +120% of trial progression using a 1% moving window, and

447   smoothed the results by averaging each data point with its two adjacent data points. When presenting

448   the population data, we further normalized the firing rate between -20% to 120% of trial progression by

449   calculating the z-scores for each neuron with its own mean and standard deviation.

450   LFP: LFPs were imported into Matlab with the Matlab custom script provided by Plexon, and then

451   analyzed with the Chronux toolbox (chronux.org). The power spectrogram of each LFP trace was

452   calculated with mtspecgramc function (moving window size: 500 ms, moving window step: 5ms, tapers:

453   [3 5]) in Chronux.

454   In a few occasions, animal movement caused artifacts in our tetrode recording, such as when the

455   tetrode devices bumped the arena walls, which create large voltage deflections in our recordings. To

456   eliminate the impact of such movement artifact in our analysis, we identified these artifact periods as

457   having the 5% maximum amplitude, either positive or negative, of the whole recording session. Trials

458   that contained these artifact periods were excluded from all analysis involving LFPs.

459   The spectrogram examples shown in Fig. 5A were log-normalized (10*log(power/max)), with the

460   maximum power of either the whole recording session (Figure 5A), or of the examined time window (2

461   second, centered at either trial start or end) (Figures 5B and 7A, averaged across trials). To compare the

462   powers at specific frequency bands across mice, the powers within the examined time window (2

463   second, centered at either trial start or end) of each trial were first normalized by converting to their z-

464   scores and then averaged within a given frequency band to obtain the power of each trial. The powers

465   of all trials from the same animal were then averaged as the power of each animal. In our analysis, we

466   examined five frequency bands, and the ranges of each frequency bands were defined as follows: alpha

467   (5-8 Hz), theta (9-14 Hz), beta (15-30 Hz), low-gamma (30-50 Hz), and high-gamma (70-100 Hz).

468   Spike-field coherence was calculated with cohgramcpt function (moving window size: 500 ms, moving

469   window step: 5ms, tapers: [3 5]) in Chronux. For each trial, we first calculated the spike-field coherence

470   for each neuron with LFPs at a specific frequency range, and then averaged across trials.

471   **Statistical testing**

472   For spike rate modulation, we used one-way ANOVA to compare the firing rates at the same trial

473   progression period for different auditory cues. For LFP power spectrum, we used a paired t-test to

474   compare the power during the 500 ms before and after either trial start or trial end. For spike-field

475   coherence, we used a non-paired t-test to compare the coherence at the same trial progression period

476   between the "discrimination task" blocks and "passive listening" blocks. All analyses were performed in

477   Matlab. Details for each test are presented throughout the Results section.

478

479   **Data Availability**

21

480   The datasets generated during and/or analysed during the current study are available from the

481   corresponding author on reasonable request.

482

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630

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632   H.T and X.H. designed all experiments and wrote the paper. H.T. performed experiments and data

633   analysis. X.H. supervised the project.

634

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639

Competing Interests

641   The author(s) declare no competing interests.

642

643   **Figure Legends**

644   **Figure 1. Recording of excitatory and inhibitory neurons during a three-choice auditory discrimination**

645   **behavioral task.** (A) During the auditory discrimination task, mice self-initiated each trial by triggering a

646   motion detector at the "initiation" location. Upon trial-start, one of three auditory stimuli were

647   presented throughout the trial. On the other end of the arena, there were three reward locations, each

648   paired with a specific auditory stimulus. Mice were given 5 seconds to reach one of the three reward

649   locations. If mice reached the correctly paired reward location, a reward was provided. If mice failed to

650   reach the correct reward location, they experienced a 5 second timeout period, with a bright light

651   illuminating the arena. (B) The behavioral performance of mice during the first three recording sessions

652   (left) and the last three recording sessions (right). All mice had correct rates above random chance of

653   33% (N=6 mice). (C) The reaction time during the first three recording sessions (left) and last three

654   recording sessions (right). Each line represents mean±std of an individual mouse. (D) Representative

655   waveforms of excitatory (D1) and inhibitory (D2) neurons recorded with tetrode bundles. (E) Binomial

656   distribution of the width of spike waveforms, with a clear separation at 0.4 milliseconds between the

657   two peaks, which was used as a threshold to identify excitatory (blue) and inhibitory (red) neurons.

658   **Figure 2. Context and stage-dependent modulation of spiking activity.** (A, B and C) Raster plots from

659   two representative excitatory neurons (A and B), and one inhibitory neuron (C) during the discrimination

660   tasks (A1, B1, C1) and during passive listening (A2, B2, C2). Normalized firing rates throughout trial

661   progression across all trials in the discrimination task (A3-C3, blue lines) and the passive listening

662   condition (A3-C3, gray lines). PFC neurons showed elevated firing rates at trial-start (A), in the middle of

663   the task (B), and at trial-end (C). (D) Normalized population firing rate during the discrimination task (D1)

29

664 and during passive listening (D2, sorted in the same order as in D1; 130 neurons tested only in

665 discrimination block, but not in passive listening block are filled with dark blue). Neurons were grouped

666 by type (excitatory and inhibitory) and sorted based on the timing of their peak firing rates. (E)

667 Distribution of excitatory (blue bars) and inhibitory neurons (red bars) based on the timing of the peak

668 firing rate.

669 **Figure 3. Heterogeneous responses of PFC neurons to different auditory cues.** (A) One representative

670 neuron with increased response to the presentation of one auditory cue (25 click/sec), but not to the

671 other two (10 kHz sine wave and 100 click/sec). *Left*: raster plot of spikes for trials with different

672 auditory cues presentation (blue: 10 kHz sine wave; red: 25 click/sec; green: 100 click/sec), and sorted

673 by trial durations. *Right*: normalized firing rate during each auditory stimulus across trials. (B) Another

674 example neuron positively modulated by two auditory cues (10 kHz sine wave and 100 click/sec), but

675 not a third (25 click/sec). (C) Modulations of each neuron presented as p-values calculated with one-way

676 ANOVA over trial progression. Neurons were grouped as excitatory (top) and inhibitory neurons

677 (bottom), and sorted by the total duration when they were modulated. Modulations only occurred

678 during the discrimination task (C1), but not during the passive-listening condition (C2, the neurons are

679 sorted in the same order as C1). (D) The percentage of modulated excitatory (blue) and inhibitory (red)

680 neurons throughout trial progression. During the task, the percentage of modulated excitatory neurons

681 (blue, dot line) and inhibitory neurons (red, solid line) increased as the trial progressed; during passive

682 listening, however, the percentage of modulated neurons remained constant (excitatory: blue, dot line.

683 inhibitory: red, dot line).

684 **Figure 4. Changes in LFP oscillation powers during auditory discrimination versus passive listening.** (A)

685 An example of LFP oscillation during the discrimination task (A1) and during passive listening (A2) from

686 the same recording session. Average spectrograms (top) aligned at trial-start (left) and trial-end (right).

687 (B) Average LFP powers during discrimination task (B1) and during passive listening (B2) at trial-start

688   (left, blue and red: 500 ms window before and after trial start, respectively) and at trial-end (right, blue

689   and red: 500 ms window before and after trial start, respectively). (C and D) Normalized population LFP

690   oscillation powers across animals (N=5) at theta (5-8 Hz), alpha (8-14 Hz), beta (15-30 Hz), low-gamma

691   (40-50 Hz), and high-gamma frequencies (70-100 Hz), aligned at trial-start (C, discrimination task: black,

692   passive listening: gray) or at trial-end (D, discrimination task: black, passive listening: gray). LFP powers

693   were normalized as z-scores to the 2 second window centered at either trial-start (C) or trial-end (D).

694   Shaded areas indicate standard error. (E and F) During discrimination task, decrease in LFP powers

695   across all frequency bands after trial initiation (E1, N=5, paired t-test, *: $p<0.05$) and again when the

696   animal completed the trial (F1, N=5, paired t-test, *: $p<0.05$). During the passive listening condition, LFP

697   powers in all frequency bands increased at trial-start (E2; N=5, paired t-test, *: $p<0.05$) and decreased at

698   trial-end (F2; N=5, paired t-test, *: $p<0.05$). Error bars indicate standard deviation.

699   **Figure 5. LFP oscillation power diverged between correct and incorrect trials at the end of the trial.**

700   (A) Spectrogram of the correct trials (A1) and the incorrect trials (A2) from one representative recording

701   session, aligned to trial-start (left) and to trial-end (right). (B) Normalized population LFP oscillation

702   powers of correct (black line) and incorrect (gray line) trials, aligned to trial-start. LFP powers at all

703   frequency bands decreased after the animals initiated the trial; however, the differences in power were

704   larger in correct trials than in incorrect trials (D1, N=5, paired t-test, *: $p<0.05$). (C) Normalized

705   population power of correct (black line) and incorrect (gray line) trials at trial end. LFP power of correct

706   trials exhibited a prolong suppression, while those of incorrect trials recovered and further increased

707   (D2, N=5 mice, paired t-test, *: $p<0.05$).

708   **Figure 6. Spike-field coherence during auditory discrimination versus during passive listening.** The

709   spike-field coherence (SFC) at trial-start (left) and trial-end (right) during the discrimination task (black

710   line, mean±s.e.m) and passive listening (gray line, mean±s.e.m) at theta (A), alpha (B), beta (C), low

711   gamma (D) and high gamma (E) frequencies. (N=422 neurons, t-test, *: $p<0.05$).

712 **Figure 7. Inhibitory neurons exhibited stronger coherence with LFP than excitatory neurons.** The spike-

713 field coherences of excitatory neurons (blue, mean±s.e.m) and inhibitory neurons (red, mean±s.e.m) at

714 trial-start (left) and trial-end (right) during the discrimination task. (A and B) At alpha (A) and theta (B)

715 frequencies, there was no difference in SFCs between excitatory and inhibitory neurons at trial-start

716 (left). Towards trial-end, inhibitory neurons showed higher SFCs than excitatory neurons, and the

717 elevation sustained beyond trial-end (right). (C) At beta frequencies, inhibitory neurons transiently

718 showed higher SFCs at trial-end. (D) At low-gamma frequencies, inhibitory neurons showed higher SFCs

719 at trial-start (left) and trial-end (right), but not in the middle of the trial. (E) At high-gamma frequencies,

720 inhibitory neurons showed higher SFCs at trial-start (left), while excitatory neurons showed higher SFCs

721 in the middle of the trial (right). (Excitatory = 331 neurons, inhibitory= 91 neurons, t-test, *: $p<0.05$).

722

723

724 **Supplemental Figure Legends**

725 **Figure S1. PFC spiking activity during correct versus incorrect trials.** (Related to Figure 2) Normalized

726 population firing rates of excitatory (blue) and inhibitory (red) neurons during correct trials (A), and

727 incorrect trials (B).

728

A

Behavior Arena

Reward Locations
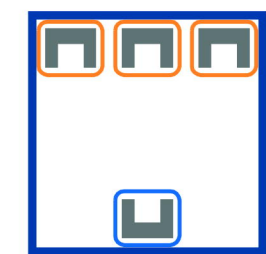
Initiation Location

Behavior Paradigm

Self-Initiation
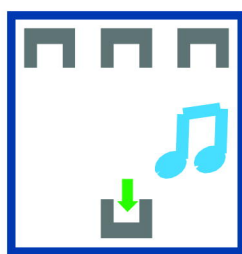
Correct: Reward
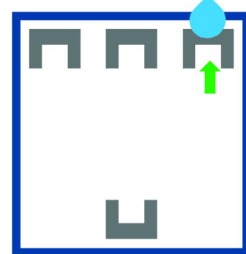
Incorrect: Timeout

B

Correct Rate (%)

100

50

Recording Session

1 2 3   -3 -2 -1

C

Reaction Time (s)

4

2

Recording Session

1 2 3   -3 -2 -1
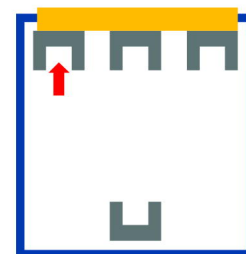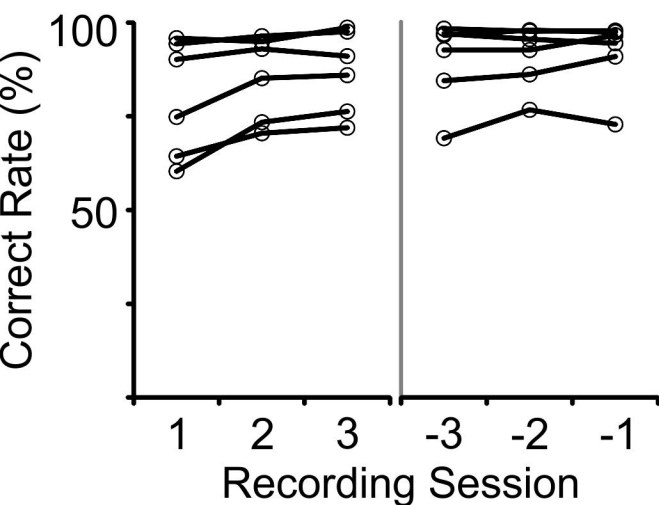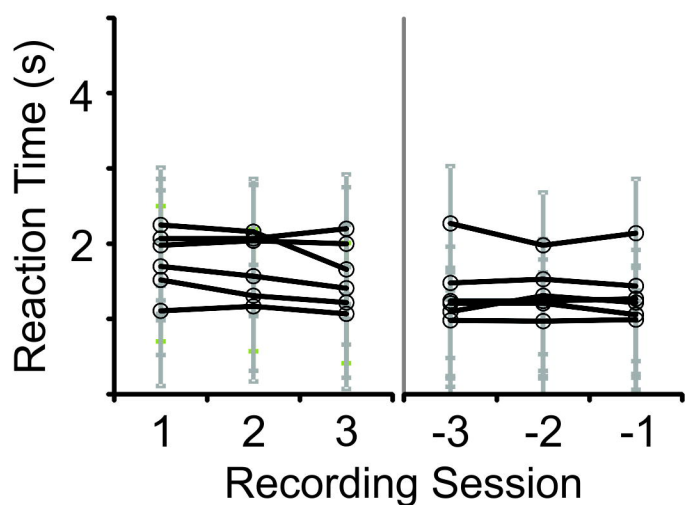
D1 Excitatory

D2 Inhibitory
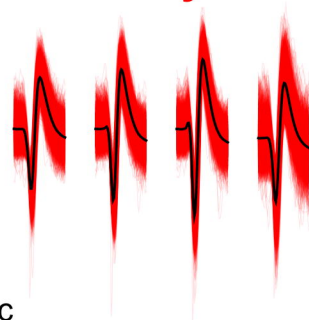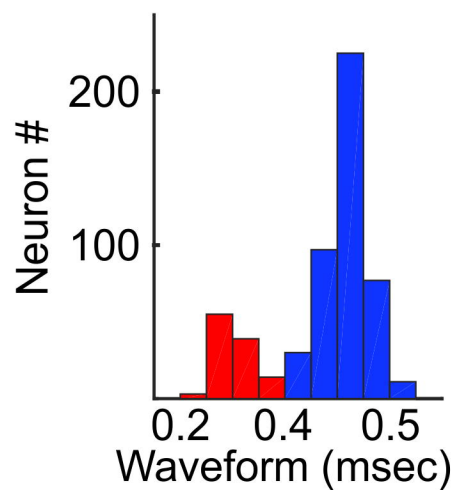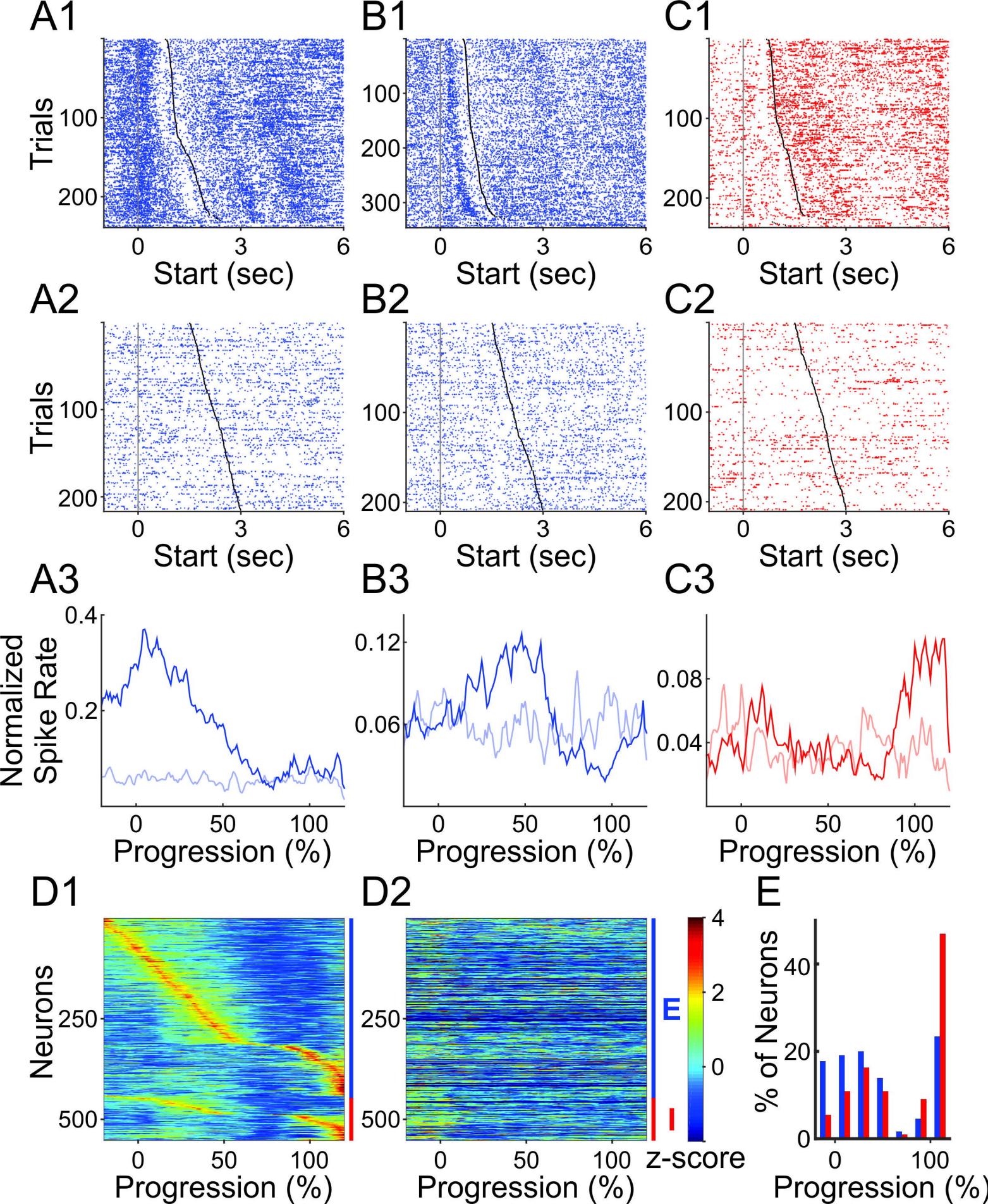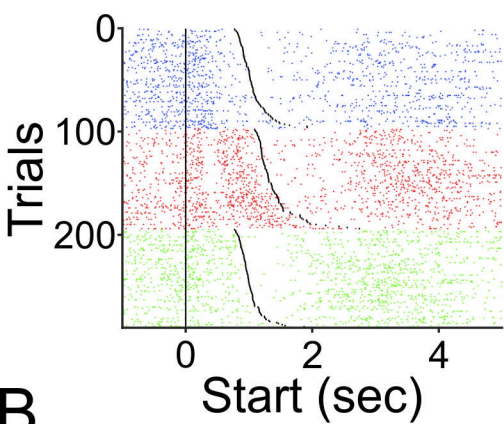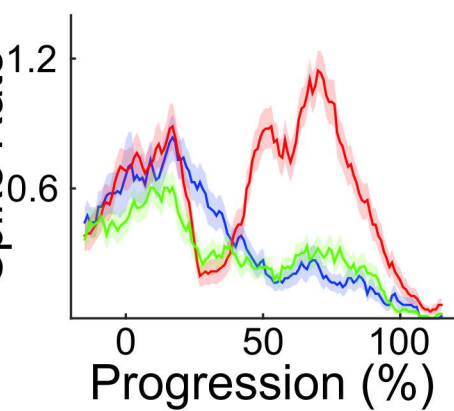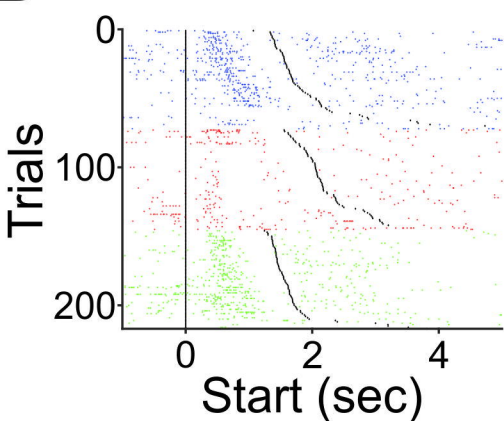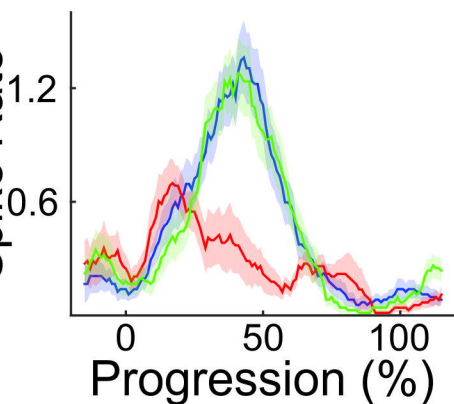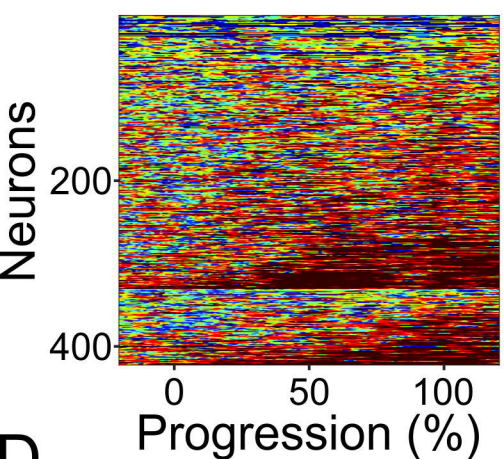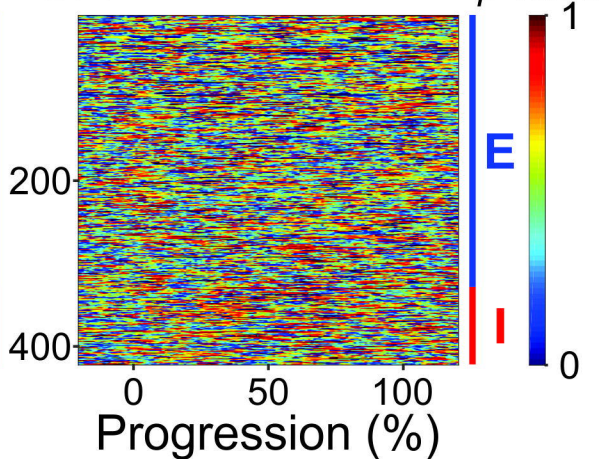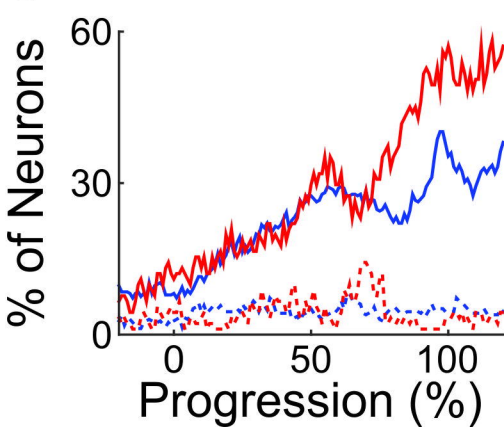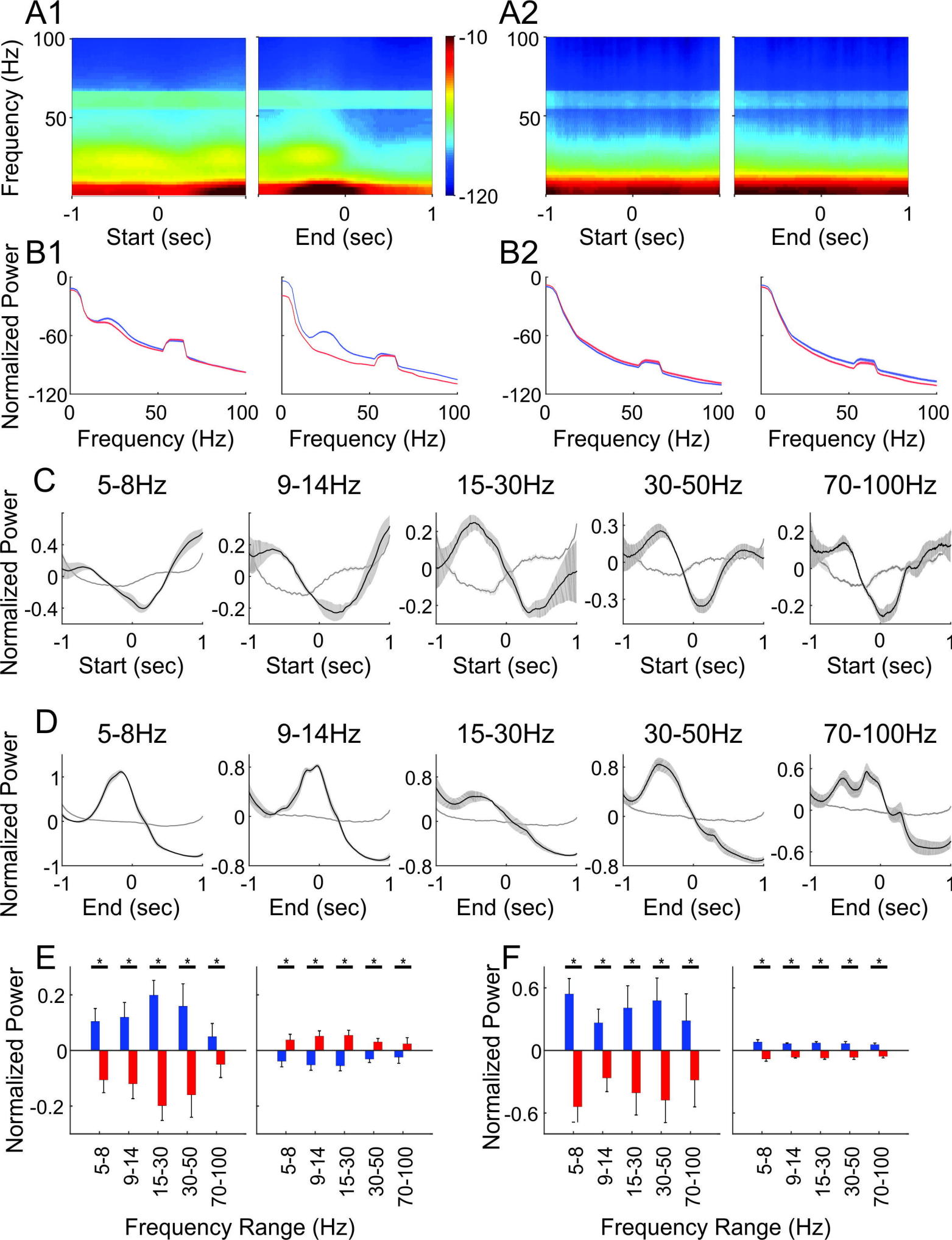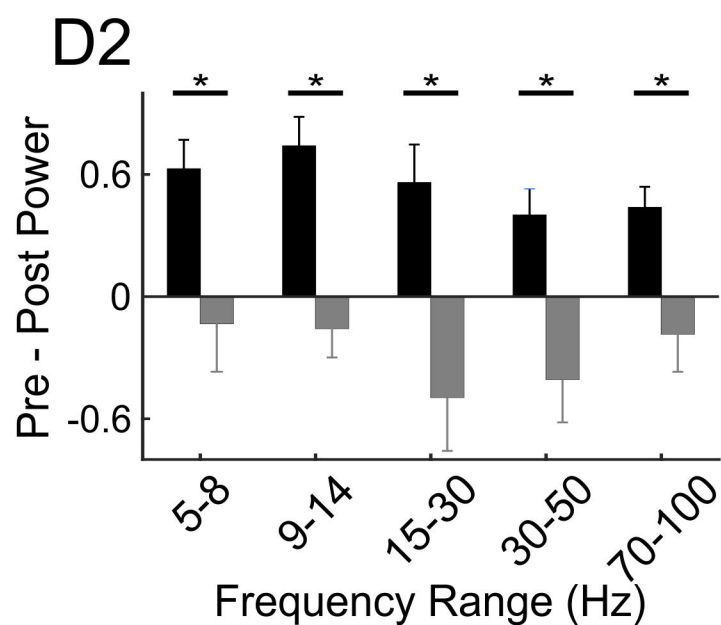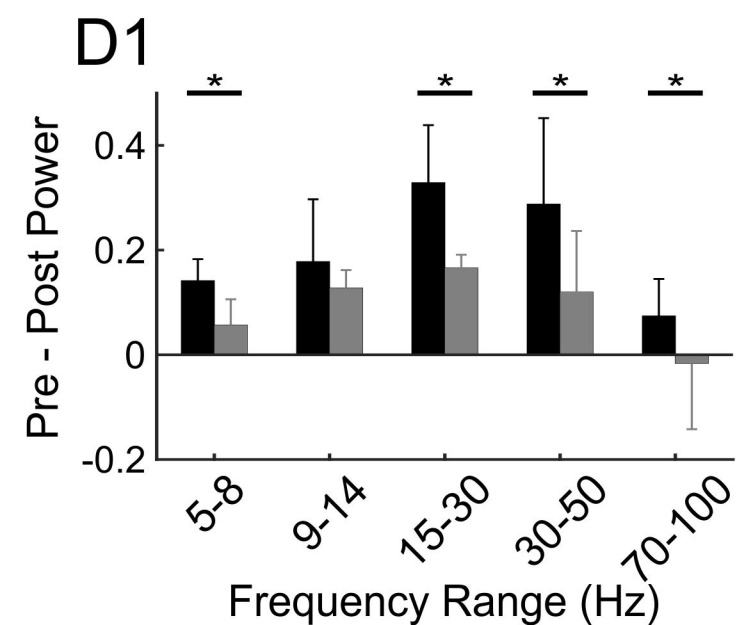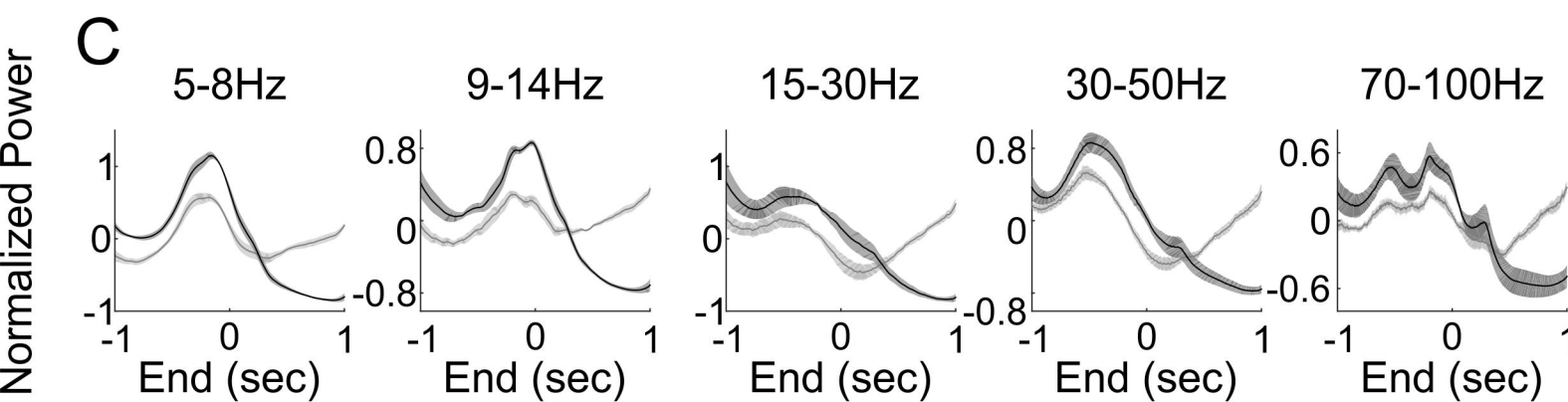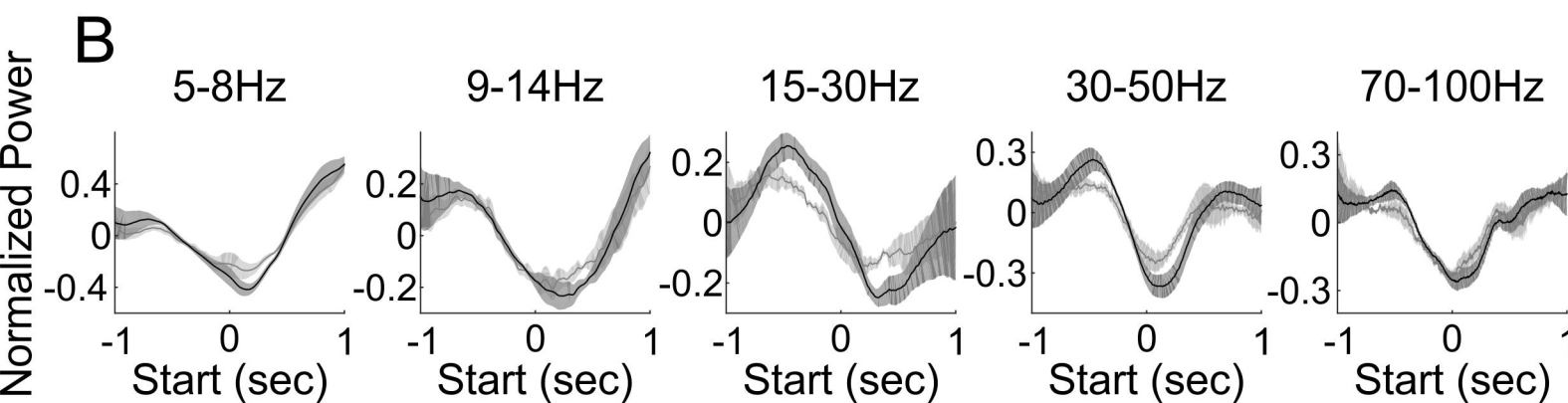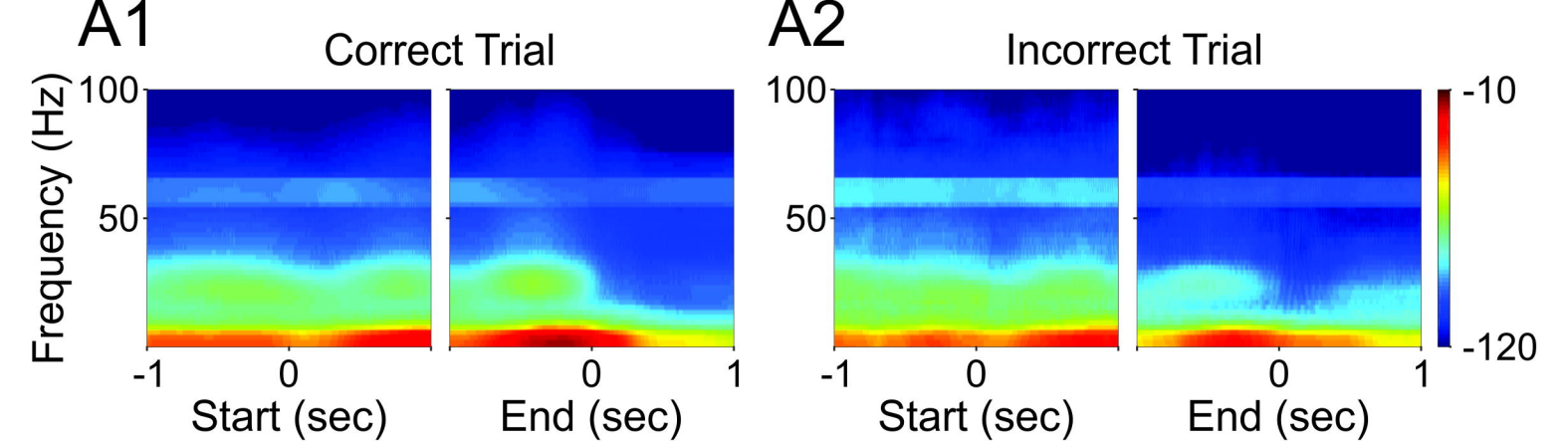
2 msec

E

Neuron #

200

100

Waveform (msec)

0.2   0.4   0.5

A 5-8 Hz
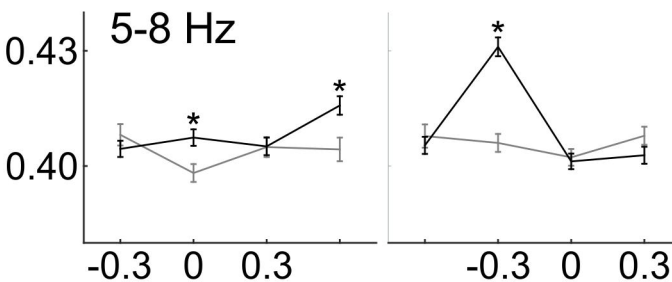
B 9-14 Hz
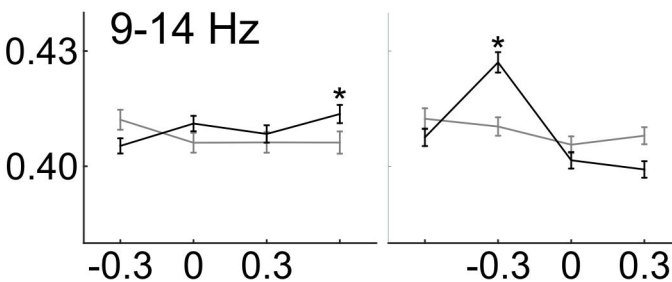
C 15-30 Hz
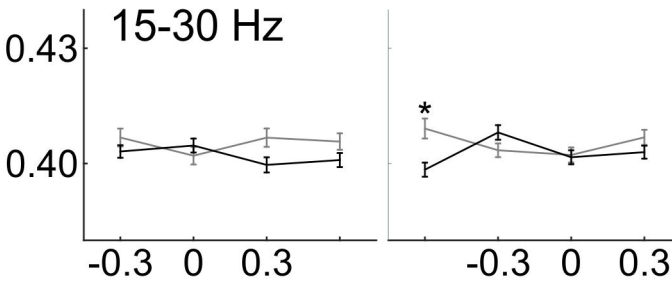
D 30-50 Hz
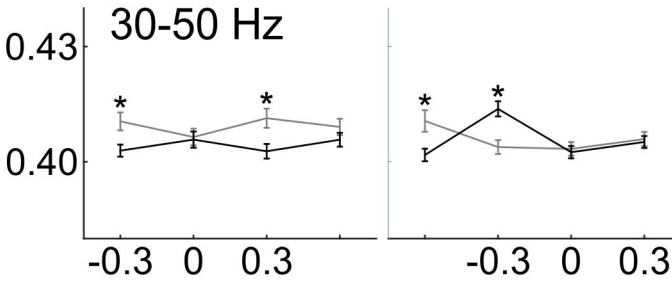
E 70-100 Hz

Start (sec)   End (sec)