

1 **Supplementary Data**

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4 **Medial prefrontal cortex–pontine nuclei projections modulate suboptimal cue induced**
5 **associative motor learning**
6

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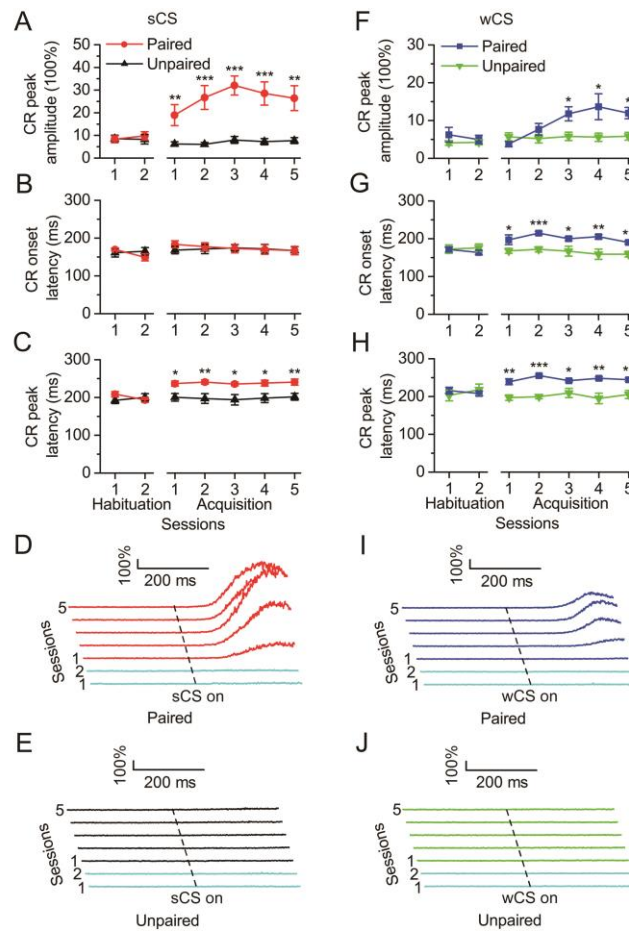
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24 **Supplementary Figures**

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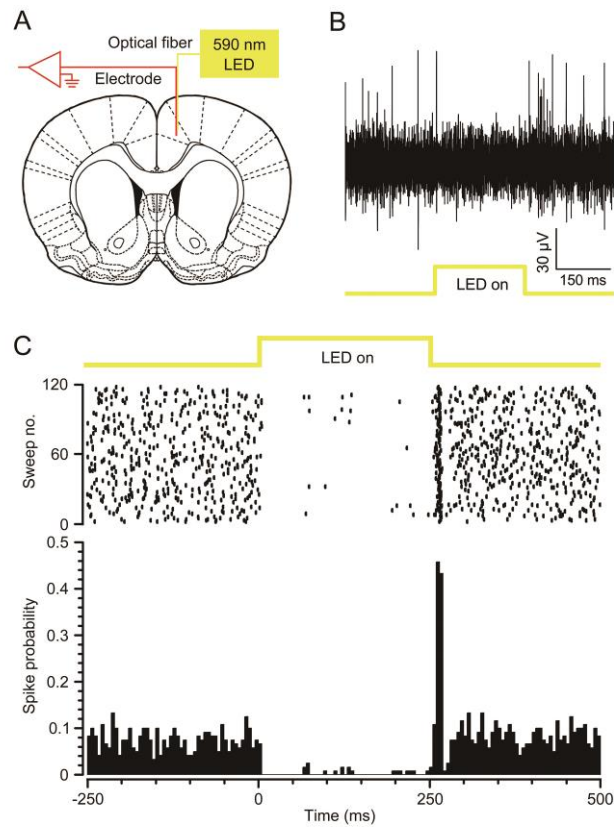


26

27 **Supplementary Figure 1.** The peak amplitude (A,F), onset latency (B,G), and peak latency (C,H)
 28 of CR and EMG response topographies (D,E,I,J) in DEC with the sCS or wCS, respectively, across
 29 two habituation and five acquisition sessions (related to Fig. 1C,E; N.S., not significant, * $P < 0.05$,
 30 ** $P < 0.01$, *** $P < 0.001$; two-way ANOVA with repeated measures followed by Tukey post-hoc
 31 test). The EMG amplitude is given as a percentage of the baseline (100%) averaged EMG amplitude.

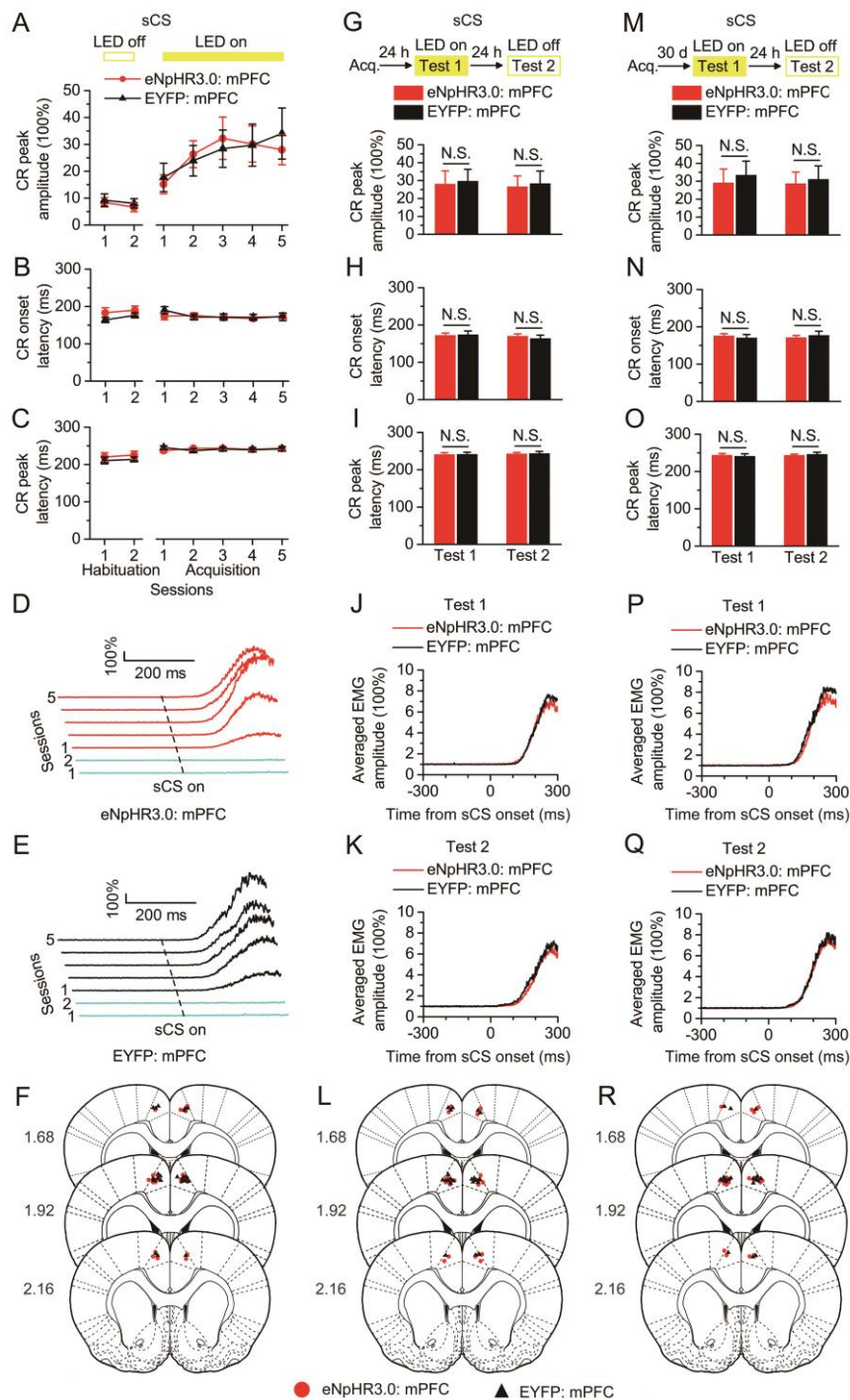
32 Data are represented as mean \pm s.e.m.

33



34

35 **Supplementary Figure 2.** 590-nmLED illumination suppressed eNpHR3.0-EYFP-expressing cells
 36 in the right caudal mPFC. (A) Schematic of *in vivo* optogenetic inhibition and recording in the right
 37 caudal mPFC. (B,C) Continuous 590-nm LED illumination (250 ms, 15 mW/mm²) of the mPFC
 38 expressing eNpHR3.0-EYFP in anesthetized rats inhibited neuronal firing in a temporally precise,
 39 stable, and reversible manner.



40

41 **Supplementary Figure 3.** Effects of optogenetic inhibition of the bilateral caudal mPFC on the

42 peak amplitude, onset latency, and peak latency of CR with the sCS (related to Fig. 2F–H). (A–C)

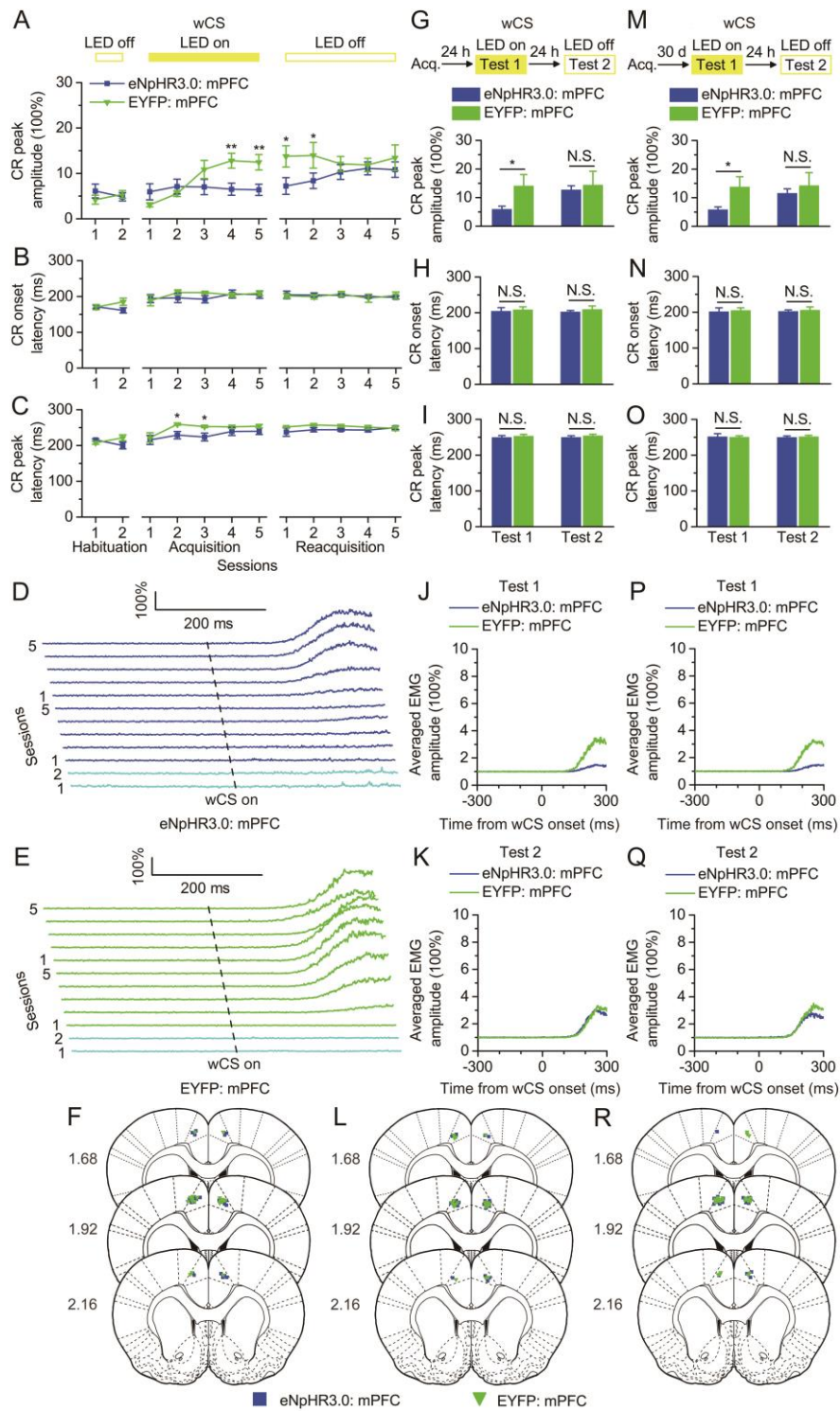
43 Top: Training and illumination protocol. Bottom: Optogenetic inhibition of the bilateral caudal

44 mPFC during acquisition training failed to impair the peak amplitude (A), onset latency (B), or peak

45 latency (C) of CR with the sCS (two-way ANOVA with repeated measures). (D,E) The EMG

46 response topographies of eNpHR3.0: mPFC (D) and EYFP: mPFC (E) groups were shown across

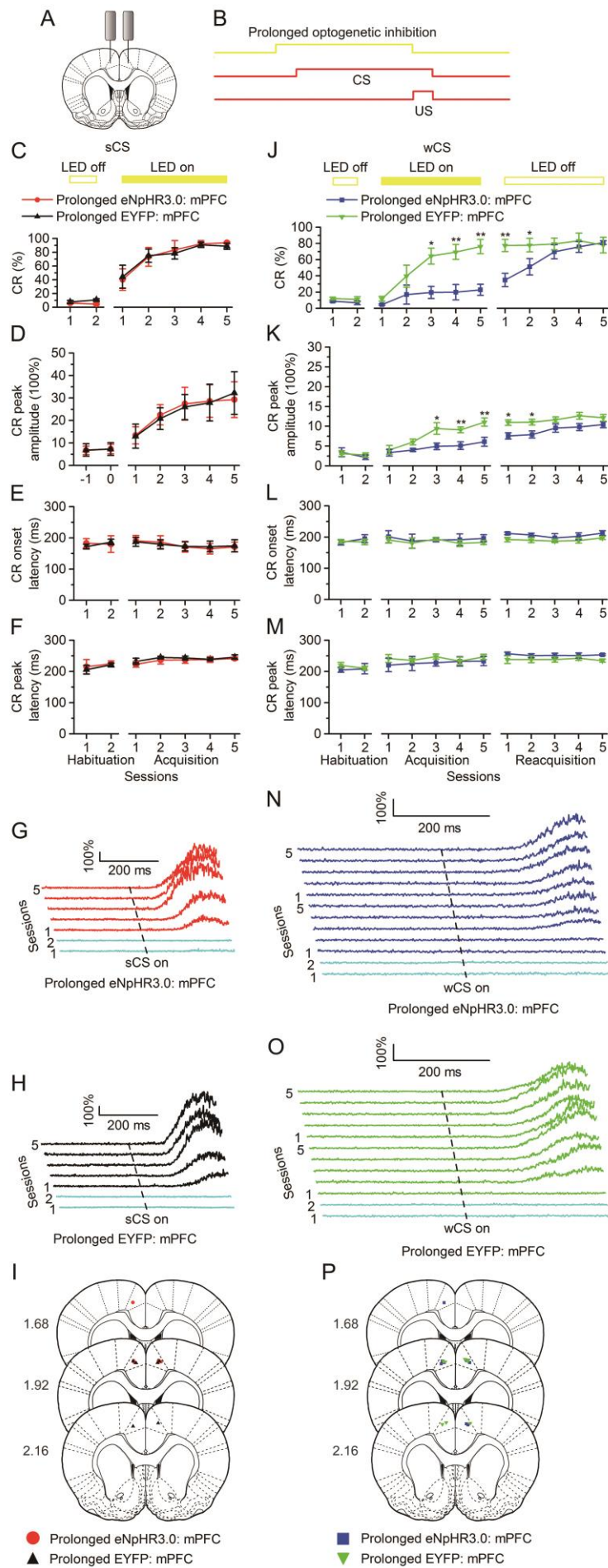
47 two habituation and five acquisition sessions. (*F*) Approximate locations of fiber optic cannula tips
48 for optogenetic inhibition experiment (related to Fig. 2*F*) among rats of eNpHR3.0: mPFC and
49 EYFP: mPFC groups. Numbers indicate the anteroposterior coordinates from bregma. (*G–I*) Top:
50 Training and illumination protocol. Rats were eyeblink conditioned in the absence of optogenetic
51 inhibition and tested 24 h later. Bottom: Twenty-four hours after the acquisition training,
52 optogenetic inhibition of the bilateral caudal mPFC did not affect the peak amplitude (*G*), onset
53 latency (*H*), or peak latency (*I*) of CR with the sCS (N.S., not significant; two-tailed unpaired
54 Student's t-test). (*J,K*) The EMG response topographies for eNpHR3.0: mPFC and EYFP: mPFC
55 groups during test 1 (*J*) and test 2 (*K*) were shown. (*L*) Same as (*F*), except rats from the other
56 optogenetic inhibition experiment (related to Fig. 2*G*). (*M–R*) Same as (*G–L*), except the optogenetic
57 inhibition was applied at thirty days after the acquisition training (related to Fig. 2*H*; N.S., not
58 significant; two-tailed unpaired Student's t-test). The EMG amplitude is given as a percentage of
59 the baseline (100%) averaged EMG amplitude. Data are represented as mean \pm s.e.m.



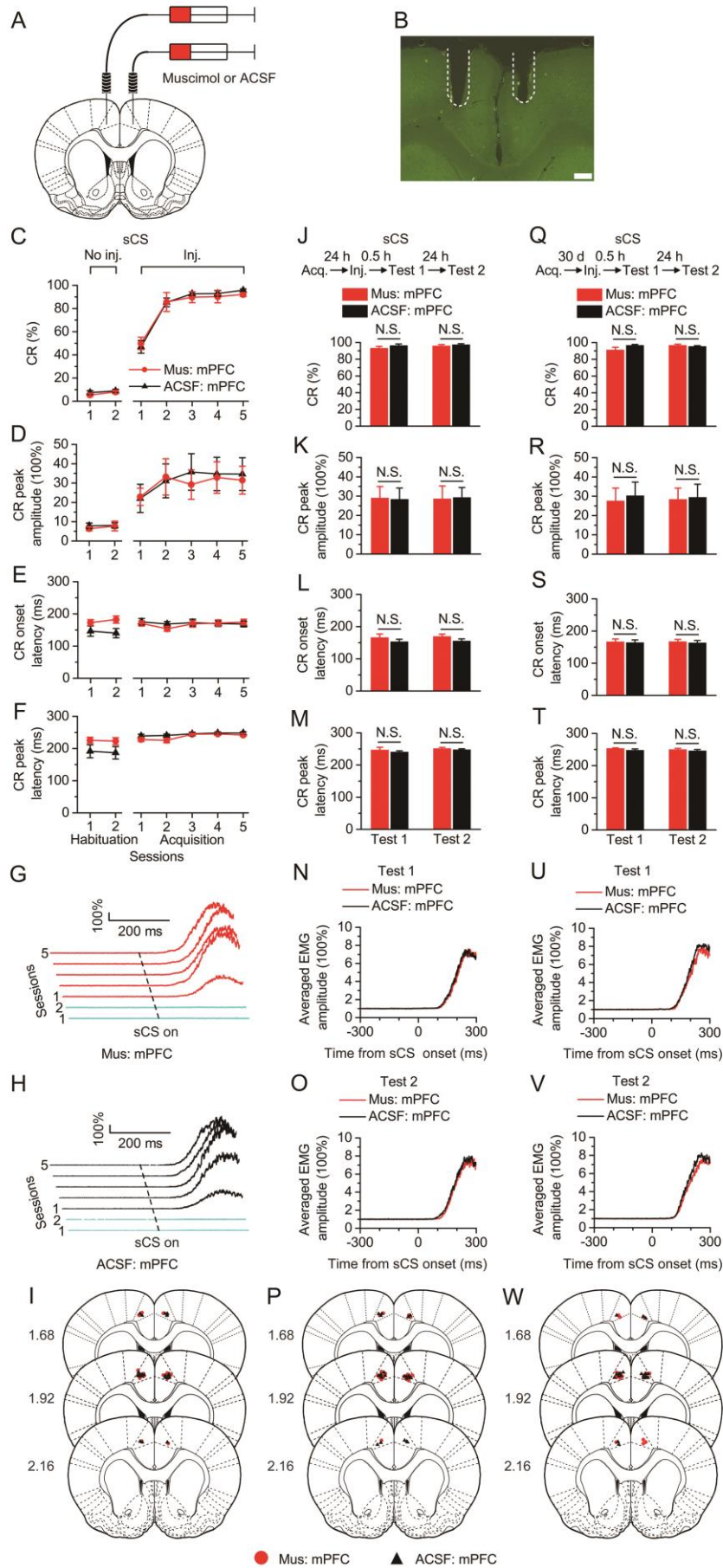
60

61 **Supplementary Figure 4.** Effects of optogenetic inhibition of the bilateral caudal mPFC on the
 62 peak amplitude, onset latency, and peak latency of CR with the wCS (related to Fig. 2I–K). (A–C)
 63 Top: Training and illumination protocol. Bottom: Optogenetic inhibition of the bilateral caudal
 64 mPFC during acquisition training affected the peak amplitude (A) and peak latency (C), but not
 65 onset latency (B) of CR with the wCS (* $P < 0.05$, ** $P < 0.01$; two-way ANOVA with repeated

66 measures followed by Tukey post-hoc test). (*D,E*) The EMG response topographies of eNpHR3.0:
67 mPFC (*D*) and EYFP: mPFC (*E*) groups were shown across two habituation, five acquisition, and
68 five reacquisition sessions. (*F*) Approximate locations of fiber optic cannula tips for optogenetic
69 inhibition experiment (related to Fig. 2*I*) among rats of eNpHR3.0: mPFC and EYFP: mPFC groups.
70 Numbers indicate the anteroposterior coordinates from bregma. (*G-I*) Top: Training and
71 illumination protocol. Rats were eyeblink conditioned in the absence of optogenetic inhibition and
72 tested 24 h later. Bottom: Twenty-four hours after the acquisition training, optogenetic inhibition of
73 the bilateral caudal mPFC affected the peak amplitude (*G*), but not onset latency (*H*) or peak
74 latency (*I*) of CR with the sCS (N.S., not significant, $*P < 0.05$; two-tailed unpaired Student's t-test).
75 (*J,K*) The EMG response topographies for eNpHR3.0: mPFC and EYFP: mPFC groups during test
76 1 (*J*) and test 2 (*K*) were shown. (*L*) Same as (*F*), except rats from the other optogenetic inhibition
77 experiment (related to Fig. 2*J*). (*M-R*) Same as (*G-L*), except the optogenetic inhibition was
78 applied at thirty days after the acquisition training (related to Fig. 2*K*; N.S., not significant, $*P <$
79 0.05 ; two-tailed unpaired Student's t-test). The EMG amplitude is given as a percentage of the
80 baseline (100%) averaged EMG amplitude. Data are represented as mean \pm s.e.m.



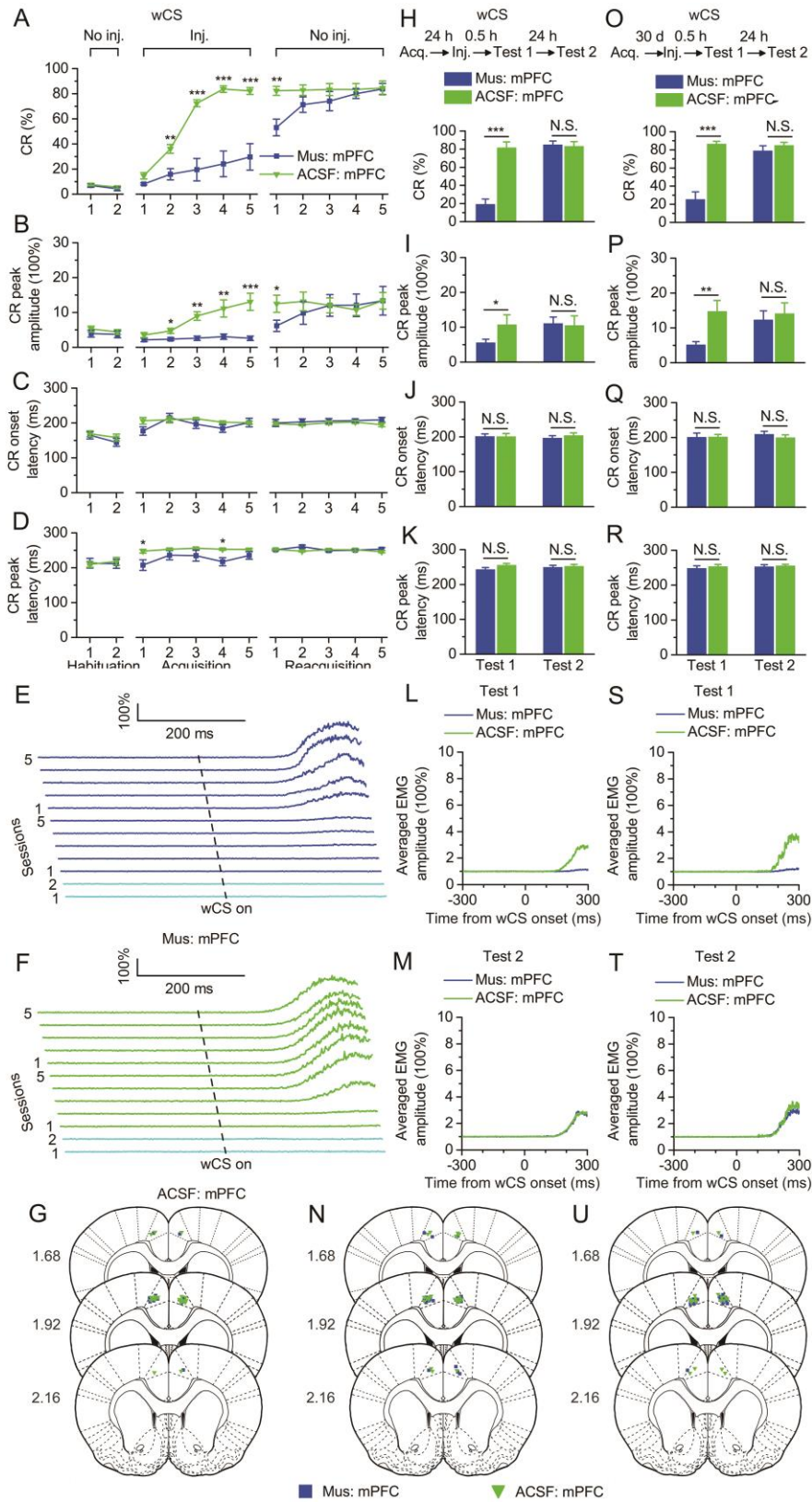
82 **Supplementary Figure 5.** Effects of prolonged optogenetic inhibition of the bilateral caudal mPFC
83 on the percentage, peak amplitude, onset latency, and peak latency of CR with the sCS or wCS.
84 (A,B) Scheme for optical fiber implant site and 590-nm LED illumination pattern to bilateral caudal
85 mPFC during each trial. (C–F) Top: Training and illumination phase protocol. Bottom: Prolonged
86 optogenetic inhibition of the bilateral caudal mPFC during acquisition training did not affect the
87 percentage (C), peak amplitude (D), onset latency (E), or peak latency (F) of CR with the sCS ($n =$
88 3 each; two-way ANOVA with repeated measures). (G,H) The EMG response topographies of
89 Prolonged eNpHR3.0: mPFC (G) and Prolonged EYFP: mPFC (H) groups with sCS were shown
90 across two habituation and five acquisition sessions. (I) Approximate locations of fiber optic
91 cannula tips for Prolonged optogenetic inhibition experiment with sCS (related to C–H) among rats
92 of Prolonged eNpHR3.0: mPFC and Prolonged EYFP: mPFC groups. Numbers indicate the
93 anteroposterior coordinates from bregma. (J–M) Top: Training and illumination phase protocol.
94 Bottom: Prolonged optogenetic inhibition of the bilateral caudal mPFC during acquisition training
95 significantly impaired the percentage (J) and peak amplitude (K), but not peak latency (L) or onset
96 latency (M) of CR with the wCS ($n = 4$ each; $*P < 0.05$, $**P < 0.01$; two-way ANOVA with repeated
97 measures followed by Tukey post-hoc test). (N,O) The EMG response topographies of Prolonged
98 eNpHR3.0: mPFC (N) and Prolonged EYFP: mPFC (O) groups with wCS were shown across two
99 habituation, five acquisition, and five reacquisition sessions. (P) Same as (I), except rats from the
100 Prolonged optogenetic inhibition experiment with wCS (related to J–O). The EMG amplitude is
101 given as a percentage of the baseline (100%) averaged EMG amplitude. Data are represented as
102 mean \pm s.e.m.



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104 **Supplementary Figure 6.** Effects of muscimol inactivation of the bilateral caudal mPFC on the

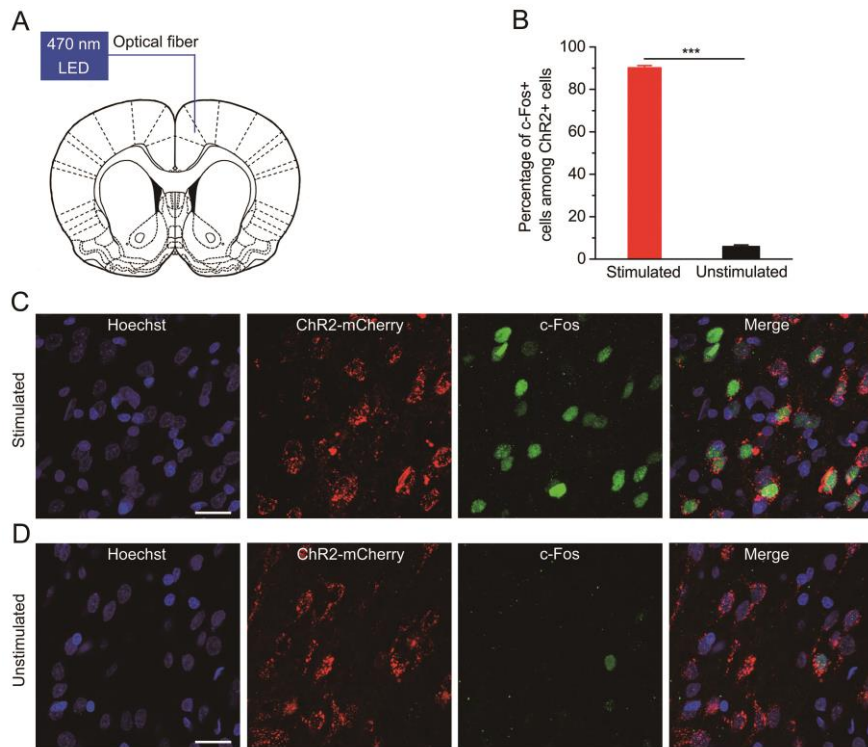
105 percentage, peak amplitude, onset latency, and peak latency of CR with the sCS. (A) Schematic of
106 *in vivo* muscimol or ACSF injection into the bilateral caudal. (B) Representative image showing
107 locations of infusion cannula tips in the bilateral caudal. (C–F) Top: Training and infusion protocol.
108 Bottom: Muscimol inactivation of the bilateral caudal mPFC during acquisition training did not
109 affect the percentage (C), peak amplitude (D), onset latency (E), or peak latency (F) of CR with the
110 sCS ($n = 11$ each; two-way ANOVA with repeated measures). (G,H) The EMG response
111 topographies of Mus: mPFC (G) and ACSF: mPFC (H) groups were shown across two habituation
112 and five acquisition sessions. (I) Approximate locations of infusion cannula tips for muscimol
113 inactivation experiment (related to C–H) among rats of Mus: mPFC and ACSF: mPFC groups.
114 Numbers indicate the anteroposterior coordinates from bregma. (J–M) Top: Training and infusion
115 protocol. Rats were eyeblink conditioned in the absence of muscimol and tested 24 h later. Bottom:
116 Twenty-four hours after the acquisition training, muscimol inactivation of the bilateral caudal
117 mPFC did not affect the percentage (J), peak amplitude (K), onset latency (L), or peak latency (M)
118 of CR with the sCS ($n = 11$ each; N.S., not significant; two-tailed unpaired Student’s t-test). (N,O)
119 The EMG response topographies for Mus: mPFC and ACSF: mPFC groups during test 1 (N) and
120 test 2 (O) were shown. (P) Same as (I), except rats from the other muscimol inactivation experiment
121 (related to J–O). (Q–W) Same as (I–P), except the muscimol inactivation was applied at thirty days
122 after the acquisition training ($n = 11$ Mus: mPFC, $n = 10$ ACSF: mPFC groups; N.S., not significant;
123 two-tailed unpaired Student’s t-test). The EMG amplitude is given as a percentage of the baseline
124 (100%) averaged EMG amplitude. Data are represented as mean \pm s.e.m.



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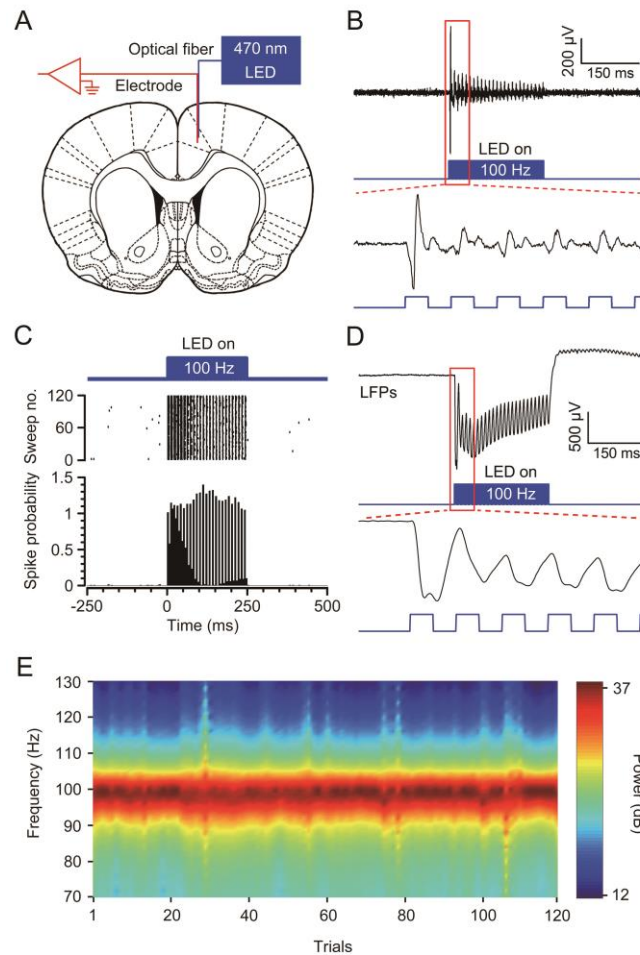
126 **Supplementary Figure 7.** Effects of muscimol inactivation of the bilateral caudal mPFC on the
 127 percentage, peak amplitude, onset latency, and peak latency of CR with the wCS. (A–D) Top:
 128 Training and infusion protocol. Bottom: Muscimol inactivation of the bilateral caudal mPFC during

129 acquisition training significantly affected the percentage (A), peak amplitude (B), and peak latency
130 (D), but not onset latency (C) of CR with the wCS ($n = 8$ Mus: mPFC, $n = 10$ ACSF: mPFC; $*P <$
131 0.05 , $**P < 0.01$, $***P < 0.001$; two-way ANOVA with repeated measures followed by Tukey
132 post-hoc test). (E, F) The EMG response topographies of Mus: mPFC (E) and ACSF: mPFC (F)
133 groups were shown across two habituation, five acquisition, and five reacquisition sessions. (G)
134 Approximate locations of infusion cannula tips for muscimol inactivation experiment (related to A–
135 F) among rats of Mus: mPFC and ACSF: mPFC groups. Numbers indicate the anteroposterior
136 coordinates from bregma. (H–K) Top: Training and infusion protocol. Rats were eyeblink
137 conditioned in the absence of muscimol and tested 24 h later. Bottom: Twenty-four hours after the
138 acquisition training, muscimol inactivation of the bilateral caudal mPFC substantially affected the
139 percentage (H) and peak amplitude (I), but not onset latency (J) or peak latency (K) of CR with the
140 wCS ($n = 11$ Mus: mPFC, $n = 10$ ACSF: mPFC; N.S., not significant, $*P < 0.05$, $***P < 0.001$;
141 two-tailed unpaired Student's t-test). (L,M) The EMG response topographies for Mus: mPFC and
142 ACSF: mPFC groups during test 1 (L) and test 2 (M) were shown. (N) Same as (G), except rats
143 from the other muscimol inactivation experiment (related to H–M). (O–U) Same as (H–N), except
144 the muscimol inactivation was applied at thirty days after the acquisition training ($n = 10$ subjects
145 for Mus: mPFC, and $n = 9$ subjects for ACSF: mPFC groups; N.S., not significant, $**P < 0.01$,
146 $***P < 0.001$; two-tailed unpaired Student's t-test). The EMG amplitude is given as a percentage of
147 the baseline (100%) averaged EMG amplitude. Data are represented as mean \pm s.e.m.



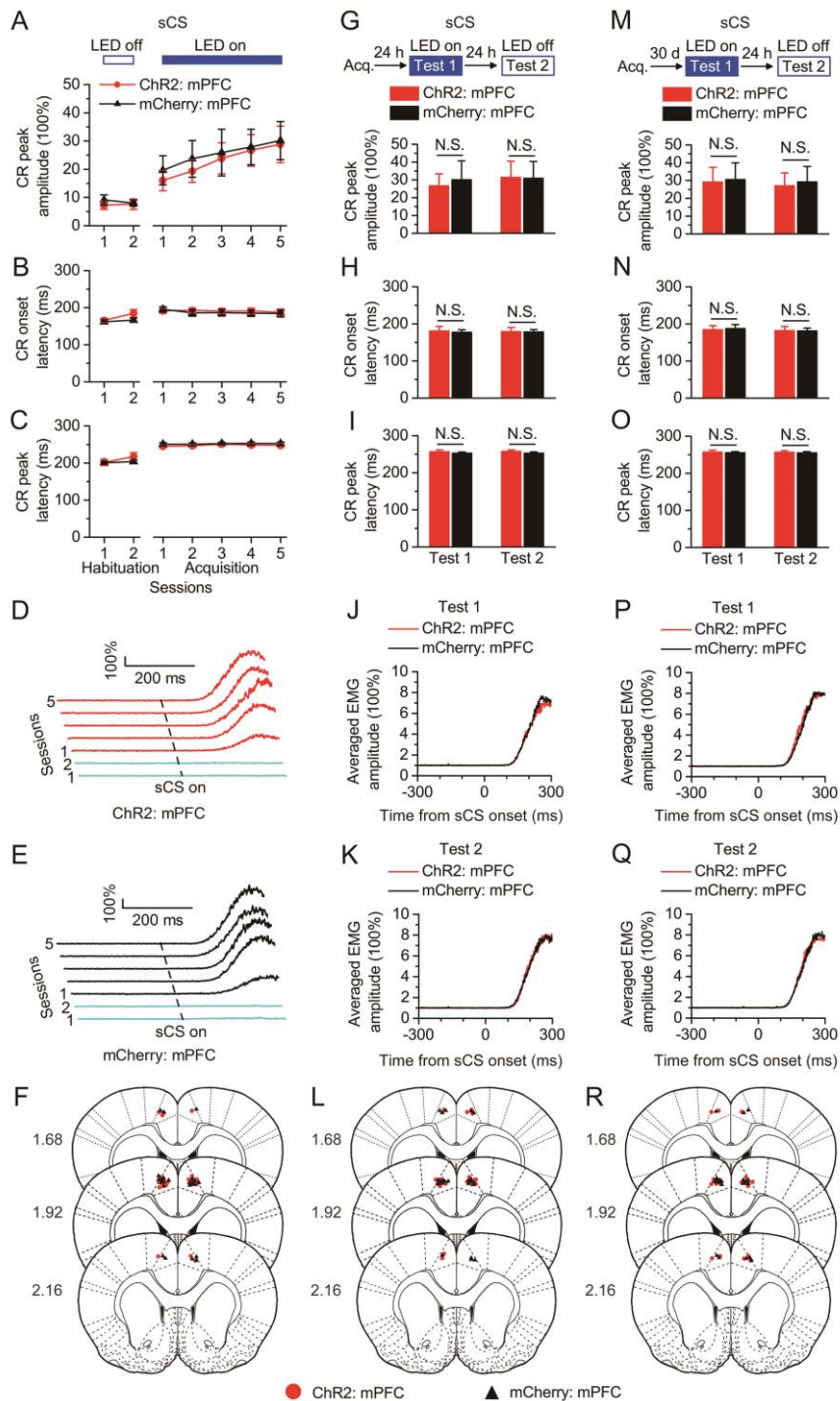
148

149 **Supplementary Figure 8.** 470-nm LED stimulation induced c-Fos expression in cells expressing
 150 ChR2-mCherry in the mPFC. (A) Schematic diagram of optical stimulation of
 151 ChR2-mCherry-expressing cells in right caudal mPFC. (B–D) Rats expressing ChR2-mCherry (red)
 152 in the mPFC were treated with or without 470-nm LED stimulation and the expression of c-Fos
 153 (green) was examined. Representative magnification images of mPFC for the light stimulated group
 154 (C) and unstimulated group (D) are shown. Scale bar, 20 μ m. Quantification of c-Fos positive cells
 155 among ChR2 positive cells is shown in (B).



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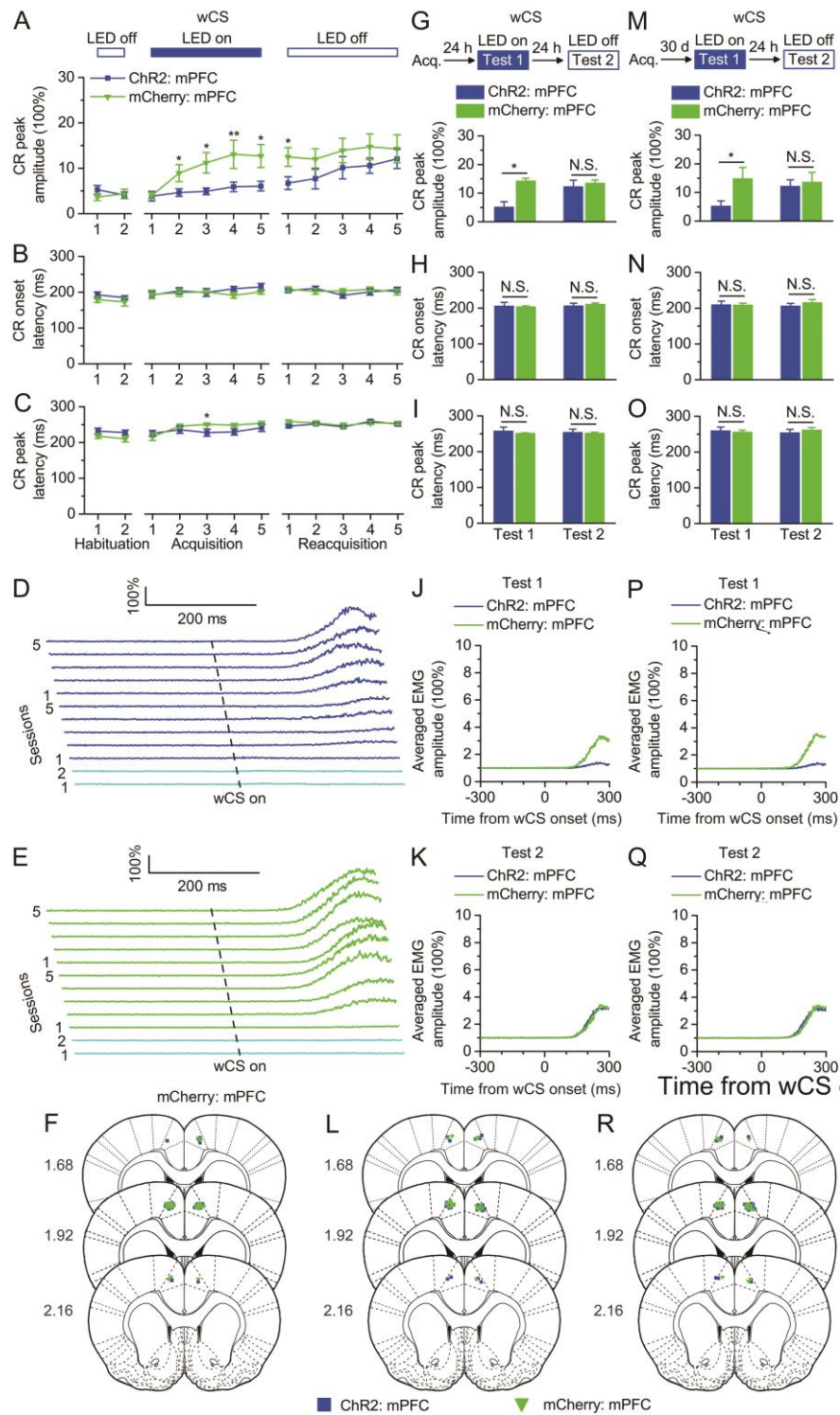
157 **Supplementary Figure 9.** 470-nm LED illumination evoked activation of
 158 ChR2-mCherry-expressing cells in the right caudal mPFC. (A) Schematic showing *in vivo* optical
 159 stimulation and recording in the right caudal mPFC. (B,C) Trains of 25 light pulses (470 nm, 15
 160 mW/mm^2 , 100 Hz, 5 ms pulse duration) induced reliable spikes in the right caudal mPFC neurons
 161 recorded from head-fixed anesthetized rats injected with pAAV 2/9-CaMKII α -ChR2-mCherry. (D,E)
 162 Trains of 25 light pulses (470 nm, 15 mW/mm^2 , 100 Hz, 5 ms pulse duration) also evoked robust
 163 LFP responses in right caudal mPFC in awake behaving rats. Note that the (D) graph illustrates an
 164 example of the mean value of 120 light-induced LFPs.



165

166 **Supplementary Figure 10.** Effects of optogenetic activation of the bilateral caudal mPFC on the
 167 peak amplitude, onset latency, and peak latency of CR with the sCS (related to Fig. 3F–H). (A–C)
 168 Top: Training and illumination protocol. Bottom: Optogenetic activation of the bilateral caudal
 169 mPFC during acquisition training failed to affect the peak amplitude (A), onset latency (B), or peak
 170 latency (C) of CR with the sCS (two-way ANOVA with repeated measures). (D,E) The EMG
 171 response topographies of ChR2: mPFC (D) and mCherry: mPFC (E) groups were shown across two

172 habituation and five acquisition sessions. (*F*) Approximate locations of optrode tips for optogenetic
173 activation experiment (related to Fig. 3*F*) among rats of ChR2: mPFC and mCherry: mPFC groups.
174 Numbers indicate the anteroposterior coordinates from bregma. (*G–I*) Top: Training and
175 illumination protocol. Rats were eyeblink conditioned in the absence of optogenetic activation and
176 tested 24 h later. Bottom: Twenty-four hours after the acquisition training, optogenetic activation of
177 the bilateral caudal mPFC did not affect the peak amplitude (*G*), onset latency (*H*), or peak latency
178 (*I*) of CR with the sCS (N.S., not significant; two-tailed unpaired Student's t-test). (*J,K*) The EMG
179 response topographies for ChR2: mPFC and mCherry: mPFC groups during test 1 (*J*) and test 2 (*K*)
180 were shown. (*L*) Same as (*F*), except rats from the other optogenetic activation experiment (related
181 to Fig. 3*G*). (*M–R*) Same as (*G–L*), except the optogenetic activation was applied at thirty days after
182 the acquisition training (related to Fig. 3*H*; N.S., not significant; two-tailed unpaired Student's
183 t-test). The EMG amplitude is given as a percentage of the baseline (100%) averaged EMG
184 amplitude. Data are represented as mean \pm s.e.m.



185

186 **Supplementary Figure 11.** Effects of optogenetic activation of the bilateral caudal mPFC on the

187 peak amplitude, onset latency, and peak latency of CR with the wCS (related to Fig. 3I–K). (A–C)

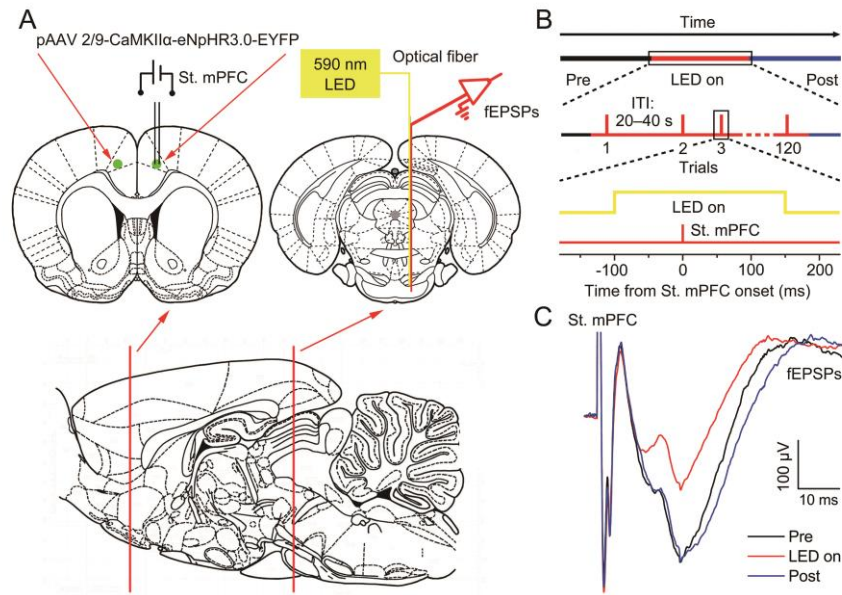
188 Top: Training and illumination protocol. Bottom: Optogenetic activation of the bilateral caudal

189 mPFC during acquisition training affected the peak amplitude (A) and peak latency (C), but not

190 onset latency (B) of CR with the wCS (* $P < 0.05$, ** $P < 0.01$; two-way ANOVA with repeated

191 measures followed by Tukey post-hoc test). (*D,E*) The EMG response topographies of ChR2:
192 mPFC (*D*) and mCherry: mPFC (*E*) groups were shown across two habituation, five acquisition,
193 and five reacquisition sessions. (*F*) Approximate locations of optrode tips for optogenetic activation
194 experiment (related to Fig. 3I) among rats of ChR2: mPFC and mCherry: mPFC groups. Numbers
195 indicate the anteroposterior coordinates from bregma. (*G–I*) Top: Training and illumination protocol.
196 Rats were eyeblink conditioned in the absence of optogenetic activation and tested 24 h later.
197 Bottom: Twenty-four hours after the acquisition training, optogenetic activation of the bilateral
198 caudal mPFC affected the peak amplitude (*G*), but not onset latency (*H*) or peak latency (*I*) of CR
199 with the sCS (N.S., not significant, $*P < 0.05$; two-tailed unpaired Student's t-test). (*J,K*) The EMG
200 response topographies for ChR2: mPFC and mCherry: mPFC groups during test 1 (*J*) and test 2 (*K*)
201 were shown. (*L*) Same as (*F*), except rats from the other optogenetic activation experiment (related
202 to Fig. 3J). (*M–R*) Same as (*G–L*), except the optogenetic activation was applied at thirty days after
203 the acquisition training (related to Fig. 3K; N.S., not significant, $*P < 0.05$; two-tailed unpaired
204 Student's t-test). The EMG amplitude is given as a percentage of the baseline (100%) averaged
205 EMG amplitude. Data are represented as mean \pm s.e.m.

206



207

208 **Supplementary Figure 12.** 590-nm LED illumination of eNpHR3.0-EYFP-expressing mPFC axon

209 terminals in the right PN significantly decreased the slope of fEPSPs evoked by the electrical

210 stimulation of the right caudal mPFC. (A) Schematic depicting a concentric stimulating electrode

211 and an optrode implanted in vivo for stimulating neurons in the right caudal mPFC, optogenetic

212 inhibition of mPFC axon terminals in the right PN, and for recording in fEPSPs in the right PN,

213 respectively. (B) Time line for examination of the effect of optogenetic inhibition on fEPSPs. Top:

214 The full experiment was divided into three epochs, i.e., pre epoch, LED on epoch, and post LED on

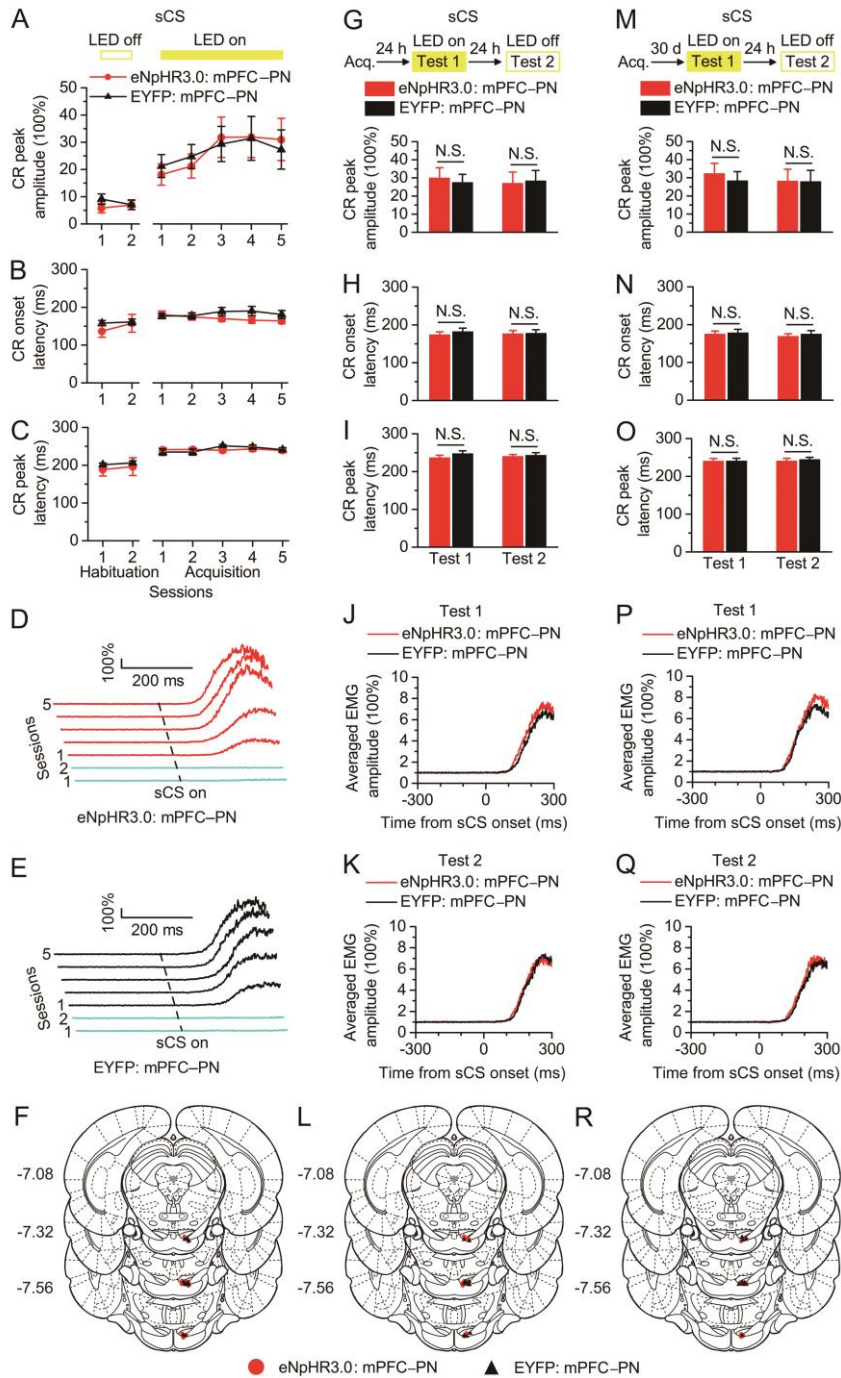
215 epoch. Middle: Each epoch consisted of 120 trials, separated by a variable interval of 20–40 s.

216 Bottom: Only during LED on epoch, the continuous 590-nm illumination (25 mW/mm^2) was

217 delivered 100 ms before electrical stimulation of the right caudal mPFC and lasted 250 ms. (C) The

218 slope of the fEPSPs evoked at the right caudal mPFC was significantly decreased by optogenetic

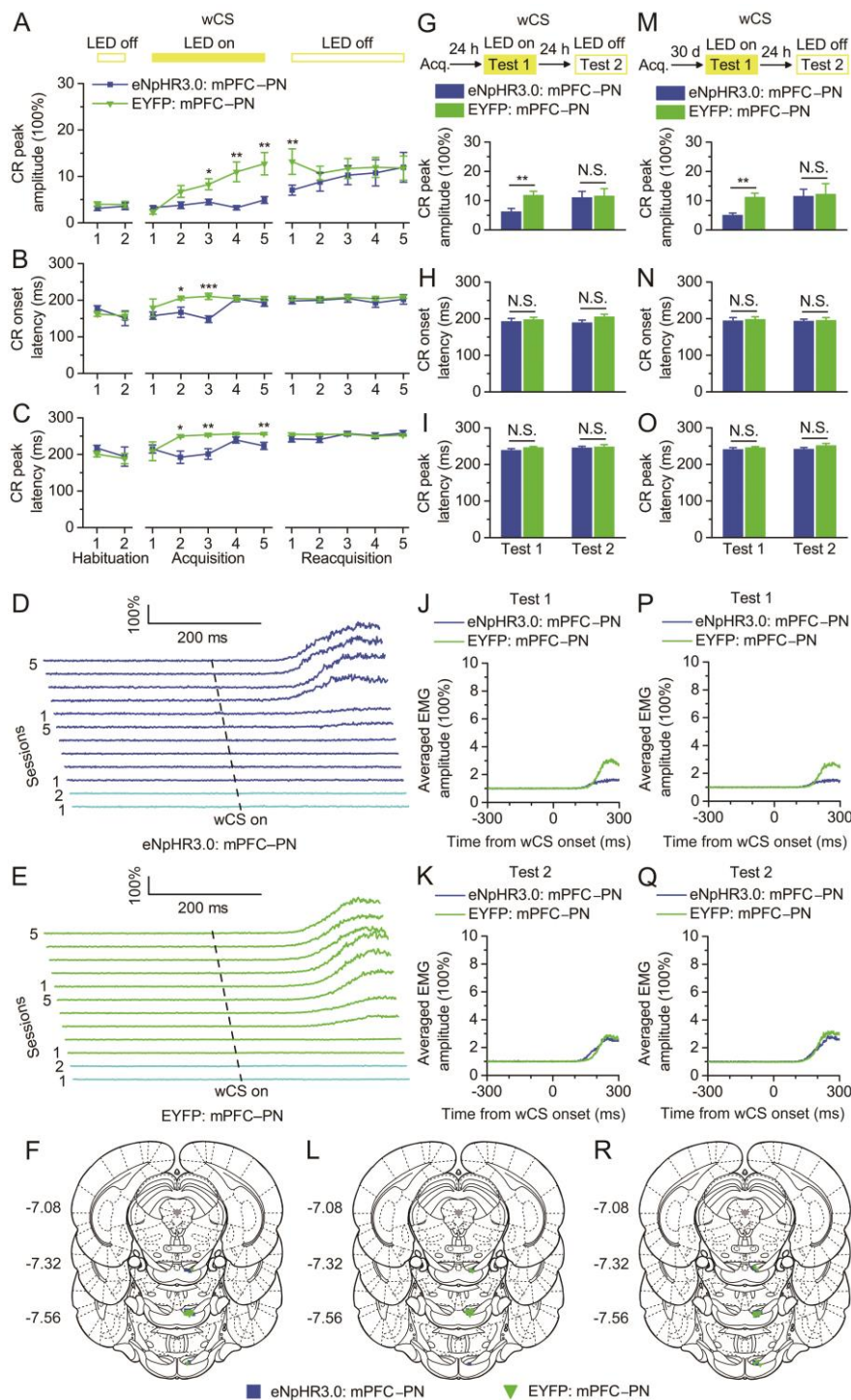
219 illumination of the caudal mPFC axon terminals in the right PN.



220

221 **Supplementary Figure 13.** Effects of optogenetic inhibition of the caudal mPFC axon terminals in
 222 the right PN on the peak amplitude, onset latency, and peak latency of CR with the sCS (related to
 223 Fig. 4E–G). (A–C) Top: Training and illumination protocol. Bottom: Optogenetic inhibition of the
 224 caudal mPFC axon terminals in the right PN during acquisition training failed to impair the peak
 225 amplitude (A), onset latency (B), or peak latency (C) of CR with the sCS (two-way ANOVA with
 226 repeated measures). (D,E) The EMG response topographies of eNpHR3.0: mPFC-PN (D) and

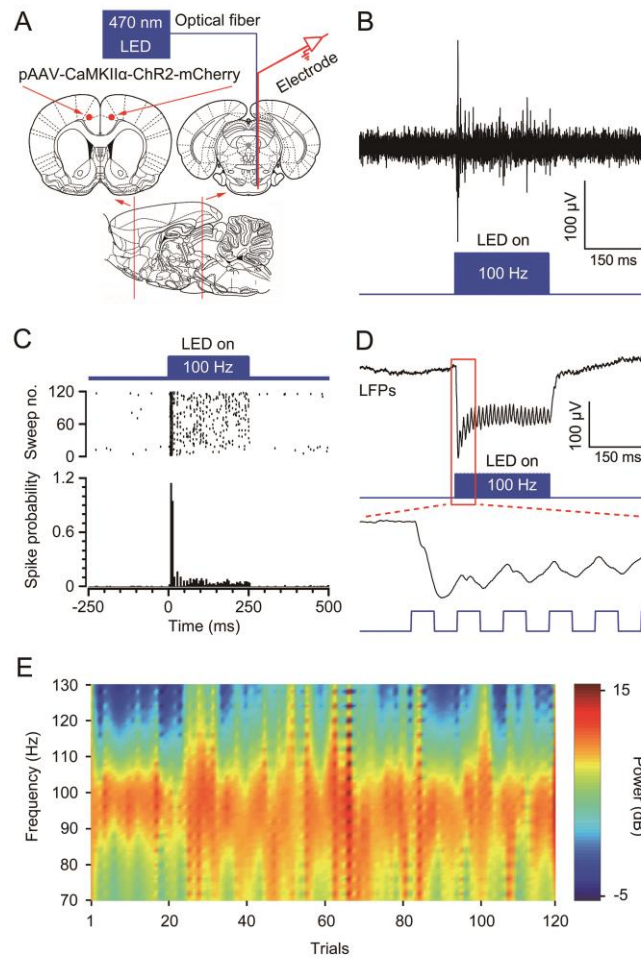
227 EYFP: mPFC–PN (*E*) groups were shown across two habituation and five acquisition sessions. (*F*)
228 Approximate locations of fiber optic cannula tips for optogenetic inhibition experiment (related to
229 Fig. 4*E*) among rats of eNpHR3.0: mPFC–PN and EYFP: mPFC–PN groups. Numbers indicate the
230 anteroposterior coordinates from bregma. (*G–I*) Top: Training and illumination protocol. Rats were
231 eyeblink conditioned in the absence of optogenetic inhibition and tested 24 h later. Bottom:
232 Twenty-four hours after the acquisition training, optogenetic inhibition of the caudal mPFC axon
233 terminals in the right PN did not affect the peak amplitude (*G*), onset latency (*H*), or peak latency (*I*)
234 of CR with the sCS (N.S., not significant; two-tailed unpaired Student’s t-test). (*J,K*) The EMG
235 response topographies for eNpHR3.0: mPFC–PN and EYFP: mPFC–PN groups during test 1 (*J*)
236 and test 2 (*K*) were shown. (*L*) Same as (*F*), except rats from the other optogenetic inhibition
237 experiment (related to Fig. 4*F*). (*M–R*) Same as (*G–L*), except the optogenetic inhibition was
238 applied at thirty days after the acquisition training (related to Fig. 4*G*; N.S., not significant;
239 two-tailed unpaired Student’s t-test). The EMG amplitude is given as a percentage of the baseline
240 (100%) averaged EMG amplitude. Data are represented as mean \pm s.e.m.



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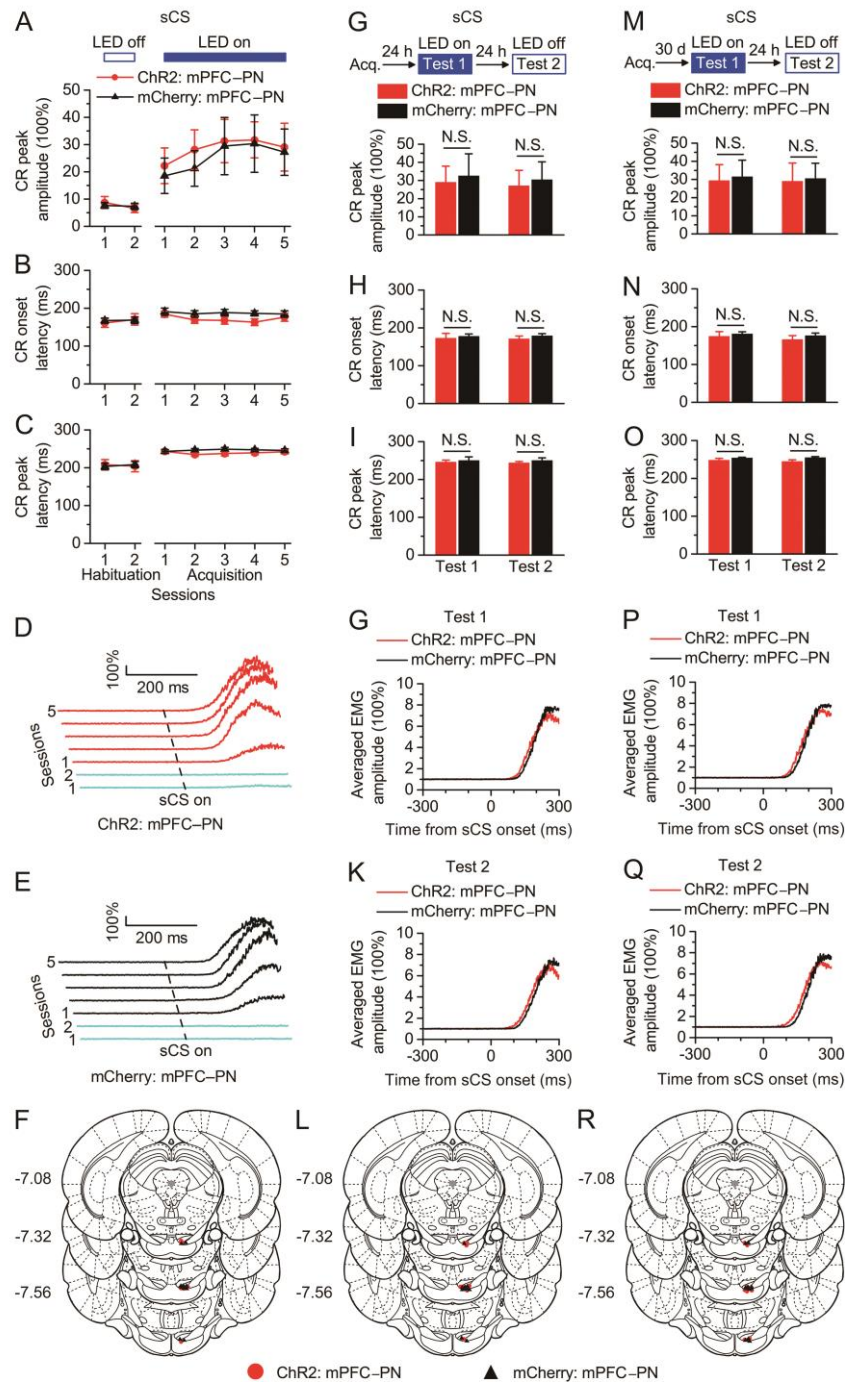
242 **Supplementary Figure 14.** Effects of optogenetic inhibition of the caudal mPFC axon terminals in
 243 the right PN on the peak amplitude, onset latency, and peak latency of CR with the wCS (related to
 244 Fig. 4H–J). (A–C) Top: Training and illumination protocol. Bottom: Optogenetic inhibition of the
 245 caudal mPFC axon terminals in the right PN during acquisition training affected the peak amplitude
 246 (A), onset latency (B), and peak latency (C) of CR with the wCS (* $P < 0.05$, ** $P < 0.01$, *** $P <$
 247 0.001 ; two-way ANOVA with repeated measures followed by Tukey post-hoc test). (D,E) The

248 EMG response topographies of eNpHR3.0: mPFC–PN (*D*) and EYFP: mPFC–PN (*E*) groups were
249 shown across two habituation, five acquisition, and five reacquisition sessions. (*F*) Approximate
250 locations of fiber optic cannula tips for optogenetic inhibition experiment (related to Fig. 4*H*)
251 among rats of eNpHR3.0: mPFC–PN and EYFP: mPFC–PN groups. Numbers indicate the
252 anteroposterior coordinates from bregma. (*G–I*) Top: Training and illumination protocol. Rats were
253 eyeblink conditioned in the absence of optogenetic inhibition and tested 24 h later. Bottom:
254 Twenty-four hours after the acquisition training, optogenetic inhibition of the bilateral caudal mPFC
255 affected the peak amplitude (*G*), but not onset latency (*H*) or peak latency (*I*) of CR with the sCS
256 (N.S., not significant, $**P < 0.01$; two-tailed unpaired Student's t-test). (*J,K*) The EMG response
257 topographies for eNpHR3.0: mPFC–PN and EYFP: mPFC–PN groups during test 1 (*J*) and test 2
258 (*K*) were shown. (*L*) Same as (*F*), except rats from the other optogenetic inhibition experiment
259 (related to Fig. 4*I*). (*M–R*) Same as (*G–L*), except the optogenetic inhibition was applied at thirty
260 days after the acquisition training (related to Fig. 4*J*; N.S., not significant, $**P < 0.01$; two-tailed
261 unpaired Student's t-test). The EMG amplitude is given as a percentage of the baseline (100%)
262 averaged EMG amplitude. Data are represented as mean \pm s.e.m.



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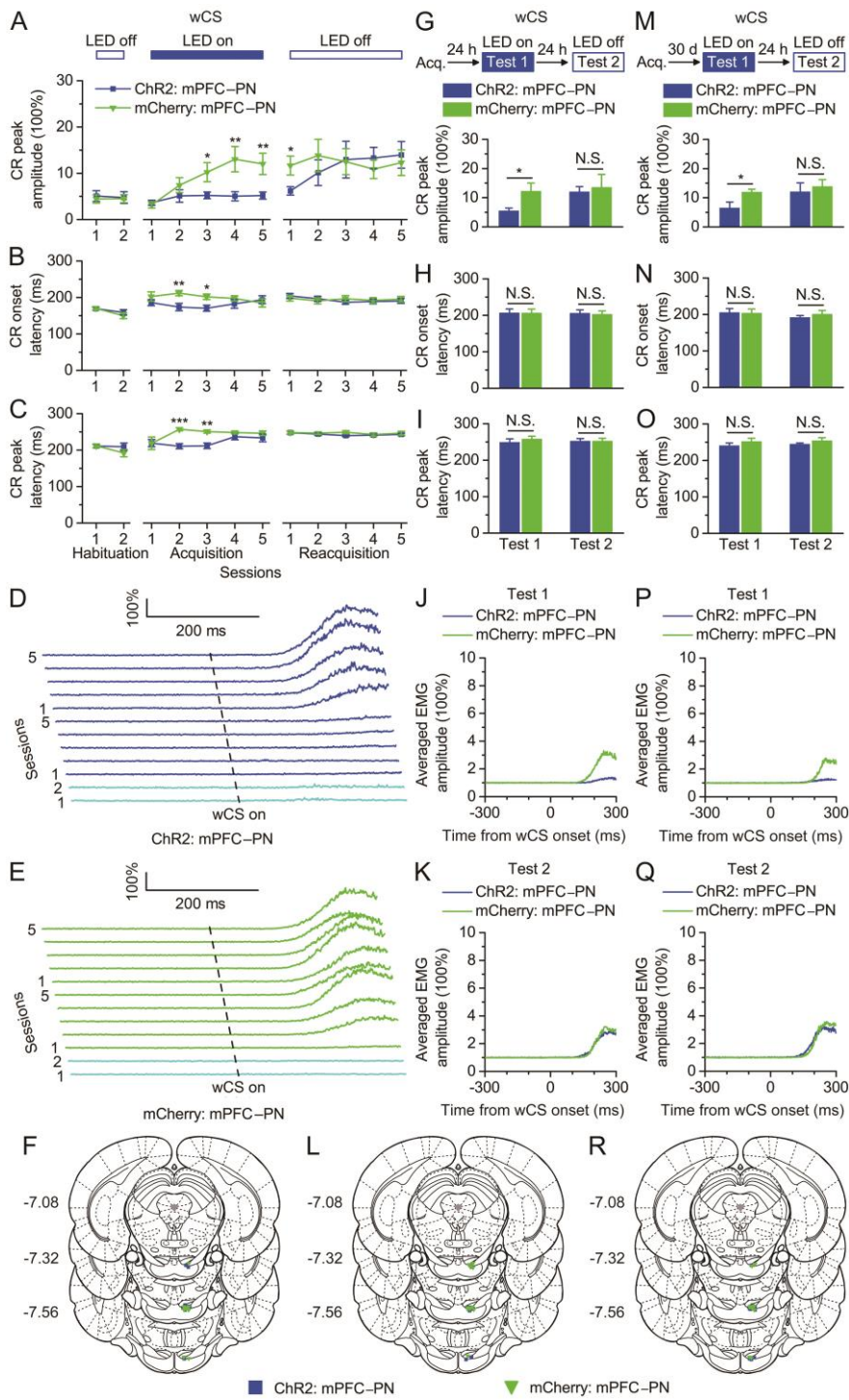
264 **Supplementary Figure 15.** 470-nm LED illumination of the axon terminals of
 265 ChR2-mCherry-expressing caudal mPFC neurons in the PN evoked activation of the right PN. (A)
 266 Schematic illustration of *in vivo* optical stimulation and recording in the right PN. (B,C) Local
 267 spikes in the right PN were induced by trains of 25 light pulses (470 nm, 25 mW/mm², 100 Hz, 5
 268 ms pulse duration) to the caudal mPFC axon terminals in the right PN of head-fixed anesthetized
 269 rats injected with pAAV 2/9-CaMKII α -ChR2-mCherry into the bilateral caudal mPFC. Note that the
 270 spikes were not elicited with every light pulse. (D,E) Trains of 25 light pulses (470 nm, 25
 271 mW/mm², 100 Hz, 5 ms pulse duration) also evoked robust LFP responses in the right PN of a
 272 wake behaving rats. Note that the graph (D) illustrates an example of the mean value of 120
 273 light-induced LFPs.



274

275 **Supplementary Figure 16.** Effects of optogenetic activation of the caudal mPFC axon terminals in
 276 the right PN on the peak amplitude, onset latency, and peak latency of CR with the sCS (related to
 277 Fig. 5E-G). (A-C) Top: Training and illumination protocol. Bottom: Optogenetic activation of the
 278 caudal mPFC axon terminals in the right PN during acquisition training also failed to produced
 279 deficits in the peak amplitude (A), onset latency (B), or peak latency (C) of CR with the sCS
 280 (two-way ANOVA with repeated measures). (D,E) The EMG response topographies of ChR2:

281 mPFC–PN (**D**) and mCherry: mPFC–PN (**E**) groups were shown across two habituation and five
282 acquisition sessions. (**F**) Approximate locations of optrode tips for optogenetic activation
283 experiment (related to Fig. 5E) among rats of Chr2: mPFC–PN and mCherry: mPFC–PN groups.
284 Numbers indicate the anteroposterior coordinates from bregma. (**G–I**) Top: Training and
285 illumination protocol. Rats were eyeblink conditioned in the absence of optogenetic activation and
286 tested 24 h later. Bottom: Twenty-four hours after the acquisition training, optogenetic activation of
287 the caudal mPFC axon terminals in the right PN also did not affect the peak amplitude (**G**), onset
288 latency (**H**), or peak latency (**I**) of CR with the sCS (N.S., not significant; two-tailed unpaired
289 Student’s t-test). (**J,K**) The EMG response topographies for Chr2: mPFC–PN and mCherry:
290 mPFC–PN groups during test 1 (**J**) and test 2 (**K**) were shown. (**L**) Same as (**F**), except rats from the
291 other optogenetic activation experiment (related to Fig. 5F). (**M–R**) Same as (**G–L**), except the
292 optogenetic activation was applied at thirty days after the acquisition training (related to Fig. 5G;
293 N.S., not significant; two-tailed unpaired Student’s t-test). The EMG amplitude is given as a
294 percentage of the baseline (100%) averaged EMG amplitude. Data are represented as mean \pm s.e.m.



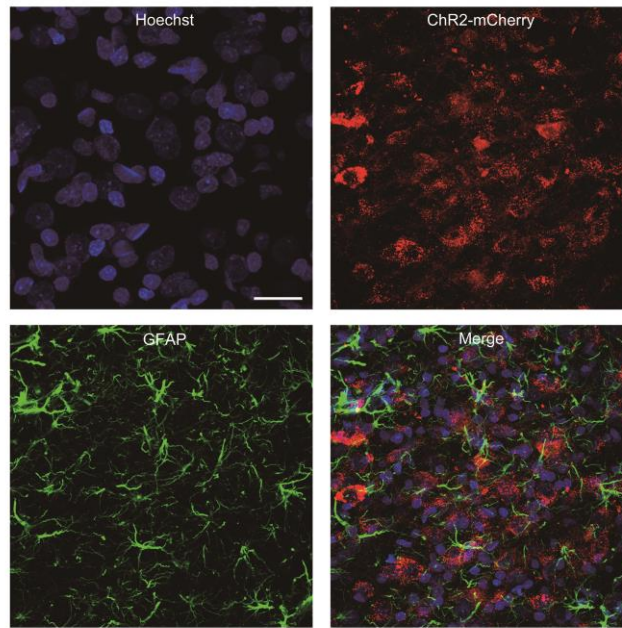
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296 **Supplementary Figure 17.** Effects of optogenetic activation of the caudal mPFC axon terminals in
 297 the right PN on the peak amplitude, onset latency, and peak latency of CR with the wCS (related to
 298 Fig. 5H–J). (A–C) Top: Training and illumination protocol. Bottom: Optogenetic activation of the
 299 caudal mPFC axon terminals in the right PN during acquisition training produced significantly
 300 deficits in the peak amplitude (A), onset latency (B), and peak latency (C) of CR with the wCS ($*P <$
 301 0.05 , $**P < 0.01$, $***P < 0.001$; two-way ANOVA with repeated measures followed by Tukey

302 post-hoc test). (*D,E*) The EMG response topographies of ChR2: mPFC–PN (*D*) and mCherry:
303 mPFC–PN (*E*) groups were shown across two habituation, five acquisition, and five reacquisition
304 sessions. (*F*) Approximate locations of optrode tips for optogenetic activation experiment (related to
305 Fig. 5*H*) among rats of ChR2: mPFC–PN and mCherry: mPFC–PN groups. Numbers indicate the
306 anteroposterior coordinates from bregma. (*G–I*) Top: Training and illumination protocol. Rats were
307 eyeblink conditioned in the absence of optogenetic activation and tested 24 h later. Bottom:
308 Twenty-four hours after the acquisition training, optogenetic activation of the caudal mPFC axon
309 terminals in the right PN affected the peak amplitude (*G*), but not onset latency (*H*), or peak latency
310 (*I*) of CR with the wCS (N.S., not significant, $*P < 0.05$; two-tailed unpaired Student's t-test). (*J,K*)
311 The EMG response topographies for ChR2: mPFC–PN and mCherry: mPFC–PN groups during test
312 1 (*J*) and test 2 (*K*) were shown. (*L*) Same as (*F*), except rats from the other optogenetic activation
313 experiment (related to Fig. 5*I*). (*M–R*) Same as (*G–L*), except the optogenetic activation was applied
314 at thirty days after the acquisition training (related to Fig. 5*J*; N.S., not significant, $*P < 0.05$;
315 two-tailed unpaired Student's t-test). The EMG amplitude is given as a percentage of the baseline
316 (100%) averaged EMG amplitude. Data are represented as mean \pm s.e.m.

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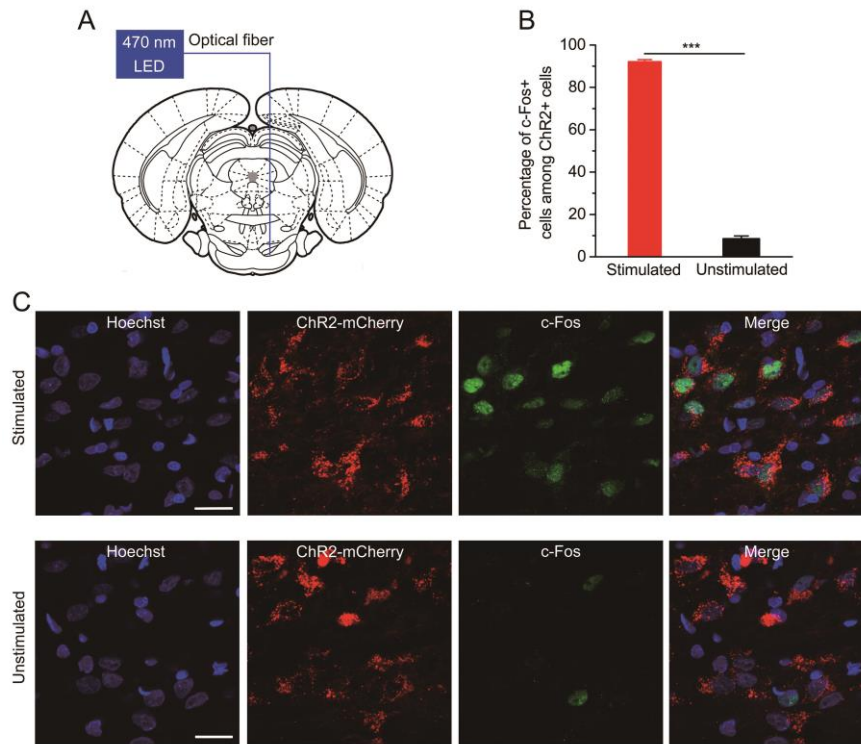


319

320 **Supplementary Figure 18.** GFAP- and ChR2-mCherry-expressing cells did not overlap
321 significantly. High-magnification view reveals membrane localization of ChR2-mCherry. No
322 obvious overlap was detected between ChR2-mCherry-expressing neurons (red) and
323 GFAP-expressing neurons (astrocytes, green). Scale bar, 20 μm .

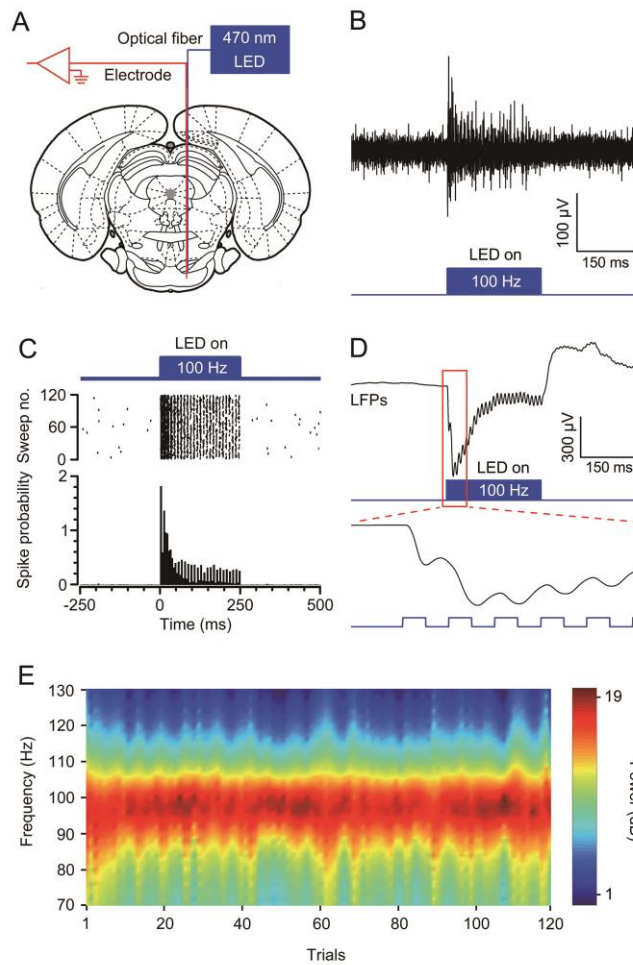
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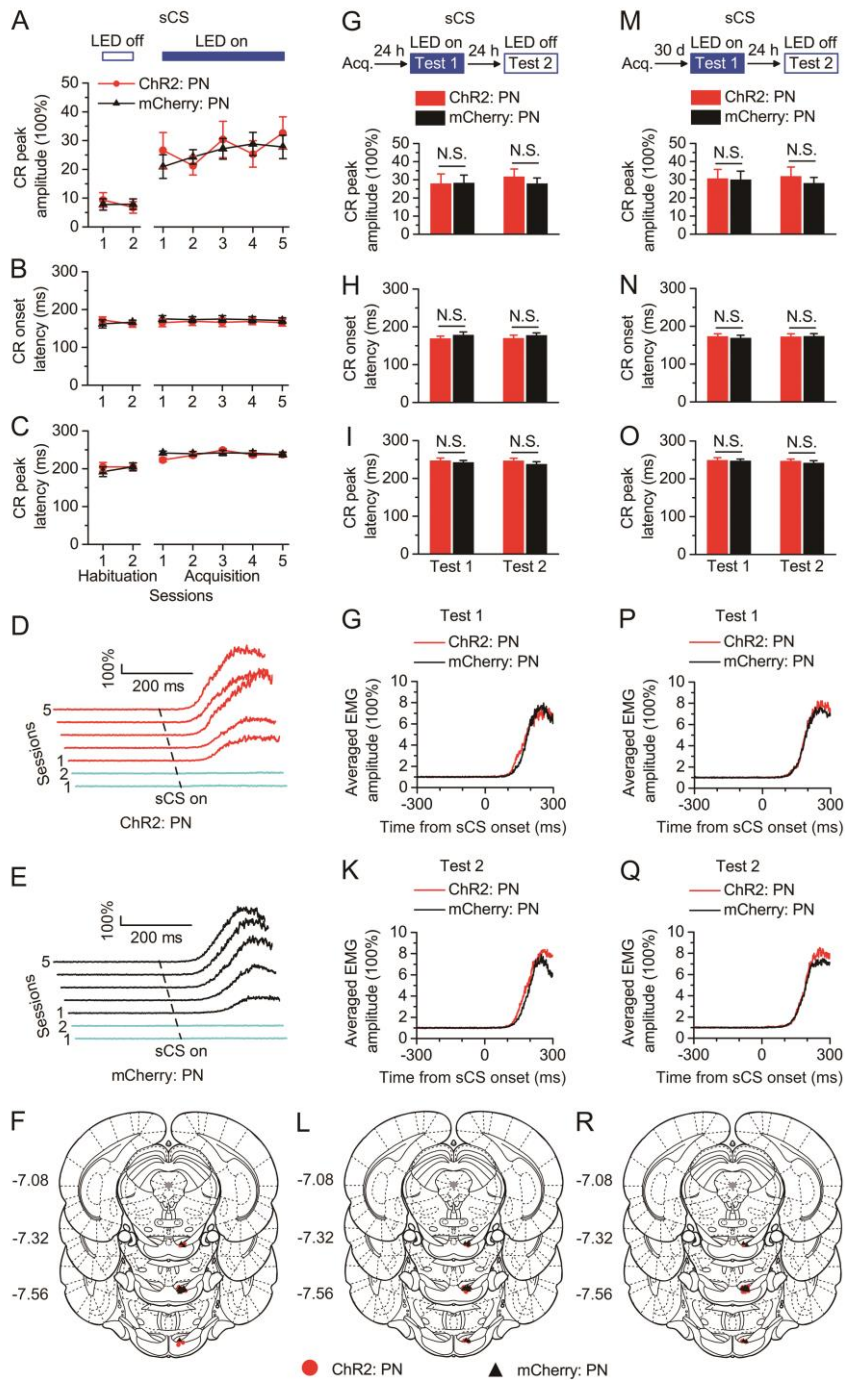
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327 **Supplementary Figure 19.** 470-nm LED stimulation induced c-Fos expression in cells expressing
 328 ChR2-mCherry in the right PN. (A) Schematic illustration of optical stimulation of
 329 ChR2-mCherry-expressing cells in right PN. (B–D) Rats expressing ChR2-mCherry (red) in the
 330 right PN were treated with or without 470-nm LED stimulation and the expression of c-Fos (green)
 331 was examined. Representative magnification images of the right PN for the light stimulated group
 332 (C) and unstimulated group (D) are shown. Scale bar, 20 μm. Quantification of c-Fos positive cells
 333 among ChR2 positive cells is shown in (B).



334

335 **Supplementary Figure 20.** 470-nm LED illumination evoked activation of
 336 ChR2-mCherry-expressing cells in the right PN. (A) Schematic showing *in vivo* optical stimulation
 337 and recording in the right PN. (B,C) Trains of 25 light pulses (470 nm, 15 mW/mm², 100 Hz, 5 ms
 338 pulse duration) induced reliable spikes in the right PN neurons recorded from head-fixed
 339 anesthetized rats injected with pAAV 2/9-hSyn-ChR2-mCherry. (D,E) Trains of 25 light pulses (470
 340 nm, 15 mW/mm², 100 Hz, 5 ms pulse duration) also evoked robust LFP responses in the right PN in
 341 awake behaving rats. Note that the (D) graph illustrates an example of the mean value of 120
 342 light-induced LFPs.



343

344 **Supplementary Figure 21.** Effects of optogenetic activation of the right PN on the peak amplitude,

345 onset latency, and peak latency of CR with the sCS (related to Fig. 6F–H). (A–C) Top: Training and

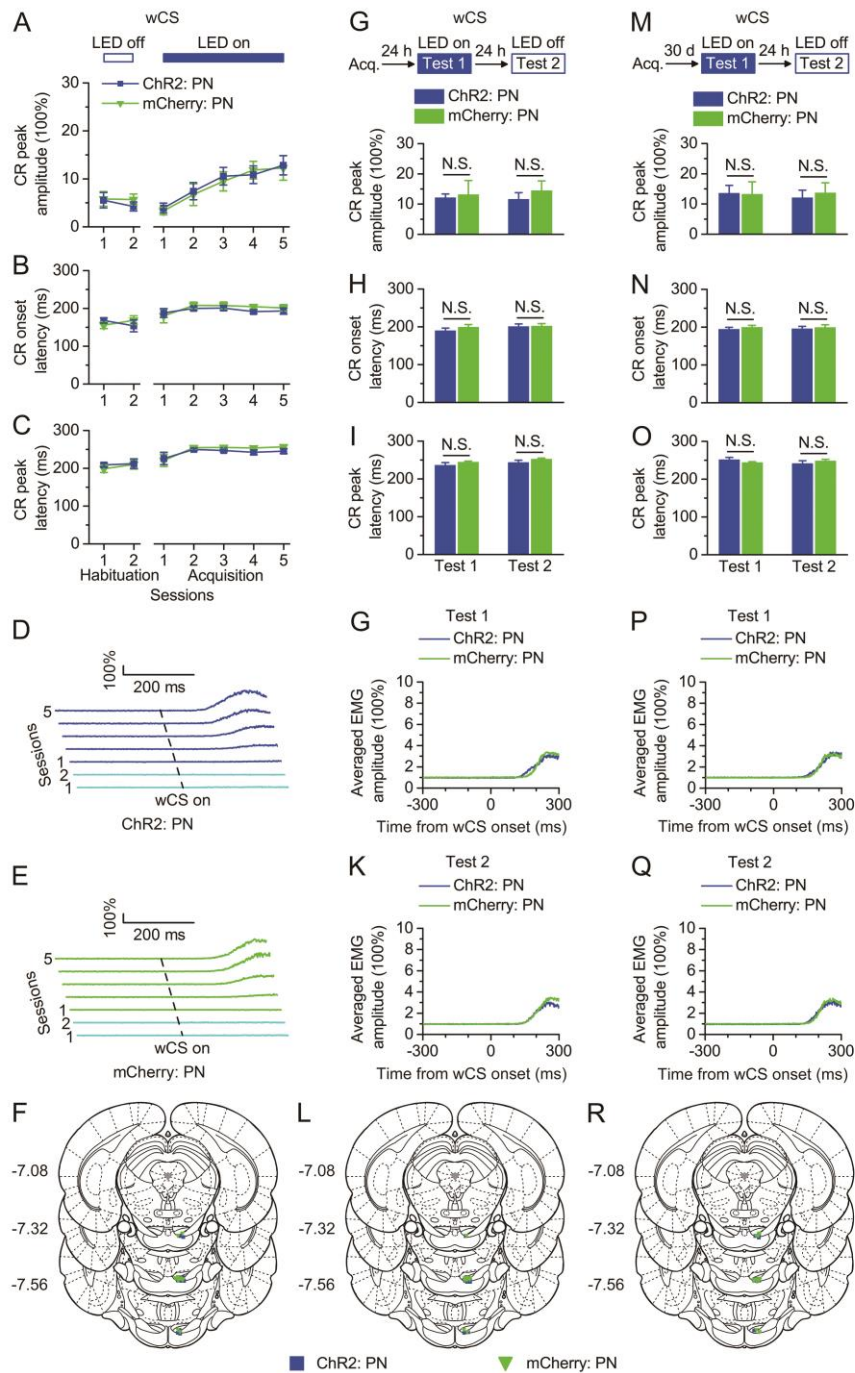
346 illumination protocol. Bottom: Optogenetic activation of the right PN during acquisition training

347 failed to caused deficits in the peak amplitude (A), onset latency (B), or peak latency (C) of CR with

348 the sCS (two-way ANOVA with repeated measures). (D,E) The EMG response topographies of

349 ChR2: PN (D) and mCherry: PN (E) groups were shown across two habituation and five acquisition

350 sessions. (*F*) Approximate locations of optrode tips for optogenetic activation experiment (related to
351 Fig. 6*F*) among rats of ChR2: PN and mCherry: PN groups. Numbers indicate the anteroposterior
352 coordinates from bregma. (*G–I*) Top: Training and illumination protocol. Rats were eyeblink
353 conditioned in the absence of optogenetic activation and tested 24 h later. Bottom: Twenty-four
354 hours after the acquisition training, optogenetic activation of the right PN also did not affect the
355 peak amplitude (*G*), onset latency (*H*), or peak latency (*I*) of CR with the sCS (N.S., not significant;
356 two-tailed unpaired Student's t-test). (*J,K*) The EMG response topographies for ChR2: PN and
357 mCherry: PN groups during test 1 (*J*) and test 2 (*K*) were shown. (*L*) Same as (*F*), except rats from
358 the other optogenetic activation experiment (related to Fig. 6*G*). (*M–R*) Same as (*G–L*), except the
359 optogenetic activation was applied at thirty days after the acquisition training (related to Fig. 6*H*;
360 N.S., not significant; two-tailed unpaired Student's t-test). The EMG amplitude is given as a
361 percentage of the baseline (100%) averaged EMG amplitude. Data are represented as mean \pm s.e.m.



362

363 **Supplementary Figure 22.** Effects of optogenetic activation of the right PN on the peak amplitude,
 364 onset latency, and peak latency of CR with the wCS (related to Fig. 6I–K). (A–F) Top: Training and
 365 illumination protocol. Bottom: Optogenetic activation of the right PN during acquisition training
 366 failed to caused deficits in the peak amplitude (A), onset latency (B), or peak latency (C) of CR with
 367 the wCS (two-way ANOVA with repeated measures). (D,E) The EMG response topographies of
 368 ChR2: PN (D) and mCherry: PN (E) groups were shown across two habituation and five acquisition

369 sessions. (*F*) Approximate locations of optrode tips for optogenetic activation experiment (related to
370 Fig. 6*J*) among rats of ChR2: PN and mCherry: PN groups. Numbers indicate the anteroposterior
371 coordinates from bregma. (*G–I*) Top: Training and illumination protocol. Rats were eyeblink
372 conditioned in the absence of optogenetic activation and tested 24 h later. Bottom: Twenty-four
373 hours after the acquisition training, optogenetic activation of the right PN also did not affect the
374 peak amplitude (*G*), onset latency (*H*), or peak latency (*I*) of CR with the wCS (N.S., not significant;
375 two-tailed unpaired Student's t-test). (*J,K*) The EMG response topographies for ChR2: PN and
376 mCherry: PN groups during test 1 (*J*) and test 2 (*K*) were shown. (*L*) Same as (*F*), except rats from
377 the other optogenetic activation experiment (related to Fig. 6*J*). (*M–R*) Same as (*G–L*), except the
378 optogenetic activation was applied at thirty days after the acquisition training (related to Fig. 6*K*;
379 N.S., not significant; two-tailed unpaired Student's t-test). The EMG amplitude is given as a
380 percentage of the baseline (100%) averaged EMG amplitude. Data are represented as mean \pm s.e.m.