

1 Cell-Specific synaptic Plasticity Induced by Network Oscillations

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19 20 Abstract

21 Gamma rhythms are known to contribute to the process of memory encoding. However, little
22 is known about the underlying mechanisms at the molecular, cellular and network levels.
23 Using local field potential recording in awake behaving mice and concomitant field-potential
24 and whole-cell recordings in slice preparations we found that gamma rhythms lead to activity-
25 dependent modification of hippocampal networks, including alterations in sharp wave-ripple
26 complexes. Network plasticity, expressed as long-lasting increases in sharp wave-associated
27 synaptic currents, exhibits enhanced excitatory synaptic strength in pyramidal cells that is
28 induced postsynaptically and depends on metabotropic glutamate receptor-5 activation. In

29 sharp contrast, alteration of inhibitory synaptic strength is independent of postsynaptic
30 activation and less pronounced. Further, we found a cell type-specific, directionally biased
31 synaptic plasticity of two major types of GABAergic cells, parvalbumin- and cholecystokinin-
32 expressing interneurons. Thus, we propose that gamma frequency oscillations represent a
33 network state that introduces long-lasting synaptic plasticity in a cell-specific manner.

34

35 **Introduction**

36 Neural oscillations are thought to play an important role in learning and memory processing
37 (Axmacher et al., 2006; Düzel et al., 2010; Nyhus and Curran, 2010). Learning is based on
38 activity-dependent modification of synaptic strength in order to incorporate transient
39 experiences into persistent memory traces (Citri and Malenka, 2008). Gamma-band and sharp
40 wave-associated ripple oscillations (SWRs), involved in memory encoding (Jutras and
41 Buffalo, 2010) and consolidation (Buzsáki, 1989; Girardeau et al., 2009; Jadhav et al., 2012),
42 respectively, appear to be interlinked in the course of memory processing. However, to date, it
43 had been unclear whether gamma rhythms itself represent a network state that can directly
44 promote the formation of long-lasting synaptic plasticity within the cortical network.

45 The hippocampus is an important structure for memory acquisition, consolidation and
46 spatial orientation (Buzsáki and Moser, 2013; Eichenbaum and Cohen, 2014). Within the
47 hippocampus, the CA3 area constitutes an autoassociative neural network, in which three
48 pathways converge: mossy fibers and associational-commissural (A/C) and perforant path
49 (PP) projections. Each of these pathways display plasticity (Berzhanskaya et al., 1998;
50 Malenka and Bear, 2004; Nicoll and Schmitz, 2005), which has seen the CA3 area harnessed
51 as a well-suited and popular model for studying activity-dependent modification of synaptic
52 transmission. However, hippocampal neural plasticity phenomena have traditionally been
53 studied using tetanus-, pairing- or chemically induced plasticity-protocols, while the

54 applicability of network oscillations, such as used in our study, as investigative tool had been
55 unproven to date.

56 GABAergic interneurons (INs) have been shown to play a major role in controlling
57 oscillatory activity, as well as synaptic transmission and plasticity in cortical networks (Ben-
58 Ari, 2006; Klausberger and Somogyi, 2008). Major inhibitory cell types, parvalbumin- (PV)
59 and cholecystokinin (CCK)-expressing INs provide distinct forms of inhibition and have
60 complementary roles in cortical circuits. In fact, PV-expressing INs are assumed to act as
61 “fast signaling devices” (Jonas et al., 2004) and provide precisely-timed phase-modulated
62 inhibition to control the timing of discharge in individual neurons as well as the
63 synchronization and emergence of oscillations at the level of the network (Cobb et al., 1995;
64 Gloveli et al., 2005a; Bartos et al., 2007; Sohal et al., 2009). In contrast, CCK-expressing INs
65 show a slower but variable discharge pattern and asynchronous GABA release (Hefft and
66 Jonas, 2005; Daw et al., 2009). High expression of various receptors for neuromodulators
67 (Freund, 2003; Armstrong and Soltesz, 2012) post- and presynaptically, suggests that these
68 INs may regulate excitability in the network by mediating inhibition in a behavioral state-
69 dependent manner.

70 We investigated the interaction and interdependence of two classical hippocampal
71 network patterns, gamma frequency oscillations and SWRs, important in memory encoding
72 and consolidation, respectively. We found that *in vivo* theta-nested gamma oscillations have
73 an enhancing effect on subsequent SWRs in awake behaving mice. Analysis of the underlying
74 molecular, cellular and synaptic mechanisms in *in vitro* slice preparations showed changes in
75 SWR-associated excitatory synaptic strength between pyramidal cells (PC) that are mediated
76 postsynaptically and depend on metabotropic glutamate receptor-5 (mGluR5) activation. In
77 stark contrast to excitation, alteration of inhibitory synaptic strength was independent of
78 postsynaptic activation and less pronounced, reflecting an IN-specific, directionally biased
79 synaptic plasticity, as demonstrated in our study for two major GABAergic inhibitory cell

80 types, PV- and CCK-expressing INs. Our results suggest that gamma frequency oscillations
81 represent a network state that promotes the formation of long-lasting synaptic plasticity in the
82 hippocampal area CA3, leading to modification of synaptic strengths in a cell-specific
83 manner.

84

85 **Results**

86 **Theta-nested gamma frequency oscillations reinforce subsequent SWRs in awake** 87 **behaving mice**

88

89 We investigated two major context-dependent activity patterns, SWRs and gamma frequency
90 oscillations, in awake behaving mice. Using local field potential (LFP) recordings from dorsal
91 hippocampus we found spontaneous SWRs in resting states of quietly sitting mice, while
92 running behavior was accompanied by theta-nested gamma oscillations (**Figure 1A**). As both
93 rhythms have been proposed as closely linked to memory processing (Axmacher et al., 2006;
94 Girardeau et al., 2009; Jadhav et al., 2012; Jutras and Buffalo, 2010), we studied the general
95 interdependence of these two network patterns in a behavioral paradigm.

96 As the two rhythms are each correlated with separate behavioral states (resting or
97 running), control of behavioral expression is a means of targeting the corresponding network
98 pattern (**Figure 1A and 1B**). Thus, we used prolonged running episodes, at an average of 3
99 minutes, (mean $3:06 \pm 0:32$ min, $n = 11$, 4 mice; range from 2:28 to 4:30 min depending on
100 running performance), to investigate the interaction between the two network patterns. The
101 impact of running-associated theta-nested gamma frequency oscillations on subsequent SWRs
102 was evaluated by comparing the areas of SWRs in the two-minute time windows directly
103 preceding and following a theta-nested gamma episode. The post-gamma SWR (p-SWR)
104 areas exhibited significant enlargement at an average of 25.7% ($n = 11$, $P = 0.0006$, **Figure**
105 **1C**) and no significant change in frequency (SWR: 0.18 ± 0.10 Hz; p-SWR: 0.16 ± 0.10 Hz, n
106 = 11, $P = 0.23$). This result indicates an enhanced network activity and suggests a surprisingly

107 direct effect of the running associated theta-nested gamma oscillations. But the fact that the
108 internal state might not be fixed for an extended time period hampered conclusive
109 interpretation of this result. In particular, the altered p-SWR could also reflect a change of the
110 animals internal state including the contribution of a different set of cell assemblies (Buzsáki,
111 2015), which is difficult to control *in vivo*. Consequently, in order to carry out the
112 experiments under well-controlled conditions with the option to investigate the underlying
113 mechanism in detail, we switched to a well-established *in vitro* model.

114

115 **Gamma frequency oscillations promote long-lasting network changes in acute slice** 116 **preparations**

117

118 We subsequently investigated the synaptic, cellular and molecular mechanisms of gamma
119 oscillation-induced effects on SWR activity in *in vitro* acute hippocampal slices, a model that
120 permits the reproduction of both oscillatory network patterns (Dugladze et al., 2012; Gloveli
121 et al., 2005a, 2005b; Maier et al., 2009).

122 Prompted by our *in vivo* results, we investigated the interaction and interdependence of
123 gamma frequency oscillations and SWRs by monitoring LFPs in the stratum pyramidale of
124 the hippocampal area CA3. In good agreement with our *in vivo* results, SWRs and gamma
125 frequency oscillation patterns represented two ‘competing’, mutually exclusive network states
126 *in vitro*: spontaneously occurring SWRs (mean frequency: 1.33 ± 0.10 Hz, $n = 30$)
127 disappeared shortly (31.0 ± 2.8 s) after bath application of kainic acid (KA, 400 nM) and
128 reappeared within a few minutes (14.6 ± 0.7 min) after KA washout (**Figure 2A**). However,
129 also in line with our *in vivo* data, the two network patterns were not fully independent –
130 plastic changes initiated in the network by means of persistent gamma activity altered the
131 subsequent SWR pattern (**Figure 2A and 2B**). The p-SWRs exhibited a significantly
132 increased area (by $69.7 \pm 15.1\%$, $n = 30$, $P < 0.0001$, **Figure 2C**) and a small decrease in
133 incidence (to 0.90 ± 0.10 Hz, $n = 30$, $P < 0.0001$). In addition, we found a slight but significant

134 increase in average ripple number (by $8.5 \pm 2.5\%$, $n = 12$, $P = 0.0065$) and an elevated
135 oscillatory ripple frequency (by $4.1 \pm 1.8\%$, $n = 12$, $P = 0.041$). These changes were
136 accompanied by a significant increase of post-gamma sharp wave amplitude (by $44.6 \pm$
137 19.3% , $n = 12$, $P = 0.042$) and a non-significantly altered sharp wave duration (increased by
138 $2.5 \pm 2.9\%$, $n = 12$, $P = 0.41$). In good agreement with these data, gamma oscillations induced
139 by bath application of carbachol ($20 \mu\text{M}$), an alternative drug to trigger persistent gamma
140 oscillations based on a different mechanisms (Fisahn et al., 1998; Fisahn et al., 2002; Hajos et
141 al., 2004), also resulted in a significant increase in SWR area (by $21.0 \pm 4.2\%$, $n = 16$, $P =$
142 0.0002 , see also Zylla et al., 2013), indicating that gamma activity itself, and not the
143 pharmacological agent, is responsible for the network alterations. Moreover, we found a
144 highly significant positive correlation of the network gamma oscillations (power \times duration)
145 with the SWR-area increase ($R = 0.58$, $n = 30$, $P = 0.0007$) and no correlation with the SWR
146 incidence ($R = -0.28$, $n = 30$, $P = 0.14$). In line with this, in a few cases where KA application
147 failed to introduce gamma frequency oscillations, no changes in SWR-area were observed
148 (reduction by $6.0 \pm 19.4\%$, $n = 8$, $P = 0.35$). Notably, in comparison to KA, carbachol-
149 triggered gamma oscillations exhibited less spectral power ($P = 0.007$) and accordingly
150 induced smaller SWR area changes ($P = 0.02$), suggesting an activity-dependent mechanism
151 of oscillation-induced neuronal network plasticity. Furthermore, we also found a reinforcing
152 effect of gamma rhythms on subsequent gamma episodes (**Figure 3**). Together, these results
153 emphasize the general potential of gamma oscillations to modify network activities.

154 To reveal the molecular mechanism underlying the network plasticity we examined the
155 effects of activation of metabotropic glutamate (mGluR)5- and/or N-methyl-D-aspartate
156 receptors (NMDAR) that have been proposed to play an important role in hippocampal
157 synaptic plasticity and memory (Zalutsky and Nicoll, 1990; Nakazawa et al., 2002; Naie and
158 Manahan-Vaughan, 2004). Administration of mGluR5 antagonist, 2-methyl-6-
159 (phenylethynyl)pyridine (MPEP) largely reduced the SWR area increase (by $83.4 \pm 6.2\%$, $n =$

160 16, $P < 0.0001$, **Figure 2C and 2E**). A similar, albeit less pronounced effect was observed for
161 the NMDAR antagonist, DL-2-Amino-5-phosphonopentanoic acid (AP5) (reduction by $51.7 \pm$
162 8.9% , $n = 13$, $P < 0.0001$, **Figure 2D and 2E**). Finally, this form of plasticity was abolished
163 by joint application of the mGluR5 and NMDAR antagonists (reduction by $91.8 \pm 12.2\%$, $n =$
164 14 , $P < 0.0001$, **Figure 2D and 2E**). Taken together, these results suggest that in the
165 hippocampal area CA3, gamma frequency oscillations influence the subsequent network
166 activity through mGluR5- and NMDAR-dependent mechanisms.

167

168 **Gamma frequency oscillations support long-lasting synaptic plasticity in the CA3** 169 **network**

170

171 We next studied activity-dependent alteration in synaptic transmission, defined here by
172 synaptic strength, in CA3 PC by examining long-lasting changes in p-SWR-associated
173 excitatory and inhibitory synaptic currents (p-EPSCs and p-IPSCs, **Figure 4**). Our data
174 demonstrate that parallel to the field p-SWR (**Figure 2**), cells held in current-clamp mode
175 during gamma oscillations (see Materials and Methods) exhibit long-lasting increase in the
176 area of p-EPSCs (by $104.2 \pm 21.2\%$, $n = 14$, $P = 0.0003$, **Figure 4B**). The change in the EPSC
177 strength correlates positively with the magnitude of SWR-area increase ($R = 0.56$, $n = 14$, $P =$
178 0.040). Conversely, p-EPSCs recorded in PCs held in voltage-clamp mode at -70 mV during
179 gamma oscillations decreased rather than increased (by $40.6 \pm 7.7\%$, $n = 13$, $P = 0.0002$,
180 **Figure 4B**), suggesting that the increase of EPSC area depend on PC postsynaptic
181 depolarization. The p-EPSC in voltage-clamp mode might also be affected by altered
182 inhibition (see below).

183 To clarify the underlying molecular mechanism, we compared the properties of these
184 currents in the presence and absence of mGluR5 antagonist MPEP. Similar to the effects on
185 the LFP, bath application of MPEP strongly reduced the increase in p-EPSC area of CA3 PCs
186 (reduction by $90.3 \pm 7.1\%$, $n = 6$, $P = 0.0001$, **Figure 4B**). These data provide direct evidence

187 for an mGluR5-dependent increase of the excitatory synaptic strength onto PCs as an effect of
188 an intermediate gamma episode. Close temporal correlation existed between these changes
189 and the network alterations described above.

190 We further investigated whether oscillatory gamma activity-dependent modification of
191 the hippocampal network also includes alteration in inhibitory synaptic strength. In contrast to
192 the strongly potentiated p-EPSC, p-IPSCs showed a less pronounced but still significant
193 increase ($32.8 \pm 12.9\%$, $n = 8$, $P = 0.039$, **Figure 4C**). Altogether, p-EPSC and p-IPSC
194 alterations resulted in a significant increase in the PC EPSC-to-IPSC ratio (by $44.4 \pm 14.6\%$,
195 $P = 0.019$, **Figure 4D**). In stark contrast to the EPSC potentiation, changes in IPSCs were
196 independent of postsynaptic activation. PCs held in voltage-clamp mode during gamma
197 oscillations exhibited a similar IPSC increase of $30.3 \pm 7.3\%$ ($n = 6$, $P = 0.0089$, **Figure 4C**),
198 indicating activity-dependent changes in presynaptic inhibitory INs.

199

200 **Gamma rhythms promote cell type-specific synaptic plasticity in CA3 interneurons**

201

202 To further elucidate the cellular mechanisms underlying the differential alterations in PC
203 excitatory and inhibitory synaptic strength we examined the gamma frequency-dependent
204 changes in two major inhibitory cell types: fast spiking PV-expressing INs targeting
205 perisomatic or proximal dendritic domains of PCs and regular spiking CCK-containing
206 perisomatic targeting cells. Fast-spiking PV-positive cells (**Figure 5A**) showed strong
207 potentiation of EPSCs (increased by $89.8 \pm 20.2\%$, $n = 12$, $P = 0.0010$), whereas IPSCs
208 decreased slightly but significantly ($n = 5$, $P = 0.038$, **Figure 5B**). Combined, the latter
209 translated into a significant rise in the EPSC-to-IPSC ratio (from 3.78 ± 0.83 to 7.71 ± 1.39 , n
210 $= 5$, $P = 0.012$, **Figure 5B**). Similarly to the effect on PCs, application of mGluR5 antagonist
211 MPEP strongly reduced the increase in p-EPSC area of PV-expressing INs (reduction by 84.6
212 $\pm 4.2\%$, $n = 5$, $P = 0.0001$, **Figure 5B**). Markedly different, even inverse alterations were
213 observed in the regular firing CCK-containing perisomatic targeting INs (**Figure 6A**): IPSCs

214 were increased (by $61.01 \pm 16.0\%$, $n = 5$, $P = 0.019$), whereas EPSCs showed no change
215 (reduction by $0.1 \pm 10.6\%$, $n = 8$, $P = 0.99$). As such, the EPSC-to-IPSC ratio was
216 significantly reduced in these INs (from 2.14 ± 0.39 to 1.21 ± 0.26 , $n = 5$, $P = 0.024$, **Figure**
217 **6B**). These data, quantified by alterations in the EPSC-to-IPSC ratio, demonstrate that gamma
218 frequency oscillations induce cell type-specific synaptic plasticity in the CA3 inhibitory
219 network, with enhanced net excitation of PV-expressing INs, but reduced activation of CCK-
220 expressing INs.

221

222 **Discussion**

223 We have demonstrated that gamma frequency oscillations induce activity-dependent and cell
224 type-specific synaptic plasticity in hippocampal area CA3. Moreover, our results illustrate the
225 impact of oscillatory gamma activity on SWRs, a network state associated with the process of
226 memory consolidation (Girardeau et al., 2009; Jadhav et al., 2012). The plastic changes
227 require mGluR5 mediated activation, indicating that this receptor might be critically involved
228 in memory processing.

229 We found that SWRs *in vivo* displayed a significantly enlarged area after a running
230 episode, indicating a reinforcing effect of the running associated theta-nested gamma
231 oscillations. Our data are well in line with a recent publication (Bittner et al., 2015)
232 demonstrating that the induction of new place-fields initiated during active running results in
233 altered neuronal activity during subsequent SWRs. While the oscillatory theta component
234 does not seem to be essential for the induction of plastic changes in the hippocampus *in vivo*
235 (Brandon et al., 2014), gamma rhythms are thought to constitute time windows of
236 synchronized neural activity that promote spike-time-dependent synaptic plasticity
237 (Axmacher et al., 2006) and enhance signal transmission (Sohal et al., 2009). In line with this,
238 our *in vitro* results clearly demonstrate that a gamma frequency episode significantly affects
239 subsequent network activities including gamma oscillations and SWRs. The gamma activity-

240 induced effect (SWR area increase) was independent of the pharmacologic agent (KA vs.
241 carbachol) used for their induction, but did correlate with the presence and power of gamma
242 frequency oscillations. Indeed, activity dependent increase of SWR amplitude was shown by
243 high-frequency electrical stimulation (Behrens et al., 2005). Thus, our results provide
244 comprehensive data that the gamma oscillations and not the pharmacologic agents themselves
245 (Zylla et al., 2013) are responsible for the observed network plasticity.

246 Different forms of plasticity have been described for the three excitatory input systems
247 converging on CA3 PCs: mossy fibers and associational-commissural (A/C) and perforant
248 path (PP) projections (Urban and Barrionuevo, 1996; McMahon and Barrionuevo, 2002;
249 Nakazawa et al., 2002; Kobayashi and Poo, 2004; Nicoll and Schmitz, 2005; Rebola et al.,
250 2011). However, crucially, the latter publications and similar studies on hippocampal
251 neuronal plasticity have certain methodological limitations. First, they were usually elicited
252 by high-frequency electrical stimulation of neurons providing afferent input, whereas the
253 predominant firing rate of CA3 PCs and their afferent neurons *in vivo* is far less frequent
254 (Hahn et al., 2007; Jung and McNaughton, 1993). Second, electrical stimulation was usually
255 limited to one of these inputs, whereas, in the intact hippocampal network, individual inputs
256 do not act in isolation, but converge onto postsynaptic cells. Thus, during physiological
257 activity patterns, such as gamma frequency oscillations, different inputs to CA3 PCs may act
258 synergistically, with their joint activity resulting in a specific alteration of synaptic strength.
259 Consequently, the here investigated oscillatory pattern might constitute a more physiological
260 paradigm that can elucidate network-dependent mechanisms of synaptic plasticity. With our
261 approach, we reveal a unique role of gamma frequency oscillations in activity-dependent
262 modification of hippocampal network. Our results highlight this oscillatory network rhythm
263 as a fundamental mechanism to induce synaptic plasticity and a potential primary driving
264 force for memory processing. Nevertheless, the specific role of an individual input for
265 gamma-dependent plasticity in hippocampal network remains to be clarified.

266 Our data lend further support to the hypothesis that, overall, the two major memory
267 relevant oscillatory patterns, gamma frequency oscillations and SWRs that are generated
268 during different behavioral states in freely moving animals (Chrobak et al., 2000), can be
269 considered two ‘competing’, mutually exclusive network states: spontaneous occurring SWRs
270 disappeared shortly after onset of gamma rhythms and reappeared after their termination, both
271 *in vivo* and *in vitro*. However, these two network patterns are not fully independent: plastic
272 changes initiated in the network during persistent gamma activity were reflected in a
273 subsequent altered SWR activity (Fig. 1 and Fig. 2). Consistent with the here observed tight
274 link of gamma oscillations and SWR activity, sleep-dependent memory consolidation is
275 associated with increased gamma activity (Ognjanovski et al., 2014) and cells active during
276 exploratory behavior exhibit enhanced SWR-associated EPSCs in subsequent slice
277 preparations (Mizunuma et al., 2014).

278 Our data suggests that mGluR5 is a key component of the process underlying the
279 observed plastic changes in the hippocampal CA3 network. In line with our findings,
280 impairment of both LTP and spatial learning as well as place field encoding of novel
281 environments induced by mGluR5 antagonists have been reported (Naie and Manahan-
282 Vaughan, 2004; Zhang and Manahan-Vaughan, 2014). Group I mGluRs, comprising of
283 mGluR5 and mGluR1, are preferentially expressed postsynaptically in CA3 PC dendrites
284 (Shigemoto et al., 1997). Even though NMDAR might be involved, our results show that
285 mGluR5 is more central in gamma network oscillation-induced synaptic plasticity. The effect
286 can only partially be explained by NMDAR-modulation, with mGluR5 obviously exerting a
287 more complex impact on the neuronal network dynamics, affecting both PC and IN activity.
288 Interestingly, dysregulation of mGluR5 has been reported in several profound neurological
289 disorders, such as schizophrenia (Conn et al., 2009; Nickols and Conn, 2014), autistic
290 spectrum disorders (Williams, 2012) and fragile X syndrome (Michalon et al., 2012),

291 altogether pointing towards a pivotal regulatory function for this receptor. Our results
292 highlight mGluR5 now in the general context of memory processing and neuronal plasticity.

293 In contrast to the postsynaptically mediated potentiation of PCs excitatory currents,
294 inhibitory currents only underwent minimal changes that were independent of postsynaptic
295 activation. These differences could be explained by a cell-specific, directionally biased
296 synaptic plasticity at PC-IN and IN-IN synapses as demonstrated for two major types of
297 GABAergic inhibitory cells, PV- and CCK-expressing INs. Gamma network oscillations alter
298 synaptic strength within PV-expressing INs in favor of excitation (Alle et al., 2001), while
299 CCK-expressing INs are subject to stronger inhibition, as demonstrated by the EPSC-to-IPSC
300 ratio analysis. Importantly, inhibition provided by these two types of GABAergic cells is not
301 uniform. Fast-spiking PV-INs mediate a rapid, phasic-form of inhibition, which contributes to
302 the precise timing of neuronal synchronization and emergence of network oscillations
303 (Gloveli et al., 2005a; Sohal et al., 2009; Schlingloff et al., 2014). In contrast, regular firing
304 CCK-INs mediate slower inhibition (Hefft and Jonas, 2005; Daw et al., 2009) and modulate
305 excitability in cortical networks in a behavioral state-dependent manner. Thus, the two IN
306 types mediate distinct forms of inhibition and could contribute differentially to cortical
307 network activity. Our data now suggest that divergent forms of synaptic plasticity observed in
308 these two IN types could result in a reduced tonic but increased phasic inhibition onto PC.
309 These changes in turn might lead to enhanced network excitability and promote synaptic
310 plasticity within the cortical circuits.

311 In summary, we conclude that gamma frequency oscillations represent a network state
312 responsible for activity-dependent and cell type-specific synaptic plasticity, interlinking two
313 memory-relevant network patterns, namely, gamma rhythms and SWRs.

314

315 **Materials and methods**

316 **Animals**

317 Experiments were performed on P27-P33 (*in vivo*) and P18-P23 (*in vitro*) C57/Bl6 mice. All
318 animal procedures were approved by the Regional Berlin Animal Ethics Committee (Permits:
319 G0151/12 and T 0124/05) and were in full compliance with national regulations.

320

321 **Local field potentials *in vivo***

322 We recorded LFP from head-fixed mice, a well-established approach that allowed us to
323 conduct prolonged running episodes. Mice were first implanted with a head-holder and a
324 recording chamber (1.5% isoflurane anesthesia) and then habituated to a spherical treadmill
325 for around 12 days. Afterwards, a small craniotomy (approx. 2.3 mm rostral-caudal and 2.5
326 mm lateral from bregma; 1.5% isoflurane anesthesia) was performed inside the recording
327 chamber and the exposed area covered with a layer of silicone elastomer (Kwik-Sil, World
328 Precision Instruments). The mouse was allowed to recover for at least 2 h before the recording
329 session started (one recording session per mouse).

330 LFP from the left hippocampus were recorded with glass pipettes, while we were
331 using the control of behavioral expression without a task-specific reward to target certain
332 network patterns. To investigate the impact of a theta-nested gamma episode on SWRs we
333 first waited for a prolonged resting period with a quietly sitting mouse on the spherical
334 treadmill allowing us to record spontaneous SWR activity. Then, once the mouse had begun
335 to move independently, a pressurized air stream was applied to the bottom of the Styrofoam
336 ball, resulting in a smooth ball rotation that encouraged a running behavior accompanied by
337 theta-nested gamma oscillations. If the mouse stopped running and began to balance the air-
338 supported ball instead, we accelerated the ball slightly until running behavior was restored,
339 maintaining an activity phase around 3 minutes in total depending on the actual running
340 performance. Turning off the air pressure usually terminated the running behavior and
341 initiated another resting period, once again accompanied by spontaneous occurring SWRs. In

342 order to reduce the stress level while maintaining the attentional component, some dummy
343 runs were performed prior to the final recording session.

344

345 **Matlab analysis of *in vivo* data**

346 All *in vivo* LFP data were analyzed in Matlab (MathWorks Inc., Natick, Massachusetts) by
347 means of custom-made routines. We compared the SWR areas of two time periods (120s
348 each) ending 10s before and starting 30s after the prolonged running episode. LFP recordings
349 were divided into a sharp wave (filtered 2-50Hz) and a ripple (filtered 100-300Hz) trace. We
350 used the ripple trace to automatically preselect SWRs based on a voltage and a spectral
351 threshold criterion. In detail, we first used a voltage threshold (mean plus six standard
352 deviations of event-free recording) for a primary selection of individual ripples and grouped
353 adjacent single ripples to a ripple event. We then took 70ms cutouts of the event-free
354 recording preceding those ripple events and calculated each maximum absolute wavelet
355 coefficients of the complex Morlet wavelet transform (27 wavelet scales, 20 kHz sampling
356 rate). We used the mean plus one standard deviation of this distribution as a spectral threshold
357 criterion and discarded all ripple events, in which the maximum absolute wavelet coefficient
358 did not exceed the threshold value. The preceding and subsequent local minima in the sharp
359 wave trace were used to automatically identify SWR start and end points. However, the
360 bandpass (2-50Hz) filtered *in vivo* trace still exhibited a remarkable variation in the sharp
361 wave trace, leading to incorrect boundaries in some cases. Consequently, the sharp wave,
362 ripple and original recording traces were scrutinized by eye (Forro et al., 2015). We rejected
363 erroneously detected SWRs and manually adjusted the automatically identified start and end
364 points if required. Finally, the SWR area was defined in the sharp wave trace as the area
365 beneath the curve enclosed by those start and end points. However, comparing the
366 uncorrected automatically identified SWRs we also obtained a statistically significant
367 difference. Spectral power density of gamma frequency oscillations were determined with an

368 Welch algorithms (pwelch) and the complex Morlet wavelet transform (cmor2-1) was used to
369 display SWR (bandpass filter 100-300 Hz, 134 wavelet scales, 20 kHz sampling rate).

370

371 **Slice preparation**

372 The animals were anesthetized with inhaled isoflurane, decapitated and the brains removed.
373 Tissue blocks containing the hippocampal formation were mounted on a Vibratome (Leica
374 VT1200) in a chamber filled with ice-cold artificial cerebrospinal fluid (ACSF). Transverse
375 hippocampal slices were cut at 400 μm thickness and incubated for at least 1 h in a holding
376 ‘interface’ chamber (continuously oxygenized with carbogen and perfused with ACSF at ~ 2
377 mL/min) and then transferred to the recording ‘submerged’ chamber (perfused at a rate of 6
378 mL/min), both at $33 \pm 1^\circ\text{C}$. The solution used during cutting, incubation and recording
379 contained (in mM): NaCl, 129; KCl, 3; NaH_2PO_4 , 1.25; CaCl_2 , 1.6; MgSO_4 , 1.8; NaHCO_3 ,
380 21; glucose, 10; saturated with 95 % O_2 and 5 % CO_2 , pH 7.4; 290–310 mOsm.

381

382 **Local field potentials *in vitro***

383 LFP were obtained from the stratum pyramidale of the hippocampal CA3 area. KA (400 nM,
384 unless indicated otherwise) or carbachol (20 μM) were applied in the bath to induce network
385 gamma frequency oscillations. The SWR oscillations occurred spontaneously, disappeared
386 shortly after bath application of KA or carbachol and reappeared within a few minutes after
387 their washout. mGluR5 and/or NMDAR activation was blocked by MPEP (50 μM , Tocris
388 Bioscience) and/or AP5 (50 μM , Tocris Bioscience). MPEP and/or AP5 were launched
389 simultaneously to KA, but continued throughout the entire oscillatory gamma network
390 episode.

391 Field oscillations were low pass filtered at 5 kHz, digitized at 10 kHz (Digidata
392 1440A, Axon Instruments) and analyzed with the pClamp software package (notch filter 50
393 Hz; Axon Instruments). Oscillatory peak power and frequency was determined by averaging

394 several consecutive fast Fourier transforms (FFT). SWRs were identified and the area under
395 curve calculated (pClamp software, Axon Instruments). A Student's t-test was used for
396 statistical comparisons unless stated otherwise; differences were considered significant if
397 $P < 0.05$. Average values are expressed as mean \pm SEM. Spearman's rho was used to assess
398 statistical dependence. Pre- and post-gamma data values were normalized to the mean of all
399 prior gamma data. The EPSC-to-IPSC ratio was used to assess the net changes in cellular
400 excitability. We further analyzed the spectral components of the LFPs with custom routines
401 written in Matlab. Signals were zero-phase digital filtered from 2-300 Hz using a Butterworth
402 filter, 50 Hz components including their harmonics were removed using a second-order
403 infinite impulse response notch filter. A complex Morlet wavelet transform (cmor2-1) was
404 used to display SWRs (bandpass filter 100-300 Hz, 134 wavelet scales, 20 kHz sampling
405 rate).

406

407 **Whole-cell recording *in vitro***

408 The patch-clamp recordings were obtained from PCs and INs of hippocampal CA3 area
409 visualized by infrared differential interference contrast video microscopy. The intrinsic and
410 firing properties of cells were measured in whole-cell current-clamp mode as described
411 previously (Gloveli et al., 2005a). In order to follow the Hebbian plasticity rules, during
412 gamma frequency oscillations, cells were recorded in current-clamp mode enabling them to
413 generate action potentials. In an additional set of experiments, the PCs were held in voltage-
414 clamp mode at -70 mV during gamma activity to prevent their depolarization. Whole-cell
415 recording pipettes (3-5 M Ω) were filled with a solution containing (in mM): K-gluconate,
416 135; KCl, 5; ATP-Mg, 2; GTP-Na, 0.3; HEPES, 10; plus biocytin, 0.5% (pH 7.4 and 290
417 mOsm). A Multiclamp 700B amplifier and pClamp software (Axon Instruments) were used
418 for current- and voltage-clamp recordings. The holding potential in voltage-clamp mode was
419 either -70 mV or 0 mV to record the EPSCs and IPSCs, respectively. The areas under curve

420 were calculated for EPSCs and IPSCs and the EPSC-to-IPSC ratios were determined. The seal
421 resistance before establishing whole-cell mode was ≥ 2 G Ω . The series resistance (range 12-18
422 M Ω) was not compensated, but was repeatedly monitored during the experiment by
423 measuring the amplitude of the capacitive current in response to a -10 mV pulse.
424 Experiments, in which the series resistance increased by >20% were discarded. Signals were
425 low-pass filtered at 5 kHz, digitized at 10 kHz (Digidata 1440A) and analyzed using pClamp
426 software.

427 The firing properties of IN [fast (> 100 Hz), non-accommodating vs. regular] were
428 studied using intrasomatic current injection (0.5 nA). Electrophysiological identification was
429 confirmed by post hoc immunostaining and biocytin staining.

430

431 **Immunolabeling**

432 For immunolabeling of interneurons, slices were immersed overnight in a fixative solution
433 containing 4 % paraformaldehyde (PFA) in 0.1 M phosphate buffer (PB), washed three times
434 in 0.1 M PB and subsequently in 0.025 phosphate-buffered saline (PBS; pH 7.3). Slices were
435 then incubated in PBS containing 1% Triton X-100, 10% goat serum and Mouse on Mouse
436 (M.O.M) blocking reagent (2 drops per 2.5 ml solution) for 1 hour at room temperature (RT).
437 To visualize PV- and CCK-containing cells, we used antibodies against PV (mouse, Swant,
438 Marly, CH) and CCK (mouseCURE, Los Angeles, CA) diluted 1:5000 in PBS containing 5%
439 goat serum and 1% Triton X-100. Slices were incubated with primary antibodies for 48 hours
440 at RT. After rinsing three times in PBS, sections were incubated in the PBS solution
441 containing 0.5% Triton X-100, 5% goat serum, goat anti-mouse conjugated with (for PV)
442 Alexa fluor 546 (Invitrogen Corporation, Carlsbad, CA) or (for CCK) Alexa fluor 568
443 (Invitrogen Corporation, Carlsbad, CA) diluted 1:500 or (for biocytin-filled neurons) Alexa
444 fluor 647 (in some experiments 350) conjugated avidin diluted 1:500 (Invitrogen Corporation,
445 Carlsbad, CA). Slices were mounted on glass slides in the glycerol-based, aqueous mountant

446 Vectashield (Vector Laboratories) under coverslips at 48 hours after incubation with the
447 secondary antibodies. Labeled cells were visualized using 20x and/or 60x objectives on a
448 confocal microscope system (Leica). To examine the full extent of somato-dendritic
449 compartments and axonal arborization, the intensity of Z-stack projections was optimized and
450 the images were overlaid.

451

452 **Biocytin staining**

453 Slices were processed as described previously in principle (Dugladze et al., 2012). For
454 biocytin staining, slices with biocytin-filled cells were removed from the chamber and
455 immersed overnight in a fixative solution containing 4 % paraformaldehyde (PFA) in 0.1 M
456 phosphate buffer (PB). Slices were washed three times in 0.1 M PB. The avidin–biocytin
457 complex reaction (Vectastain ABC kit, Camon laboratory service) took place overnight at 4
458 °C in the presence of 0.3 % Triton X-100 (Sigma-Aldrich). Afterwards the sections were
459 rinsed several times before development with 0.02 % diaminobenzidine in 0.1 M PB. The
460 reaction product was intensified with 0.5 % OsO₄ and sections were mounted and
461 coverslipped. Stained cells were reconstructed with the aid of a Neurolucida 3D system
462 (MicroBrightField, Inc).

463

464 **Acknowledgments**

465 We thank Imre Vida for critically reading the manuscript and assisting with the
466 immunostaining.

467

468 **Additional Information**

469 Matlab source code files for the calculation of FFT, Welch’s spectrogram and the wavelet
470 transformation are available on our homepage (<https://glovelilab.wordpress.com>).

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612 **Figure 1.** Theta-nested gamma rhythms enhance hippocampal SWRs *in vivo*. (A)
613 Representative LFP recording in an awake mouse illustrates the occurrence of different
614 network states in a behavioral-dependent manner. The initial spontaneous SWRs during a
615 quiet state (left, SWR) are replaced by a running-associated theta-nested gamma rhythm
616 (middle), followed by another rapid reversal to SWRs (right, p-SWR). Note the higher
617 amplitude of the p-SWRs. Prolonged running period is marked by the black bar above. (B) (i,
618 ii, iii) Left, three example excerpts from the trace in (A) at higher temporal resolution. (i)
619 Left, the initial quiet state example band-pass filtered at 2-300 Hz and 100-300 Hz to illustrate
620 the SWRs and the corresponding ripple component, respectively. Two SWRs are accentuated
621 in red. Right, a single SWR together with its wavelet transform (color-coded power spectral
622 density with superimposed corresponding ripple trace in white). (ii) Left, the theta-nested
623 gamma example with a small excerpt shown in greater magnification above. Right, the
624 corresponding spectral analysis demonstrates the predominant theta (7.3 Hz) and gamma
625 (43.3 Hz) peak. (iii), the p-SWR with the same type of illustration as for the SWR shown in
626 (i). (C) Left, a direct comparison of the mean values of SWR areas before and after the
627 intervening gamma episode (n = 11, 4 mice) highlight a significant increase in SWR areas (P
628 = 0.001, Wilcoxon signed rank test). The respective grand means are indicated by the
629 horizontal bold bars. Right, the corresponding percentage increase of mean SWR areas.

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642 **Figure 2.** Gamma rhythms promote long-lasting alterations in the network activity. (A) SWRs
643 recorded in the stratum pyramidale of the CA3 region occurred spontaneously (left),
644 disappeared shortly after bath application of KA (middle) and reappeared with a significantly
645 higher amplitude after KA washout (right). (B) Example of the wavelet transform (color-
646 coded power spectral density) for three consecutive highlighted SWRs (white trace) before
647 (SWR) and after (p-SWR) intermediate gamma oscillations. (C) p-SWR areas (red-filled
648 squares) increased significantly compared to the SWR areas (gray-filled squares). MPEP (50
649 μM) administration largely prevented p-SWR area increase (black, open triangles). The time
650 courses of drug applications are depicted schematically. The horizontal lines mark the p-SWR
651 data points used for statistical analyses. The significance stars compare the pre-gamma data
652 with the marked post-gamma data. The number of asterisks indicates the significance level
653 (Student's t-test). Insert, examples of SWR (gray) and p-SWR (red and black) without (left)
654 and with MPEP (right) administration during gamma rhythm oscillations. (D) Effects of AP5
655 (50 μM , green open squares) and MPEP+AP5 (black filled triangles) on SWR area increase.
656 Insert, examples of corresponding SWR (gray) and p-SWR (green and black). (E) Gamma
657 oscillation-induced SWR area increase (LTP) is reduced significantly by administration of
658 AP5 (green open bar), MPEP (black open bar) and MPEP+AP5 (black filled bar).

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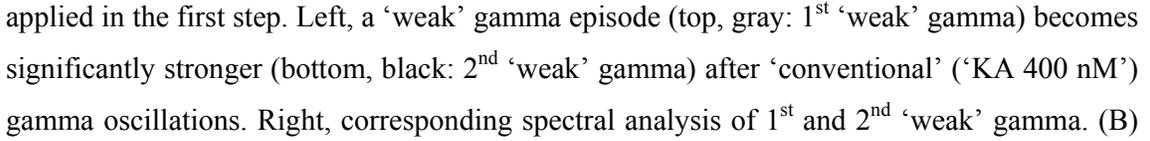
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672 **Figure 3.** Gamma frequency oscillations promote changes in network activity. (A) Brief
673 ‘weak’ field gamma episodes were induced by bath application of 50 nM KA. After this test
674 period, ‘conventional’ gamma frequency oscillations were induced by 400 nM KA
675 application, followed by KA washout achieving a complete cessation of oscillatory gamma
676 activity. In a third step, the network behavior was again tested with low KA concentration as
677 applied in the first step. Left, a ‘weak’ gamma episode (top, gray: 1st ‘weak’ gamma) becomes
678 significantly stronger (bottom, black: 2nd ‘weak’ gamma) after ‘conventional’ (‘KA 400 nM’)  gamma oscillations. Right, corresponding spectral analysis of 1st and 2nd ‘weak’ gamma. (B)
680 Summary bar charts of peak power and frequency obtained before (1st ‘weak’ gamma) and
681 after ‘conventional’ gamma (2nd ‘weak’ gamma). Spectral power of gamma rhythms increased
682 from $0.72 \times 10^{-4} \pm 0.39 \times 10^{-4}$ mV²/Hz to $1.34 \times 10^{-4} \pm 0.50 \times 10^{-4}$ mV²/Hz (n = 10, P = 0.003)
683 while frequency remained unchanged, illustrating that the intervening gamma episode has a
684 reinforcing effect.

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701 **Figure 4.** Gamma frequency oscillations support long-lasting synaptic plasticity. (A) Light
702 micrograph of an example CA3 PC. Insert: The regular firing pattern of this PC in response to
703 a depolarizing current injection. (B) The area of SWR-associated EPSC increases
704 significantly after gamma frequency oscillations (gray and red filled squares for EPSCs and p-
705 EPSC, respectively). Administration of MPEP (50 μ M) prevents the increase of p-EPSC (gray
706 and black filled triangles illustrate EPSC and p-EPSC, respectively). Holding PCs in voltage
707 clamp configuration at -70 mV during gamma frequency oscillations leads to a significant
708 decrease in EPSC area (gray and red open squares for EPSC and p-EPSC, respectively). The
709 significance stars compare the pre-gamma data with the marked post-gamma data. Insert:
710 Representative examples of EPSC (gray) and p-EPSC (red) recorded without (left) and with
711 MPEP (middle), as well as using voltage clamping of cells during gamma rhythms (right,
712 gamma-VC). (C) SWR-associated IPSCs exhibit a moderate increase in area in PCs held in
713 both current- (filled gray and blue triangles) and voltage-clamp mode (open gray and black
714 triangles for IPSC and p-IPSC, respectively) during gamma rhythms. Inserts: Representative
715 examples of corresponding IPSC (gray) and p-IPSC (gamma-CC, blue and gamma-VC,
716 black). (D) Contra-directional change in p-EPSC to p-IPSC ratio for PCs held in current-
717 (gamma-CC) vs. voltage-clamp mode (gamma-VC) during gamma rhythms (normalized to
718 pre-gamma values).

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731 **Figure 5.** Increased excitability of fast spiking PV-expressing interneurons. (A) Top: A
732 confocal image of a typical PV-expressing IN filled with biocytin. Insert: Fast firing pattern in
733 response to depolarizing current injection. Bottom: Immuno-reactivity of the biocytin filled
734 cell for PV. (B) Normalized EPSC (gray squares), IPSC (gray triangles), p-EPSC (red
735 squares) and p-IPSC (blue triangles) recorded from PV-positive INs held in current-clamp
736 mode during gamma rhythms. Administration of MPEP (50 μ M) prevents the increase of p-
737 EPSC (gray and black open triangles illustrate EPSC and p-EPSC, respectively). The
738 significance stars compare the pre-gamma data with the marked post-gamma data. Inserts:
739 left, representative examples of EPSC and IPSC (gray) with corresponding p-EPSC (red) and
740 p-IPSC (blue); right, the EPSC-to-IPSC ratio before (pre) and after (post) gamma rhythms
741 demonstrates that the excitability significantly increases in PV-positive INs.

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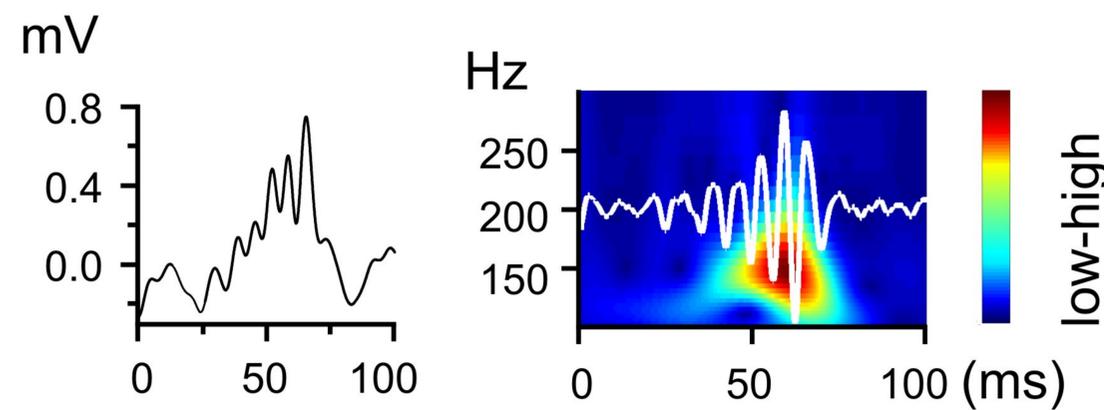
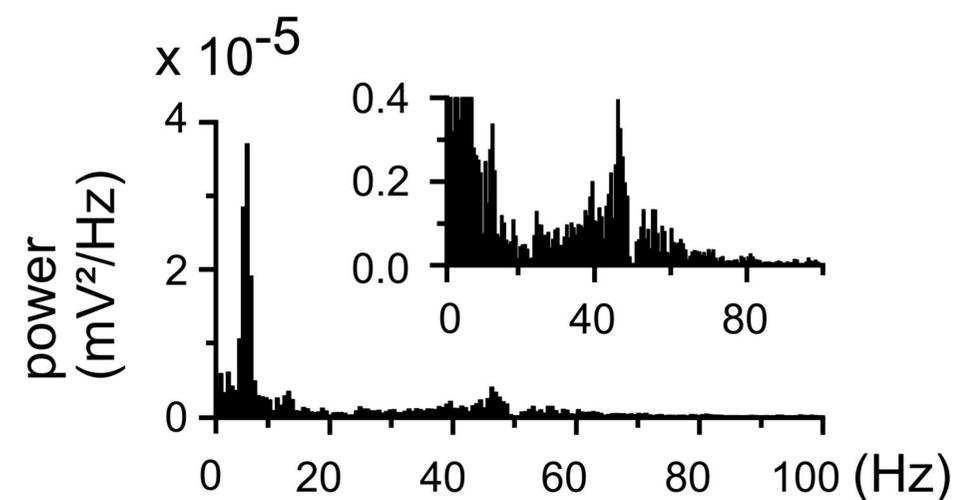
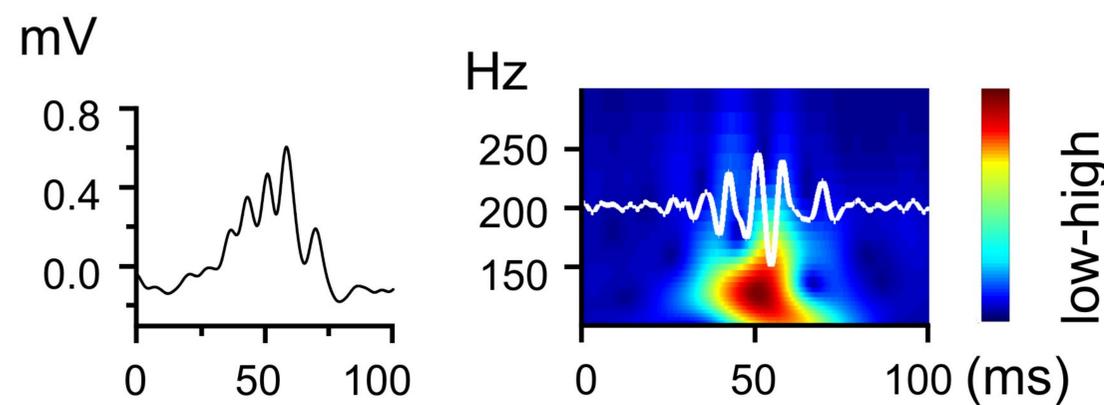
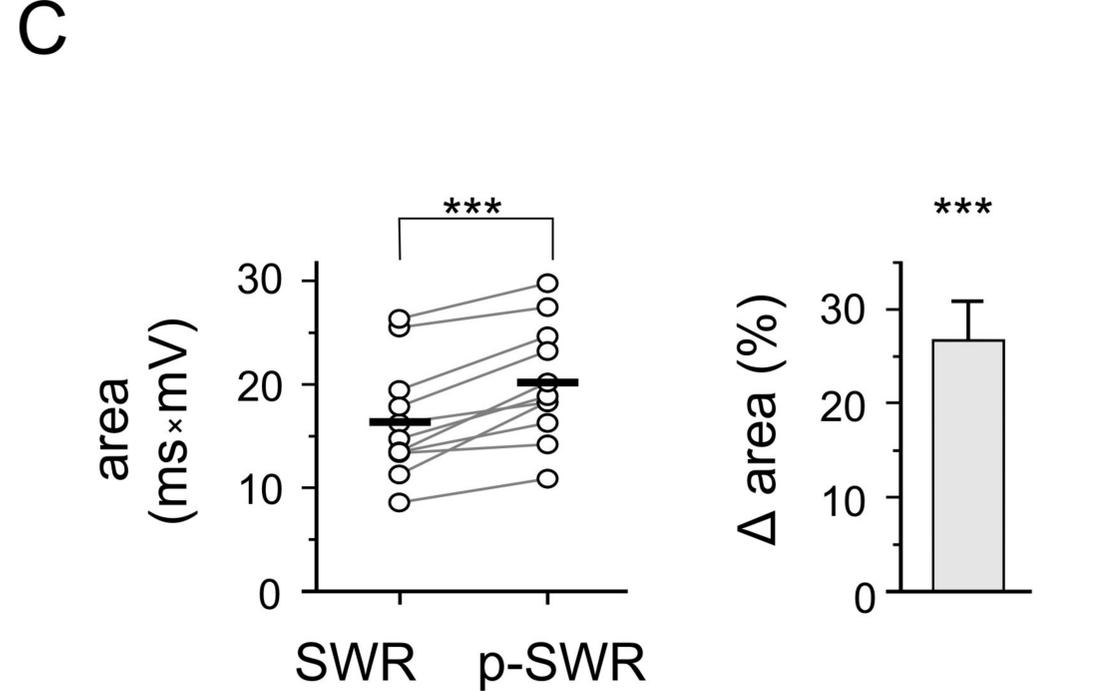
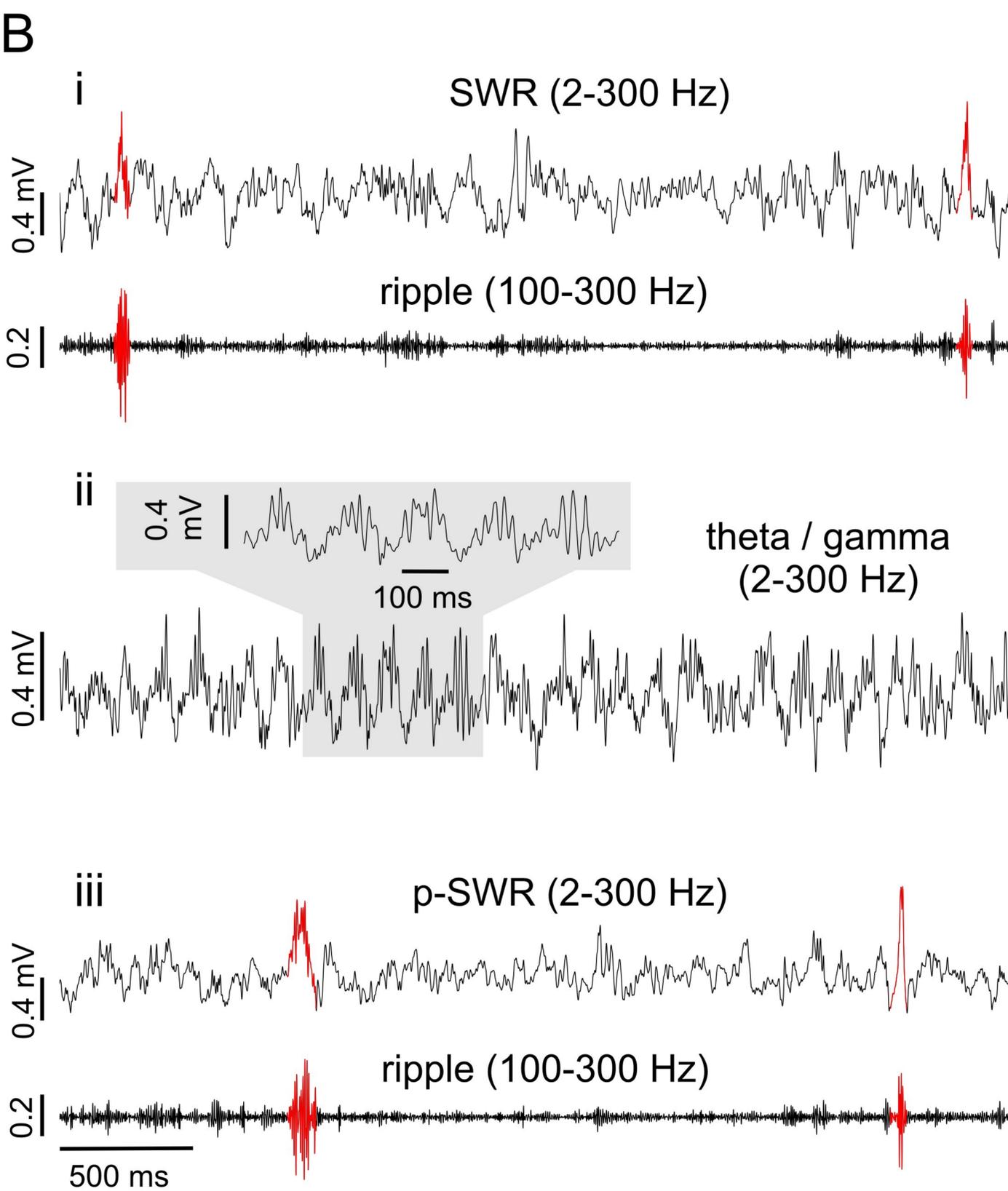
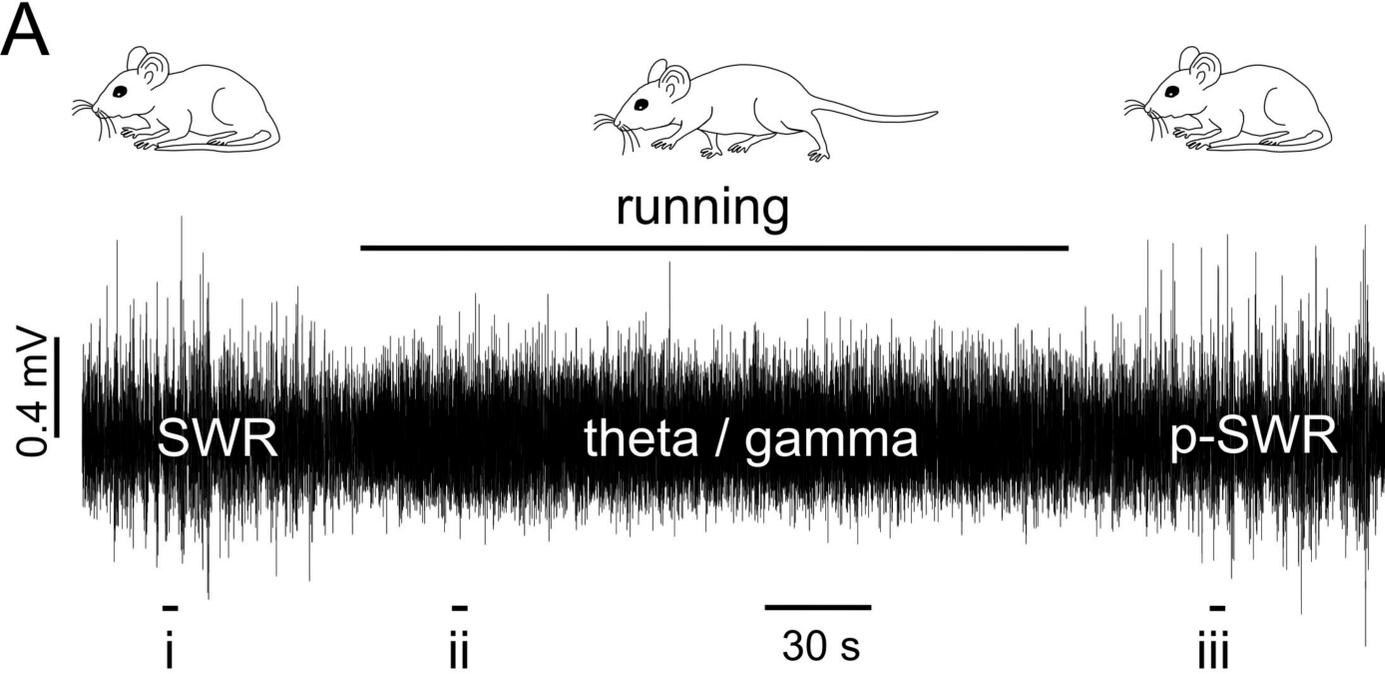
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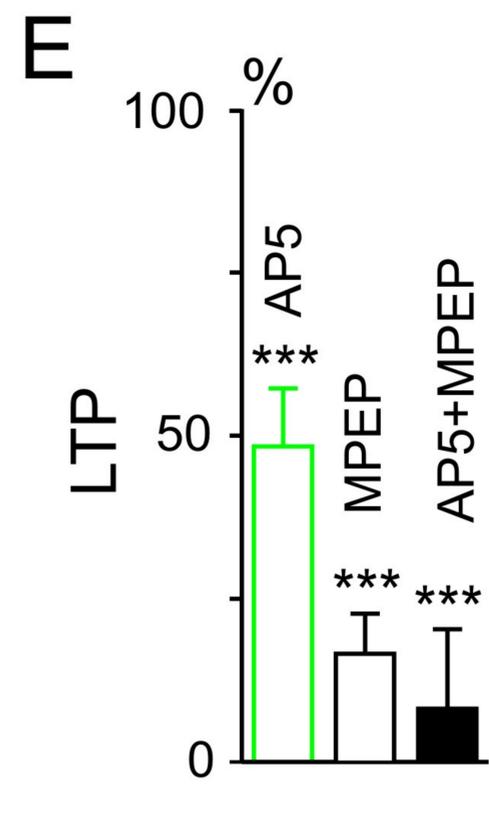
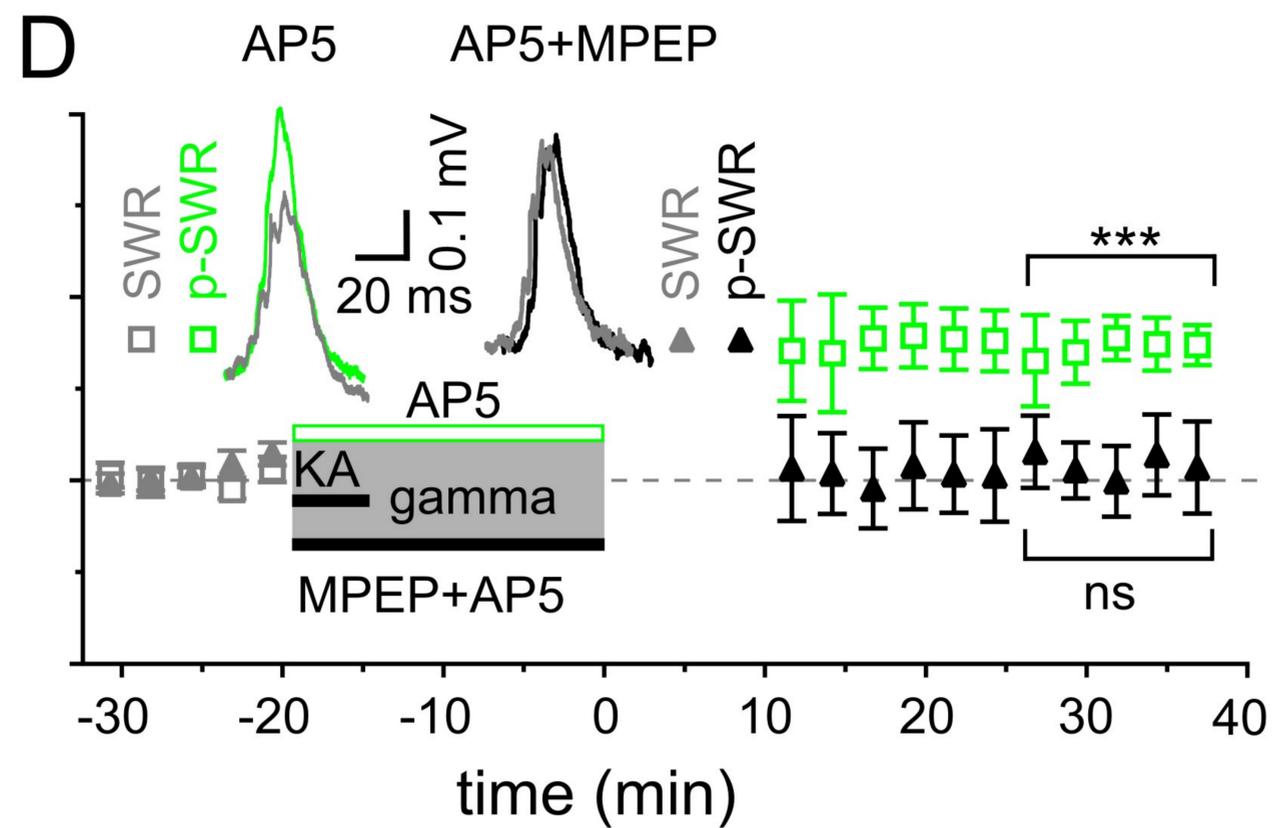
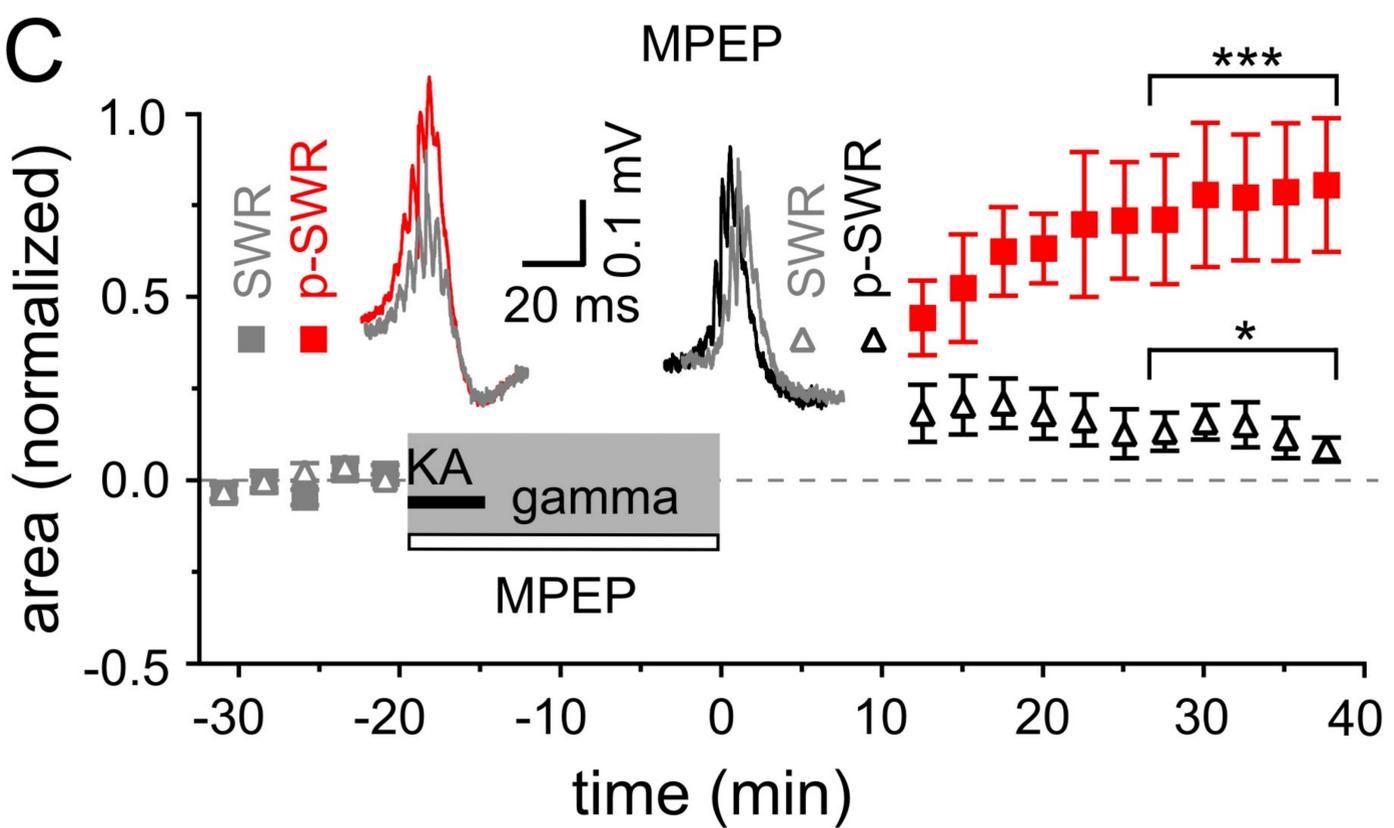
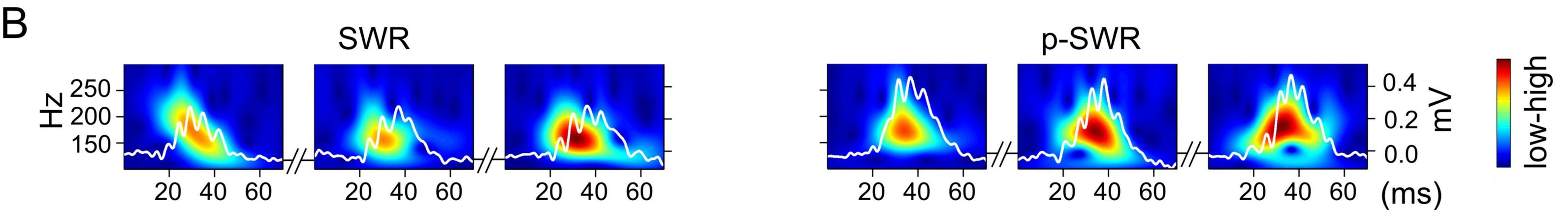
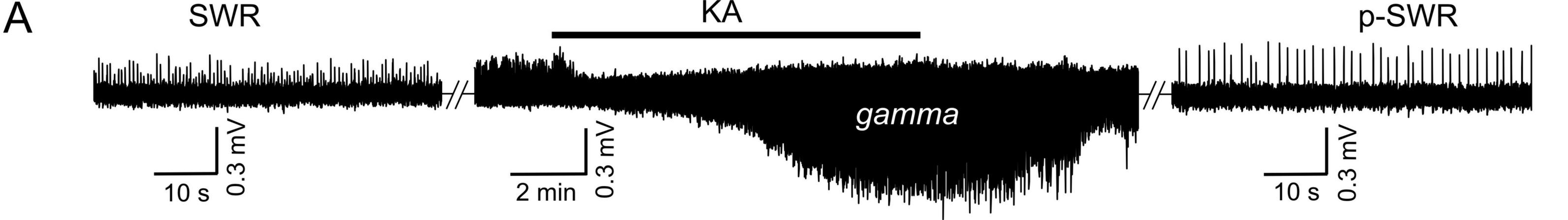
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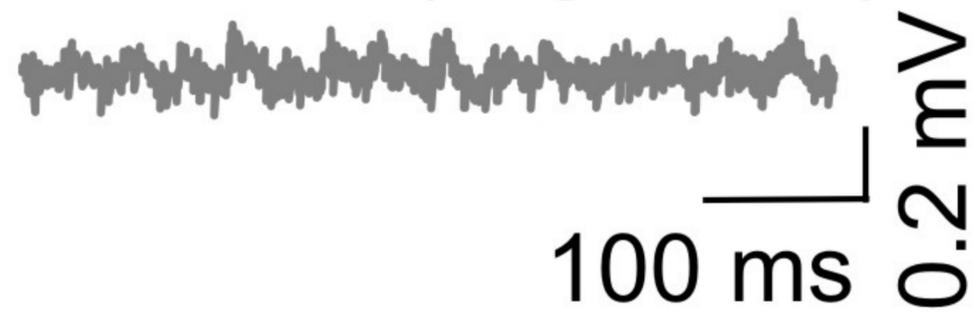
759 **Figure 6.** Reduced excitability of regular spiking CCK-expressing interneurons. (A) Top: A
760 confocal image of a typical CCK-expressing IN filled with biocytin. Insert: Regular firing
761 patterns in response to depolarizing current injection. Bottom: Immuno-reactivity of the
762 biocytin filled cell for CCK. (B) Normalized EPSC (gray squares), IPSC (gray triangles), p-
763 EPSC (red squares) and p-IPSC (blue triangles) recorded from CCK-expressing INs held in
764 current-clamp mode during gamma frequency oscillations. The significance stars compare the
765 pre-gamma data with the marked post-gamma data. Inserts: Left, representative examples of
766 EPSC and IPSC (gray) with corresponding p-EPSC (red) and p-IPSC (blue); right, the EPSC-
767 to-IPSC ratio before (pre) and after (post) gamma rhythms demonstrates that the excitability
768 significantly decreases in CCK-positive INs.
769





A

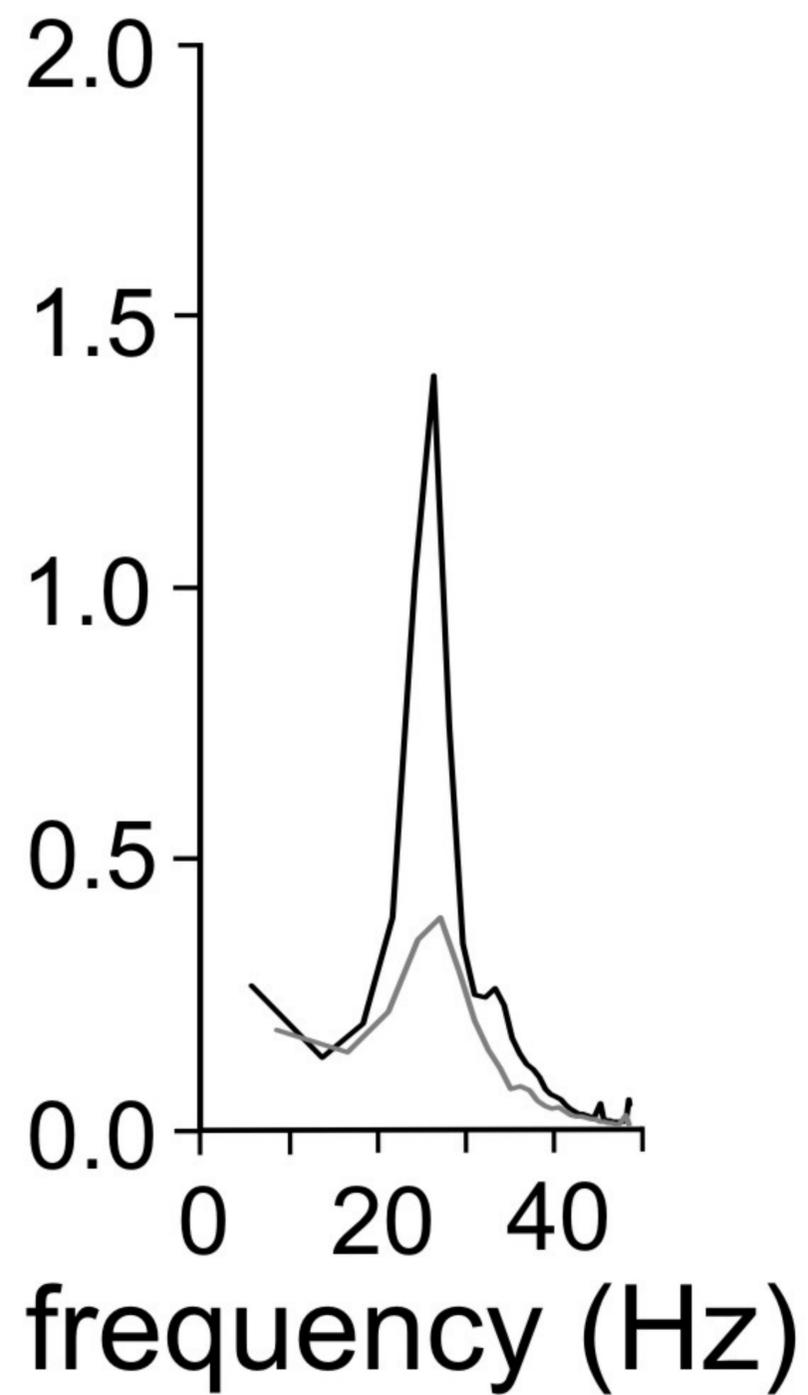
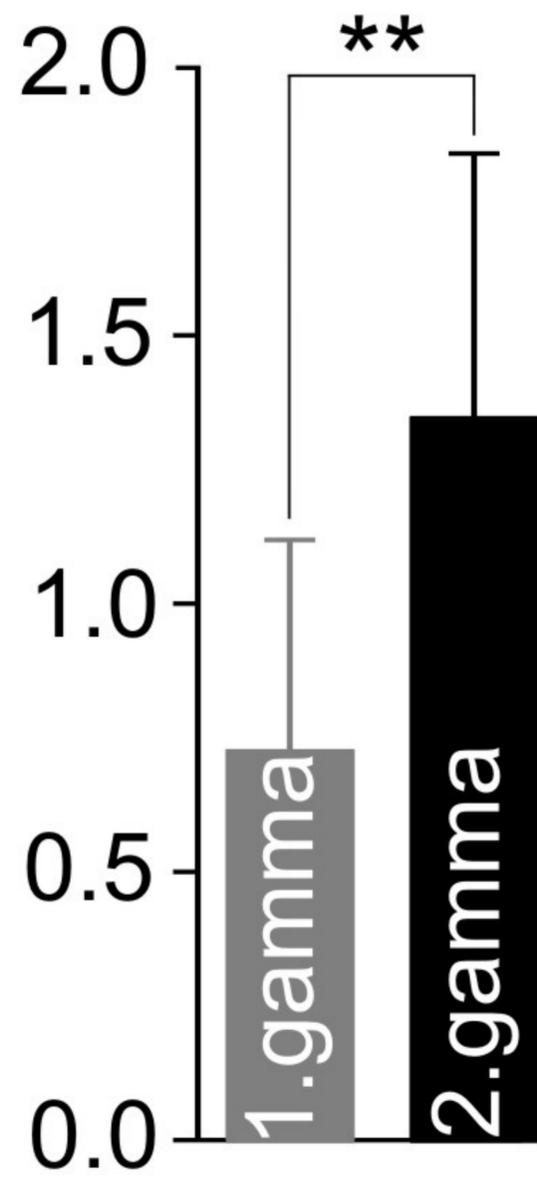
KA 50 nM (1. gamma)



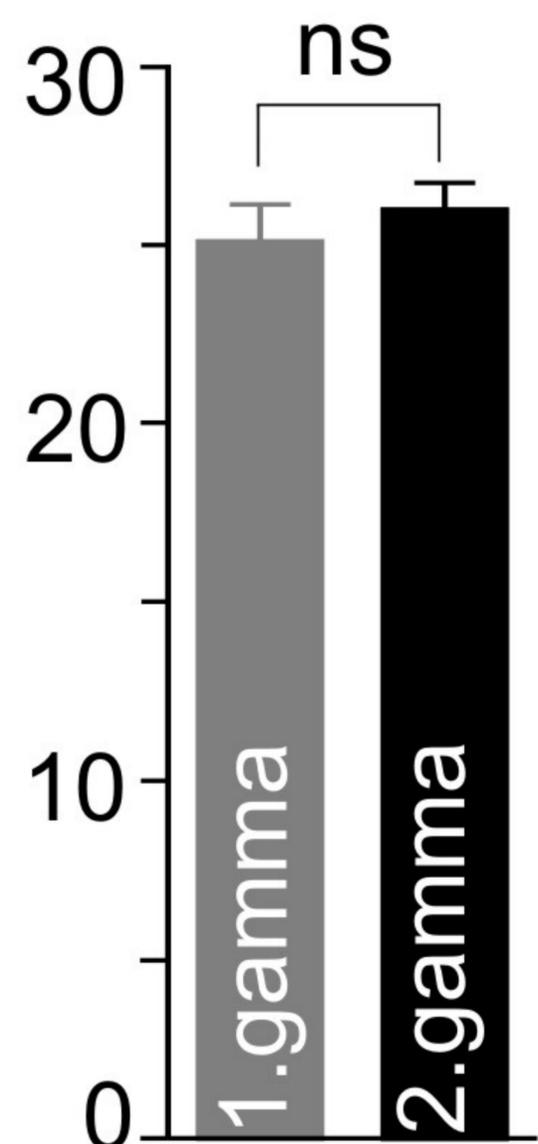
KA 400 nM



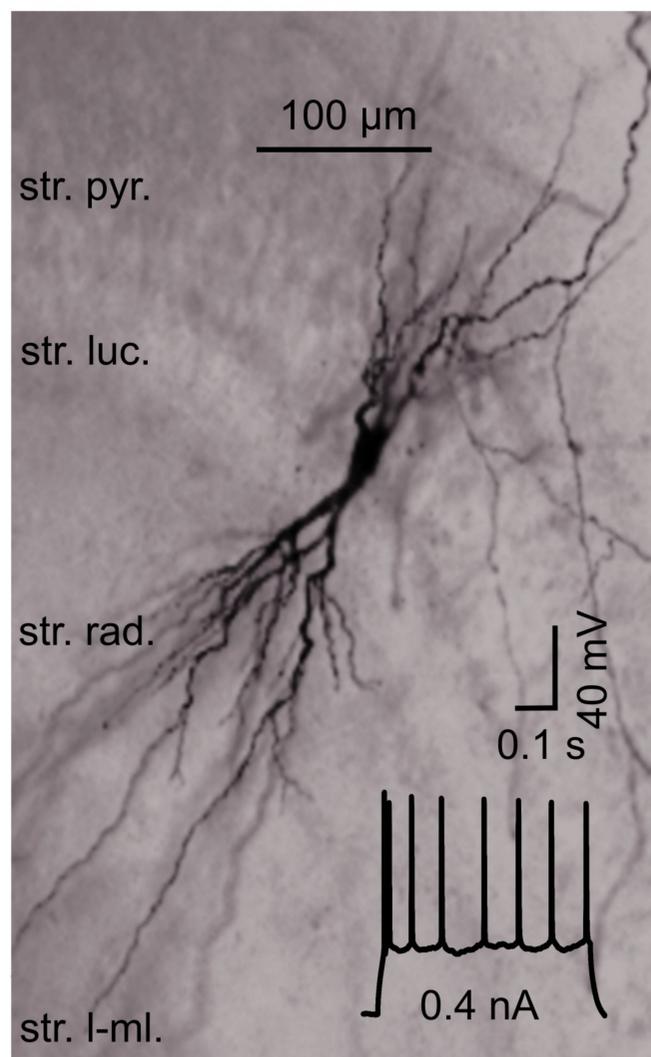
KA 50 nM (2. gamma)

power $\times 10^{-4}$ (mV^2/Hz)**B**power $\times 10^{-4}$ (mV^2/Hz)

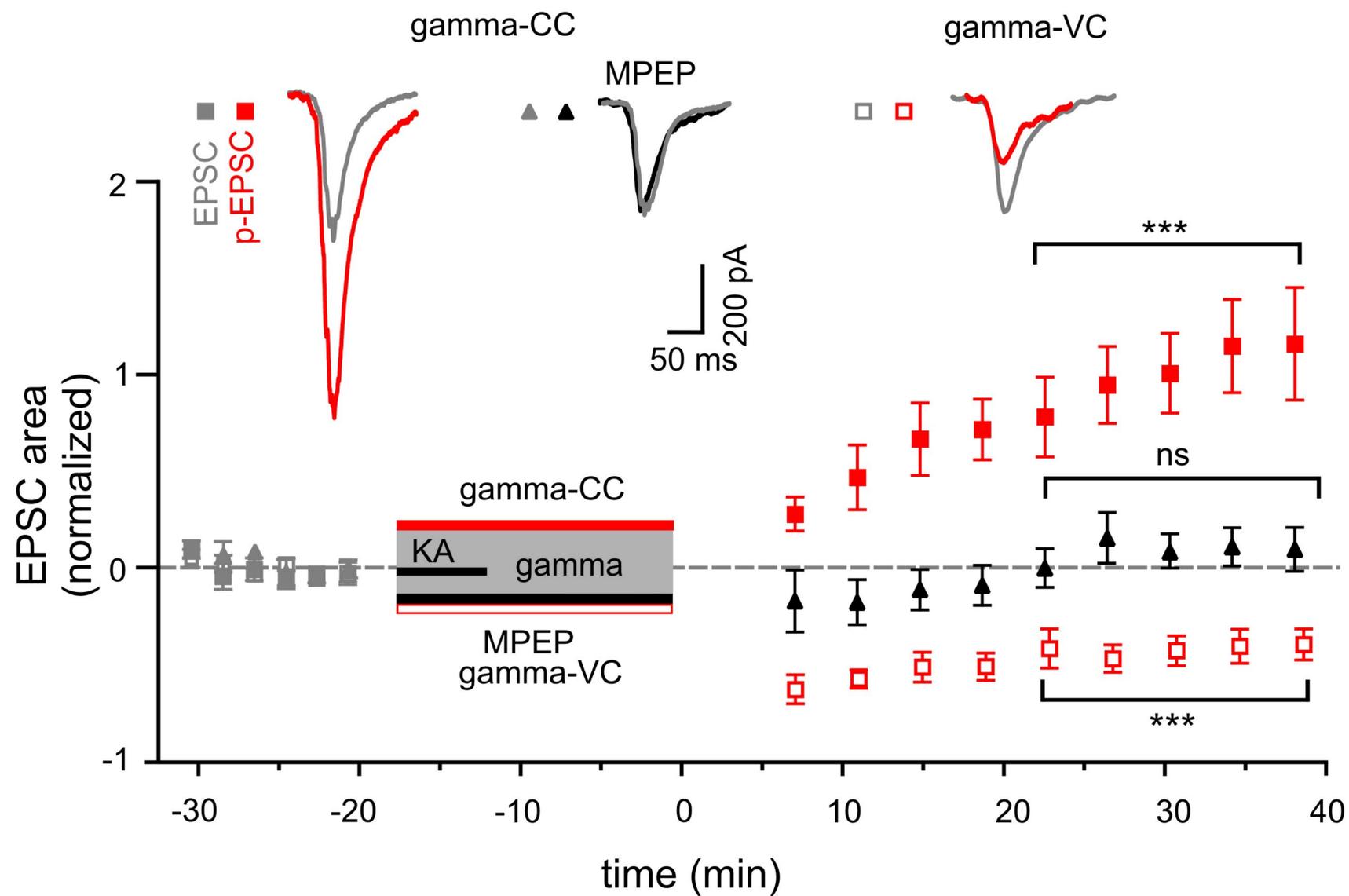
frequency (Hz)



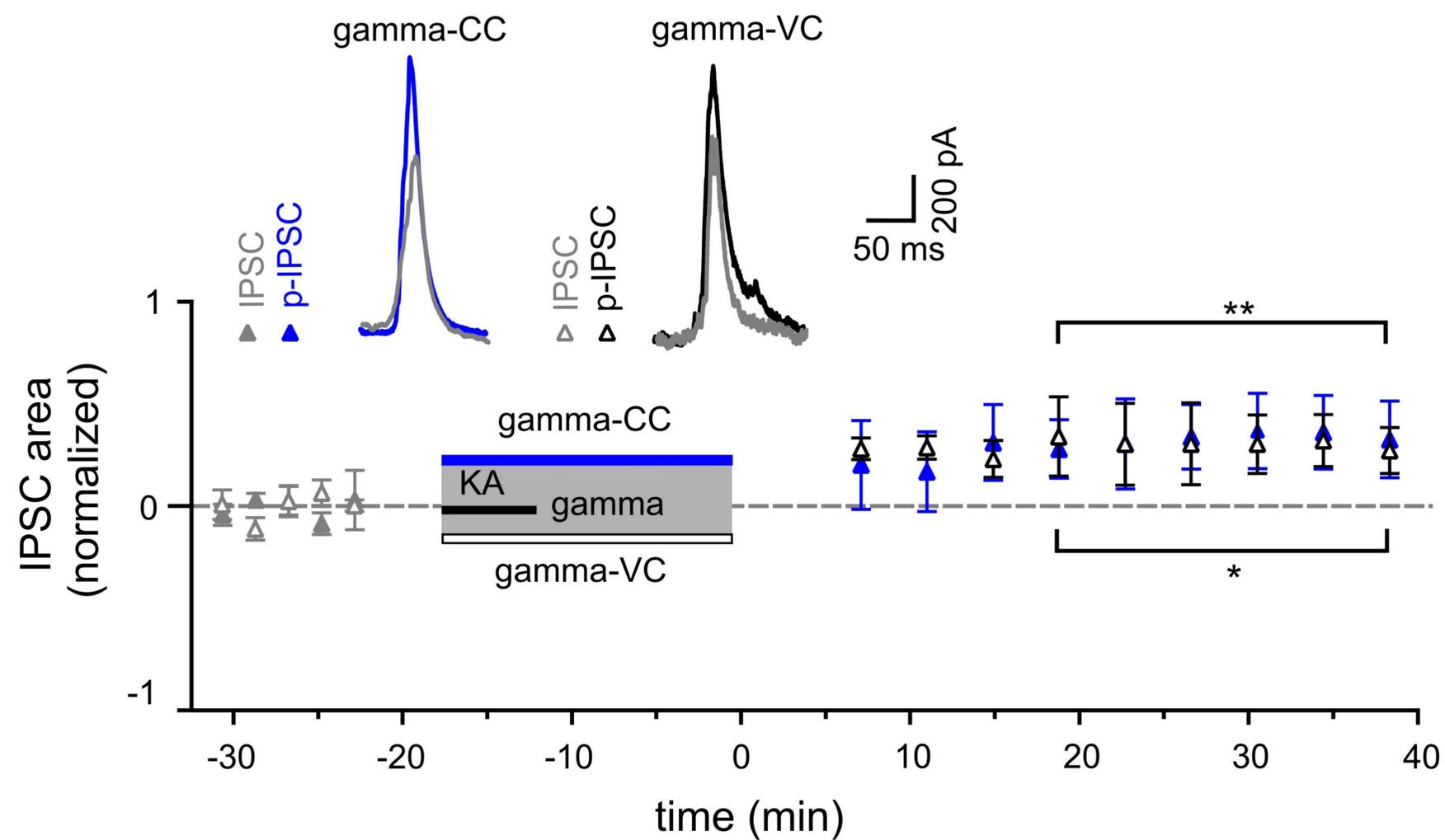
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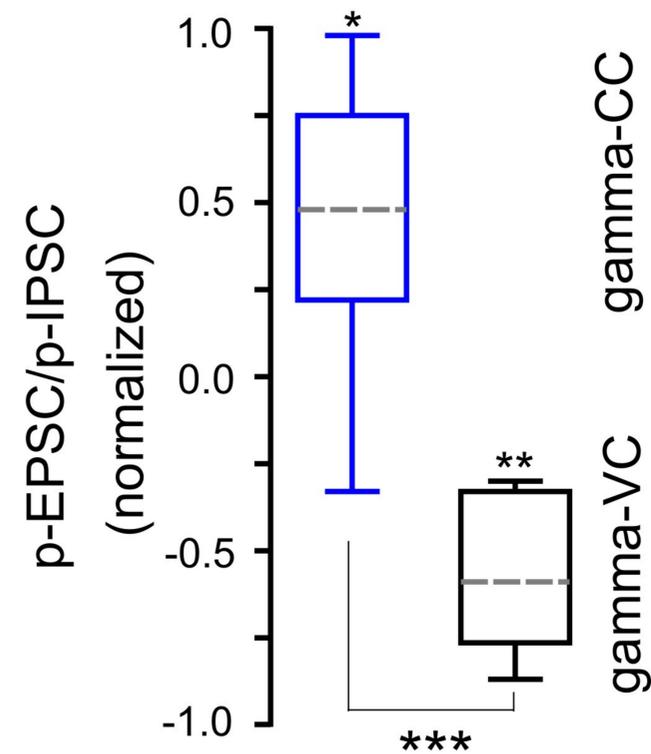
B



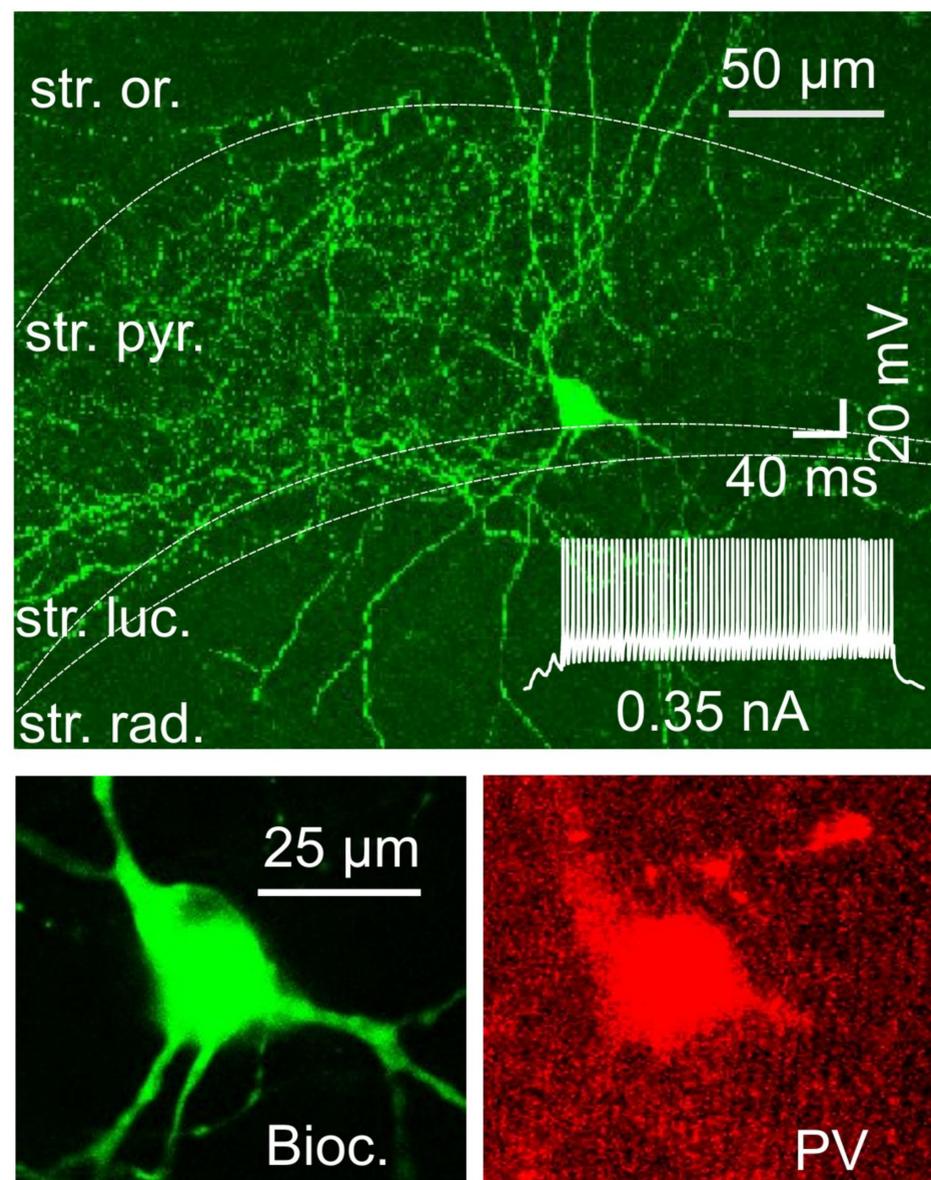
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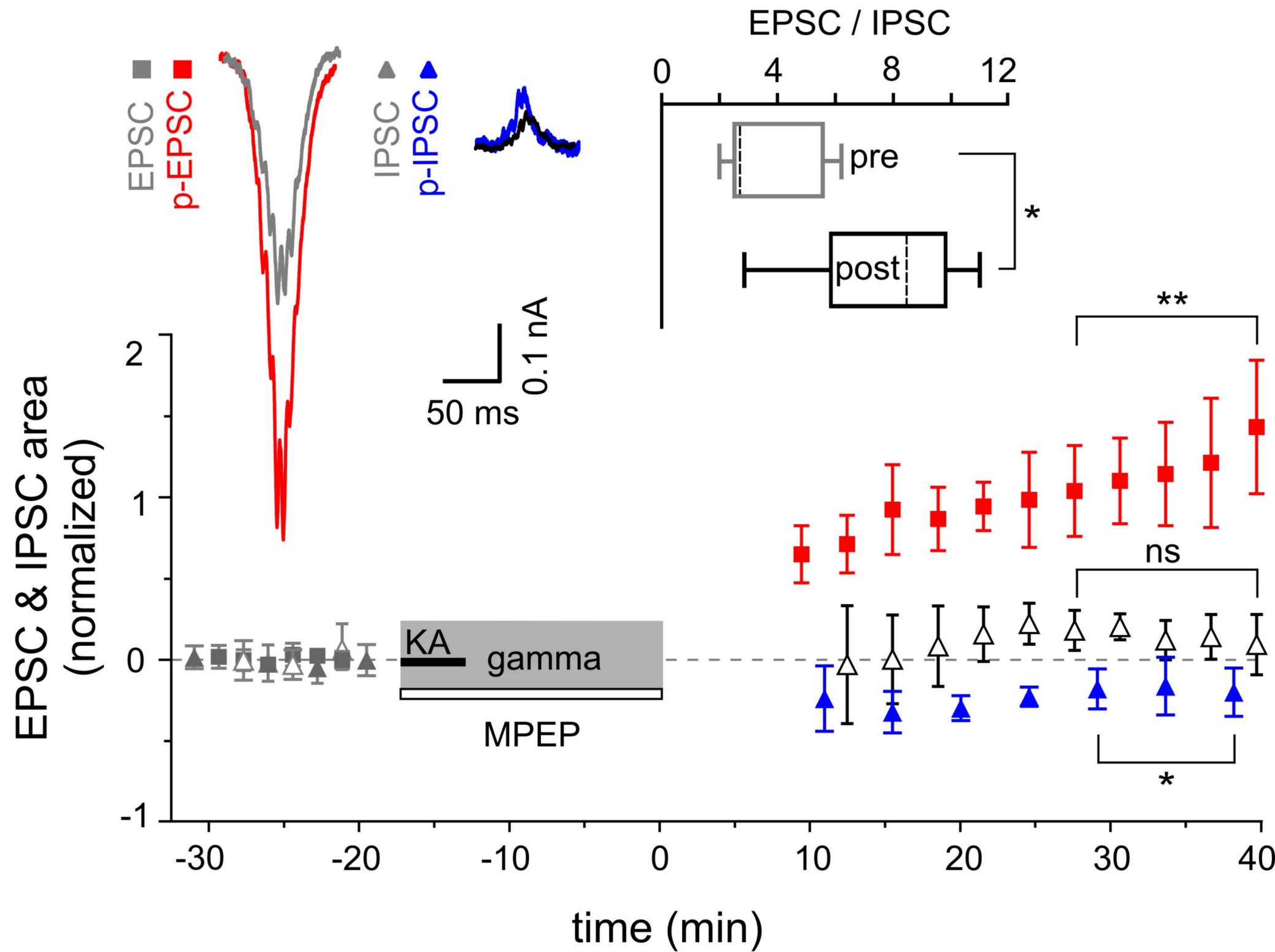
D



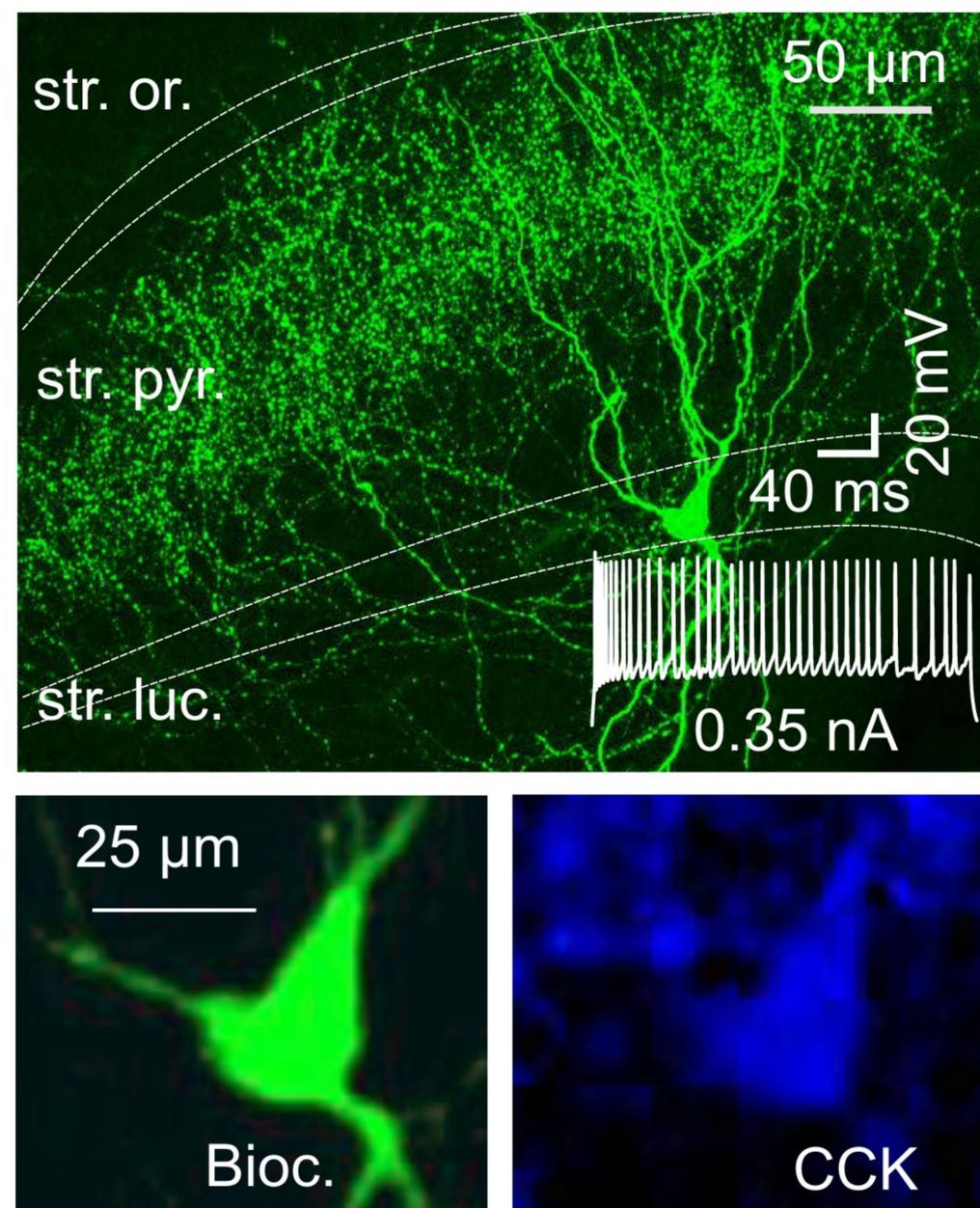
A



B



A



B

