

1 **Hippocampal inputs engage CCK+ interneurons to mediate endocannabinoid-modulated**
2 **feed-forward inhibition in the prefrontal cortex**

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27 **ABSTRACT**

28 Connections from the ventral hippocampus (vHPC) to the prefrontal cortex (PFC) regulate
29 cognition, emotion and memory. These functions are also tightly regulated by inhibitory networks
30 in the PFC, whose disruption is thought to contribute to mental health disorders. However,
31 relatively little is known about how the vHPC engages different populations of interneurons in the
32 PFC. Here we use slice physiology and optogenetics to study vHPC-evoked feed-forward
33 inhibition in the mouse PFC. We first show that cholecystokinin (CCK+), parvalbumin (PV+), and
34 somatostatin (SOM+) interneurons are prominent in layer 5 (L5) of infralimbic PFC. We then show
35 that vHPC inputs primarily activate CCK+ and PV+ interneurons, with weaker connections onto
36 SOM+ interneurons. CCK+ interneurons make stronger synapses onto pyramidal tract (PT) cells
37 over nearby intratelencephalic (IT) cells. However, CCK+ inputs undergo depolarization-induced
38 suppression of inhibition (DSI) and CB1 receptor modulation only at IT cells. Moreover, vHPC-
39 evoked feed-forward inhibition undergoes DSI only at IT cells, confirming a central role for CCK+
40 interneurons. Together, our findings show how vHPC directly engages multiple populations of
41 inhibitory cells in deep layers of the infralimbic PFC, highlighting unexpected roles for both CCK+
42 interneurons and endocannabinoid modulation in hippocampal-prefrontal communication.

43 INTRODUCTION

44 The prefrontal cortex (PFC) controls cognitive and emotional behaviors (Euston et al., 2012; Miller
45 and Cohen, 2001) and is disrupted in many neuropsychiatric disorders (Godsil et al., 2013;
46 Sigurdsson and Duvarci, 2015). PFC activity is driven and maintained by long-range
47 glutamatergic inputs from a variety of other brain regions (Hoover and Vertes, 2007; Miller and
48 Cohen, 2001). Strong, unidirectional connections from the ventral hippocampus (vHPC)
49 contribute to both working memory and threat conditioning in rodents (Jones and Wilson, 2005;
50 Sotres-Bayon et al., 2012; Spellman et al., 2015). Dysfunction of vHPC to PFC connectivity is
51 also implicated in schizophrenia, anxiety disorders, chronic stress disorders, and depression
52 (Godsil et al., 2013; Sigurdsson and Duvarci, 2015). To understand these roles, it is necessary to
53 establish how vHPC inputs engage local excitatory and inhibitory networks within the PFC.

54
55 The vHPC primarily projects to the ventral medial PFC in rodents, with axons most prominent in
56 layer 5 (L5) of infralimbic (IL) PFC (Phillips et al., 2019). These excitatory inputs contact multiple
57 populations of pyramidal cells, and are much stronger at intratelencephalic (IT) cells than nearby
58 pyramidal tract (PT) cells (Liu and Carter, 2018). vHPC inputs can drive robust firing of IT cells
59 (Liu and Carter, 2018), which may be important for maintaining activity during behavioral tasks
60 (Padilla-Coreano et al., 2016; Spellman et al., 2015). However, they also evoke prominent feed-
61 forward inhibition at pyramidal cells (Marek et al., 2018), with excitation and inhibition evolving
62 with different dynamics (Liu and Carter, 2018). Here we focus on the mechanisms responsible for
63 this inhibition by establishing which interneurons are engaged by vHPC inputs to the PFC.

64
65 As in other cortices, PFC activity is regulated by a variety of GABAergic interneurons, which have
66 distinct functions (Abbas et al., 2018; Courtin et al., 2014; Tremblay et al., 2016). Parvalbumin-
67 expressing (PV+) interneurons mediate feed-forward inhibition via strong synapses at the soma
68 of pyramidal cells (Atallah et al., 2012; Cruikshank et al., 2007; Gabernet et al., 2005). In contrast,

69 somatostatin-expressing (SOM+) interneurons mediate feed-back inhibition via facilitating
70 synapses onto the dendrites (Gentet et al., 2012; Silberberg and Markram, 2007). However,
71 during trains of repetitive activity, SOM+ interneurons can also participate in feed-forward
72 inhibition (McGarry and Carter, 2016; Tan et al., 2008). Interestingly, recent *in vivo* studies
73 suggest both PV+ and SOM+ interneurons may contribute to vHPC-evoked inhibition in the PFC
74 (Abbas et al., 2018; Marek et al., 2018).

75

76 While PV+ and SOM+ interneurons are prominent in the PFC, there is also an unusually high
77 density of cholecystokinin-expressing (CCK+) interneurons (Whissell et al., 2015). In the
78 hippocampus, these soma-targeting inhibitory cells help tune the motivation and emotional state
79 of animals (Armstrong and Soltesz, 2012; Freund, 2003; Freund and Katona, 2007). They also
80 highly express cannabinoid type 1 (CB1) receptors on their axon terminals (Katona et al., 1999),
81 and can be strongly modulated by endocannabinoids (Wilson et al., 2001; Wilson and Nicoll,
82 2001). For example, brief depolarization of postsynaptic pyramidal cells releases
83 endocannabinoids that bind to CB1 receptors on CCK+ axon terminals and inhibit presynaptic
84 GABA release, a process known as depolarization-induced suppression of inhibition (DSI) (Wilson
85 and Nicoll, 2001).

86

87 While CCK+ interneurons are prominent in the PFC and may play a role in different forms of
88 inhibition, they remain relatively understudied. A major technical reason is low-level expression
89 of CCK in pyramidal cells (Taniguchi et al., 2011), which makes CCK+ interneurons challenging
90 to specifically target. For example, expressing Cre-dependent reporters in CCK-Cre transgenics
91 also label pyramidal cells in the cortex (Taniguchi et al., 2011). Fortunately, this challenge can be
92 overcome with intersectional viruses using the Dlx enhancer, which restricts expression to
93 interneurons (Dimidschstein et al., 2016). This approach allows identification of CCK+

94 interneurons, enabling targeted recordings and optogenetic access to study their connectivity and
95 modulation in the PFC.

96

97 Here we examine vHPC-evoked inhibition at L5 pyramidal cells in IL PFC using slice physiology,
98 optogenetics and intersectional viral tools. We find vHPC inputs activate PV+, SOM+, and CCK+
99 interneurons, with different dynamics during repetitive activity. Inputs to PV+ and CCK+
100 interneurons are strong but depressing, while those onto SOM+ interneurons are weak but
101 facilitating. CCK+ interneurons contact L5 pyramidal cells, with stronger connections onto PT cells
102 than neighboring IT cells. However, endocannabinoid modulation via DSI and direct activation of
103 CB1 receptors only occurs at synapses onto IT cells. Endocannabinoid modulation of vHPC-
104 evoked feed-forward inhibition also occurs only at IT cells, highlighting a central role for CCK+
105 interneurons. Together, our findings show how the vHPC engages interneurons to inhibit the PFC,
106 while revealing a novel property of cell-type specific endocannabinoid modulation in this circuit.

107

108 **MATERIALS AND METHODS**

109

110 **Animals:** Experiments used wild-type and transgenic mice of either sex in a C57BL/6J
111 background (all breeders from Jackson Labs). Homozygote male breeders (PV-Cre = JAX
112 008069, SOM-Cre = JAX 013044, CCK-Cre = JAX 012706) were paired with female wild-type or
113 Ai14 breeders (JAX 007914) to yield heterozygote offspring for experiments. All experimental
114 procedures were approved by the University Animal Welfare Committee of New York University.

115

116 **Viruses:** AAV viruses used in this study were: AAV1.EF1a.DIO.hChR2(H134R)-
117 eYFP.WPRE.hGH (UPenn AV-1-20298P), AAV1.hysn.hChR2(H134R)-eYFP.WPRE.hGH
118 (UPenn AV-26973P), AAV1.EF1a.DIO.eYFP.WPRE.hGH (Upenn AV-1-27056),
119 AAVrg.CAG.GFP (Addgene 37825-AAVrg), AAVrg.CAG.tdTomato (Addgene 59462-AAVrg).
120 Additional viral constructs were assembled for Cre-dependent expression of a reporter under the
121 control of the Dlx5/6 enhancer: AAV-Dlx-Flex-GFP (Addgene #83900) and AAV-Dlx-Flex-ChR2-
122 mCherry (Dimidschstein et al., 2016). These constructs take advantage of the double-floxed
123 inverted system, in which two consecutive and incompatible lox-sites are placed both in 5' and 3'
124 of the reversed coding sequences of the viral reporter, restricting expression to interneurons.

125

126 **Stereotaxic injections:** Mice aged 4-6 weeks were deeply anesthetized with either isoflurane or
127 a mixture of ketamine and xylazine, then head-fixed in a stereotaxic (Kopf Instruments). A small
128 craniotomy was made over the injection site, using these coordinates relative to Bregma: PFC =
129 ± 0.4 , -2.3 , $+2.1$ mm, PAG = -0.6 , both -2.5 and -3 , -4.0 mm, vHPC = -3.3 , both -3.6 and -4.2 , -
130 3 mm (mediolateral, dorsoventral, and rostrocaudal axes). For retrograde labeling, pipettes were
131 filled with red retrogradely transported fluorescent beads (Lumafluor), Cholera Toxin Subunit B
132 (CTB) conjugated to Alexa 647 (Life Technologies), or viruses. Borosilicate pipettes with 5 to
133 10 μ m diameter tips were back-filled with dye and/or virus, and a volume of 130-550 nl was

134 pressure-injected using a Nanoject III (Drummond) every 30 s. The pipette was left in place for
135 an additional 5 min, allowing time to diffuse away from the pipette tip, before being slowly retracted
136 from the brain. For both retrograde and viral labeling, animals were housed for 2-3 weeks before
137 slicing.

138

139 **Histology and fluorescence microscopy:** Mice were anesthetized with a lethal dose of
140 ketamine and xylazine, then perfused intracardially with 0.01 M phosphate buffered saline (PBS)
141 followed by 4% paraformaldehyde (PFA) in 0.01 M PBS. Brains were fixed in 4% PFA in 0.01 M
142 PBS overnight at 4°C. Slices were prepared at a thickness of 70 µm for imaging intrinsic
143 fluorescence or 40 µm for antibody staining (Leica VT 1000S vibratome). For
144 immunohistochemistry, slices were incubated with blocking solution (1% bovine serum albumin
145 and 0.2% Triton-X in 0.01 m PBS) for 1 hour at room temperature before primary antibodies were
146 applied in blocking solution [mouse anti-parvalbumin antibody (Millipore, MAB1572) at 1:2000
147 overnight, rat anti-somatostatin (Millipore, MAB354) at 1:200 overnight, guinea pig anti-CB1R
148 (Frontier Institute, Af530) at 1:500 for 36 hours] at 4 °C. Slices were then incubated with secondary
149 antibodies in blocking solution [goat anti-mouse 647 at 1:200, goat anti-rat 647 at 1:200, goat anti-
150 guinea pig 647 at 1:500 (Invitrogen)] for 1.5 h at room temperature before mounted under glass
151 coverslips on gelatin-coated slides using ProLong Gold antifade reagent with DAPI (Invitrogen).
152 Images were acquired using a confocal microscope (Leica SP8). Image processing involved
153 adjusting brightness, contrast, and manual cell counting using ImageJ (NIH).

154

155 **Slice preparation:** Mice aged 6-8 weeks were anesthetized with a lethal dose of ketamine and
156 xylazine, and perfused intracardially with ice-cold external solution containing the following
157 (in mM): 65 sucrose, 76 NaCl, 25 NaHCO₃, 1.4 NaH₂PO₄, 25 glucose, 2.5 KCl, 7 MgCl₂, 0.4 Na-
158 ascorbate, and 2 Na-pyruvate (295–305 mOsm), and bubbled with 95% O₂/5% CO₂. Coronal
159 slices (300 µm thick) were cut on a VS1200 vibratome (Leica) in ice-cold external solution, before

160 being transferred to ACSF containing (in mM): 120 NaCl, 25 NaHCO₃, 1.4 NaH₂PO₄, 21 glucose,
161 2.5 KCl, 2 CaCl₂, 1 MgCl₂, 0.4 Na-ascorbate, and 2 Na-pyruvate (295–305 mOsm), bubbled with
162 95% O₂/5% CO₂. Slices were kept for 30 min at 35°C, before being allowed to recover for 30 min
163 at room temperature before starting recordings. All recordings were conducted at 30–32°C.

164

165 **Electrophysiology:** Whole-cell recordings were obtained from pyramidal neurons or
166 interneurons located in layer 5 (L5) of infralimbic (IL) PFC. Neurons were identified by infrared-
167 differential interference contrast or fluorescence, as previously described (Chalifoux and Carter,
168 2010). In the case of pyramidal cells, projection target was established by the presence of
169 retrobeads or Alexa-conjugated CTB, as previously described (Little and Carter, 2013). Pairs of
170 adjacent cells were chosen for sequential recording, ensuring they received similar inputs
171 (typically < 50 μm between cells). Borosilicate pipettes (2–5 MΩ) were filled with internal solutions.
172 Three types of recording internal solutions were used. For current-clamp recordings (in mM): 135
173 K-gluconate, 7 KCl, 10 HEPES, 10 Na-phosphocreatine, 4 Mg₂-ATP, and 0.4 Na-GTP, 290–295
174 mOsm, pH 7.3, with KOH. For voltage-clamp recordings (in mM): 135 Cs-gluconate, 10 HEPES,
175 10 Na-phosphocreatine, 4 Mg₂-ATP, and 0.4 Na-GTP, 0.5 EGTA, 10 TEA-chloride, and 2 QX314,
176 290–295 mOsm, pH 7.3, with CsOH. For DSI experiments (in mM): 130 K-gluconate, 1.5 MgCl₂,
177 10 HEPES, 1.1 EGTA, 10 phosphocreatine, 2 MgATP, 0.4 NaGTP. In some experiments studying
178 cellular morphology, 5% biocytin was also included in the recording internal solution. After
179 allowing biocytin to diffuse through the recorded cell for at least 30 minutes, slices were fixed with
180 4% PFA before staining with streptavidin conjugated to Alexa 647 (Invitrogen).

181

182 Electrophysiology recordings were made with a Multiclamp 700B amplifier (Axon Instruments),
183 filtered at 4 kHz for current-clamp and 2 kHz for voltage-clamp, and sampled at 10 kHz. The initial
184 series resistance was <20 MΩ, and recordings were ended if series resistance rose above 25 MΩ.
185 In some experiments, 1 μM TTX was added to block APs, along with 100 μM 4-AP and 4 mM

186 external Ca^{2+} to restore presynaptic release. In many experiments, 10 μM CPP was used to block
187 NMDA receptors. In current-clamp experiments characterizing intrinsic properties, 10 μM NBQX,
188 10 μM CPP and 10 μM gabazine were used to block excitation and inhibition. In some
189 experiments, 10 μM AM-251 was used to block CB1 receptors or 1 μM WIN 55,212-2 was used
190 to activate CB1 receptors. All chemicals were purchased from either Sigma or Tocris Bioscience.

191
192 **Optogenetics:** Channelrhodopsin-2 (ChR2) was expressed in presynaptic neurons and activated
193 with a brief light pulse from a blue LED (473 nm) (Thorlabs). For wide-field illumination, light was
194 delivered via a 10x 0.3NA objective (Olympus) centered on the recorded cell. LED power was
195 routinely calibrated at the back aperture of the objective. LED power and duration were adjusted
196 to obtain reliable responses, with typical values of 0.4 to 10 mW and 2 ms, respectively.

197
198 **Data analysis:** Electrophysiology and imaging data were acquired using National Instruments
199 boards and custom software in MATLAB (MathWorks). Off-line analysis was performed using Igor
200 Pro (WaveMetrics). Intrinsic properties were determined as follows. Input resistance was
201 calculated from the steady-state voltage during a -50 pA, 500 ms current step. Voltage sag ratio
202 was calculated as $(V_{\text{sag}} - V_{\text{ss}}) / (V_{\text{sag}} - V_{\text{baseline}})$, where V_{sag} is average over a 1 ms window around
203 the minimum, V_{ss} is average of last 50 ms, and V_{baseline} is average of 50 ms preceding the current
204 injection. The membrane time constant (τ) was measured using exponential fits to these
205 hyperpolarizations. Adaptation was calculated as the ratio of the first and last inter-spike intervals,
206 such that a value of 1 indicates no adaptation and values <1 indicate lengthening of the inter-
207 spike interval. For experiments with a single optogenetic stimulation, the PSC amplitude was
208 measured as the average value across 1 ms around the peak subtracted by the average 100 ms
209 baseline value prior to the stimulation. For experiments with a train of optogenetic stimulation,
210 each PSC amplitude was measured as the average value in a 1 ms window around the peak,
211 minus the average 2 ms baseline value before each stimulation. Most summary data are reported

212 in the text and figures as arithmetic mean \pm SEM. Ratios of responses at pairs of cells are reported
213 as geometric mean in the text, and with \pm 95% confidence interval (CI) in the figures, unless
214 otherwise noted. Comparisons between unpaired data were performed using non-parametric
215 Mann-Whitney test. Comparisons between data recorded in pairs were performed using non-
216 parametric Wilcoxon test. Two-tailed p values <0.05 were considered significant.
217

218 **RESULTS**

219

220 **vHPC-evoked inhibition and interneurons in L5 of infralimbic PFC**

221 We studied vHPC-evoked inhibition at pyramidal neurons using whole-cell recordings and
222 optogenetics in acute slices of the mouse PFC. To allow for visualization and activation of inputs
223 to the PFC, we injected Chr2-expressing virus (AAV-ChR2-EYFP) into the ipsilateral vHPC
224 (**Fig. 1A**) (Little and Carter, 2012). In the same animals, we labeled IT cells by co-injecting
225 retrogradely transported, fluorescently-tagged cholera toxin subunit B (CTB) into the contralateral
226 PFC (cPFC) (**Fig. 1A**) (Anastasiades et al., 2018). After 2-3 weeks of expression, we prepared
227 *ex vivo* slices of the medial PFC, observing vHPC axons and IT cells in L5 of IL PFC (**Fig. 1B**)
228 (Liu and Carter, 2018). We then recorded in voltage-clamp from IT cells and activated vHPC
229 inputs using wide-field illumination (5 pulses at 20 Hz, 2 ms pulse duration). Trains of vHPC inputs
230 evoked excitatory postsynaptic currents (EPSCs) at -65 mV and inhibitory postsynaptic currents
231 (IPSCs) at +15 mV (**Fig. 1C**; EPSC₁ = 386 ± 49 pA, IPSC₁ = 961 ± 207 pA, E/I = 0.50 ± 0.08; n =
232 7 cells, 3 animals). These findings indicate that vHPC inputs drive robust feed-forward inhibition
233 in deep layers of the IL PFC, motivating us to identify which interneurons are responsible.

234

235 In principle, feed-forward inhibition could be mediated by a variety of interneurons, including PV+
236 and SOM+ interneurons (Abbas et al., 2018; Anastasiades et al., 2018; Marek et al., 2018;
237 McGarry and Carter, 2016). To visualize these cells in the PFC, we crossed PV-Cre and SOM-
238 Cre mice with reporter mice (Ai14) that express Cre-dependent tdTomato (Hippenmeyer et al.,
239 2005; Madisen et al., 2010; Taniguchi et al., 2011). We observed labeling of PV+ and SOM+
240 interneurons in L5 of IL PFC, suggesting they could be contacted by vHPC afferents (**Fig. 1D**).
241 However, the PFC also has a high density of cholecystinin-expressing (CCK+) interneurons
242 (Whissell et al., 2015), which mediate inhibition in other cortices and the hippocampus (Armstrong
243 and Soltesz, 2012; Freund and Katona, 2007), and could also participate in the PFC. To label

244 these cells, we initially injected AAV-DIO-GFP into CCK-Cre mice but observed labeling of both
245 interneurons and pyramidal neurons across multiple layers (**Fig. 1E**). To restrict labeling to CCK+
246 interneurons, we instead used AAV-Dlx-Flex-GFP, expressing Cre-dependent GFP under control
247 of the Dlx enhancer (Dimidschstein et al., 2016). Injecting AAV-Dlx-Flex-GFP selectively labeled
248 CCK+ interneurons in the IL PFC, including prominent labeling in L5 (**Fig. 1E**). Importantly, we
249 found little co-labeling of CCK+ cells with either PV (**Fig. 1F & S1A**; 11.3 % overlap, n = 308 cells
250 total, 17 slices, 6 animals) or SOM (6.6% overlap, n = 105 cells total, 8 slices, 3 animals).

251
252 To confirm targeting of CCK+ interneurons, we next used whole-cell recordings followed by post-
253 hoc reconstructions (**Fig. 1G**). We found both axons and dendrites in L5 of IL PFC, and intrinsic
254 properties similar to reports in other parts of the brain (**Fig. S1B**; $R_{in} = 130 \pm 13 \text{ M}\Omega$, $V_m = -68 \pm$
255 1 mV , $\text{Sag} = 4.1 \pm 1.0 \%$, $\text{Adaptation} = 0.81 \pm 0.04$, $\text{Tau} = 9.1 \pm 0.6 \text{ ms}$; n = 12 cells, 4 animals)
256 (Daw et al., 2009). CCK+ interneurons are also distinguished from PV+ cells by enrichment of the
257 cannabinoid type 1 receptor (CB1R) on presynaptic terminals (Bodor et al., 2005; Dudok et al.,
258 2015; Katona et al., 1999). To examine expression in the IL PFC, we used immunocytochemistry
259 and detected CB1R+ puncta located at either PV+ or CCK+ axons (**Fig. 1H**). We found CB1R+
260 puncta showed higher co-localization with CCK+ axons (based on GFP+ expression) than with
261 PV+ axons (**Fig. 1H**, puncta density in $10^3/\mu\text{m}^2$: $\text{PV+CB1R+} = 3.79 \pm 0.64$, $\text{GFP+CB1R+} = 11.27$
262 ± 1.5 , $p = 0.0003$). These results confirm our viral strategy can identify CCK+ interneurons, which
263 are present in deep layers of IL. Moreover, they suggest PV+, SOM+, and CCK+ interneurons
264 are positioned to receive vHPC inputs and may mediate feed-forward inhibition.

265

266 **vHPC inputs primarily engage PV+ and CCK+ interneurons**

267 Previous studies have suggested that vHPC inputs engage PV+ and SOM+ interneurons in the
268 PFC (Abbas et al., 2018; Marek et al., 2018). However, these connections have not been

269 examined in L5 of IL, where vHPC connections are strongest (Liu and Carter, 2018). Moreover,
270 little is known about the activation of CCK+ interneurons, which may play distinct functional roles
271 (Armstrong and Soltesz, 2012; Freund and Katona, 2007). To study connectivity, we injected
272 AAV-ChR2-YFP into the vHPC and recorded EPSCs from identified interneurons. To compare
273 across animals and cell types, we recorded from neighboring IT cells within the same slice, which
274 receive the bulk of vHPC inputs (Liu and Carter, 2018). To isolate monosynaptic connections, we
275 included TTX (1 μ M), 4-AP (10 μ M), and elevated Ca^{2+} (4 mM), which blocks action potentials
276 (APs) but restores presynaptic release (Little and Carter, 2012; Petreanu et al., 2009). We also
277 kept the light intensity and duration constant within pairs, allowing us to account for differences in
278 viral expression across animals and slices (Liu and Carter, 2018). We found that vHPC inputs
279 evoked robust EPSCs at CCK+ interneurons, which were similar in amplitude to IT cells (**Fig. 2A**;
280 IT = 674 ± 102 pA, CCK+ = 413 ± 89 pA, CCK+/IT ratio = 0.56, $p = 0.13$; $n = 9$ pairs, 4 animals).
281 We also found prominent vHPC-evoked EPSCs at PV+ interneurons, with comparable amplitudes
282 at IT cells (**Fig. 2B**; IT = 569 ± 91 pA, PV+ = 555 ± 83 pA, PV+/IT ratio = 0.94, $p = 0.82$; $n = 9$
283 pairs, 4 animals). In contrast, vHPC inputs evoked much smaller EPSCs at SOM+ interneurons
284 compared to IT cells (**Fig. 2C**; IT = 421 ± 69 pA, SOM+ = 95 ± 33 pA, SOM+/IT ratio = 0.17, $p =$
285 0.004; $n = 9$ pairs, 4 animals). These findings show that all three cell types receive direct vHPC
286 inputs, with greater responses at CCK+ and PV+ interneurons.

287
288 Having established the targeting of vHPC inputs, we next assessed their ability to drive action
289 potentials (APs) at the three classes of interneurons. We used similar viral and labeling
290 approaches, but in this case conducted current-clamp recordings in the absence of TTX and 4-AP.
291 We also kept light intensity constant within each set of experiments, making sure we evoked
292 action potentials in at least one of the recorded pair of cells. We found that single vHPC inputs
293 evoked APs in pairs of CCK+ and IT cells, but the interneurons showed higher probability of firing

294 **(Fig. 2D; AP probability: IT = 0.32 ± 0.15 , CCK+ = 0.84 ± 0.11 , $p = 0.03$; n = 9 pairs, 4 animals).**
295 Similarly, we observed that vHPC inputs also preferentially activate PV+ over IT cells **(Fig. 2E;**
296 **AP probability: IT = 0.06 ± 0.06 , PV+ = 0.65 ± 0.17 , $p = 0.04$; n = 8 pairs, 4 animals).** In contrast,
297 SOM+ interneurons remained unresponsive to vHPC inputs, even when using higher light
298 intensities that were able to activate IT cells **(Fig. 2F; AP probability: IT = 0.81 ± 0.12 , SOM+ =**
299 **0.13 ± 0.06 , $p = 0.01$; n = 7 pairs, 3 animals).** These results established a hierarchy for activation,
300 suggesting CCK+ interneurons are engaged by vHPC inputs and mediate feed-forward inhibition.

301

302 **Short-term dynamics differ between populations of interneurons**

303 Repetitive activity at vHPC to PFC connections is functionally important and depends on stimulus
304 frequency (Liu and Carter, 2018; Siapas et al., 2005). At the synaptic level, repetitive activity
305 engages short-term plasticity to change the strength of individual connections (Zucker and
306 Regehr, 2002). We next examined the response to repetitive vHPC inputs by stimulating with brief
307 trains (5 pulses at 20Hz) in the absence of TTX and 4-AP (Liu and Carter, 2018; McGarry and
308 Carter, 2016). We observed that EPSCs at CCK+ and PV+ interneurons strongly depress over
309 the course of stimulus trains **(Fig. 3A, B & D; EPSC₂ / EPSC₁: CCK+ = 0.82 ± 0.06 ; n = 6 cells, 3**
310 **animals; PV+ = 0.88 ± 0.06 ; n = 7 cells, 3 animals).** In contrast, the EPSCs at SOM+ interneurons
311 initially facilitated during trains **(Fig. 3C & D; EPSC₂ / EPSC₁ = 1.78 ± 0.23 ; n = 8 cells, 3 animals).**
312 These results indicate the vHPC engages all three cell types, with connections at PV+ and CCK+
313 interneurons strong but depressing, and those at SOM+ interneurons weak but facilitating.

314

315 The short-term dynamics of vHPC inputs suggested differential engagement of PV+, SOM+, and
316 CCK+ interneurons during repetitive activity. We observed that stimulus trains of vHPC inputs
317 generated decreasing AP probabilities at CCK+ and PV+ interneurons **(Fig. 3E, F & H; stimulus**
318 **1 & 2 AP probabilities: CCK+ = 0.97 ± 0.02 & 0.75 ± 0.06 ; n = 12 cells, 5 animals; PV+ = $0.96 \pm$**

319 0.02 & 0.70 ± 0.13 ; n = 6 cells, 3 animals;). In contrast, although SOM+ interneurons did not fire
320 with single vHPC inputs, they were activated during trains. Importantly, these responses were not
321 due to recurrent network activity, as stimulation intensity was chosen here to ensure that IT cells
322 remained quiescent (**Fig. 3G & H**; stimulus 1 & 2 AP probabilities: IT = 0 & 0, SOM+ = 0 & 0.57
323 ± 0.14 ; n = 6 pairs, 4 animals). These findings suggest the vHPC differentially engages three
324 interneurons with distinct dynamics during repetitive activity, with strong and depressing PV+ and
325 CCK+ activity contributing early, and weaker but facilitating SOM+ activity contributing later.

326

327 **CCK+ interneurons make connections onto L5 pyramidal cells**

328 Our results indicate that vHPC inputs strongly engage CCK+ interneurons in the PFC, suggesting
329 a role in feed-forward inhibition. However, the connections made by CCK+ interneurons onto
330 nearby pyramidal cells are not well established in the cortex. To study CCK+ output, we developed
331 a new virus (AAV-Dlx-Flex-ChR2-mCherry) to express ChR2 in a Cre-dependent manner under
332 the Dlx enhancer (see Methods). We injected this virus into the PFC of CCK-Cre mice to
333 selectively express ChR2 in CCK+ interneurons (**Fig. 4A**). Whole-cell current-clamp recordings
334 showed labeled cells could be rapidly and reliably activated by brief pulses of blue light (**Fig. 4A**).
335 To study inhibitory connections, we then recorded CCK+-evoked currents from unlabeled L5
336 pyramidal cells in IL PFC (**Fig. 4B**). To detect both EPSCs or IPSCs, we used a low chloride
337 internal and held at -50 mV, such that inward currents were EPSCs and outward currents were
338 IPSCs (Glickfeld and Scanziani, 2006). We observed robust CCK+-evoked IPSCs, which were
339 unaffected by blockers of AMPAR (10 μ M NBQX) and NMDAR (10 μ M CPP) but abolished by
340 blockers of GABA_AR (10 μ M gabazine) (**Fig. 4B**; ACSF = 97 ± 21 pA, NBQX+CPP = 97 ± 21 pA,
341 gabazine = 0.4 ± 0.2 pA; ACSF vs NBQX + CPP, p = 0.69; NBQX + CPP vs gabazine, p = 0.01;
342 n = 7 cells, 3 animals). These findings indicate that CCK+ interneurons make inhibitory

343 connections onto neighboring L5 pyramidal cells, and that our viral strategy avoids contamination
344 from excitatory contacts due to inadvertent activation of CCK+ pyramidal cells.

345

346 Previous studies indicate that inhibitory inputs from PV+ and SOM+ interneurons are strongly
347 biased onto PT cells over nearby IT cells (Anastasiades et al., 2018). To test if similar biases
348 occur for CCK+ interneurons, we labeled PT and IT cells by injecting retrograde tracers into
349 periaqueductal gray (PAG) and cPFC, respectively (**Fig. 4C**). Recording from pairs of pyramidal
350 cells, we found that CCK-evoked IPSCs were larger onto PT cells than IT cells (**Fig. 4D**; IPSC IT
351 = 113 ± 59 pA, PT = 245 ± 79 pA, $p = 0.01$; $n = 7$ pairs, 4 animals). Together, these findings
352 indicate our viral strategy can be used to manipulate CCK+ interneurons, and show that these
353 cells make cell-type specific connections, preferentially targeting PT cells in L5 of the IL PFC.

354

355 **CB1R-mediated DSI depends on the postsynaptic cell-type**

356 In the hippocampus and the amygdala, CCK+ inputs to pyramidal cells are strongly modulated by
357 endocannabinoids (Lee et al., 2010; Vogel et al., 2016; Wilson and Nicoll, 2001). Postsynaptic
358 depolarization releases endocannabinoids that act on presynaptic CB1 receptors to prevent
359 GABA release, a process known as depolarization-induced suppression of inhibition (DSI) (Wilson
360 and Nicoll, 2001). To study DSI at IT and PT cells, we injected retrogradely transported CTBs in
361 the cPFC and PAG, along with AAV-Dlx-Flex-ChR2 in the PFC of CCK-Cre mice (**Fig. 5A**).
362 We evoked DSI with a standard protocol (Glickfeld and Scanziani, 2006; Wilson and Nicoll, 2001),
363 recording baseline CCK-evoked IPSCs during a train, followed by a 5 s depolarization to +10 mV
364 and recording a DSI test after an initial 1 s delay, followed by a recovery test after another 30 s
365 delay (**Fig. 5B**). For these experiments, it was critical to record IPSCs at -50 mV rather than +10
366 mV, which allows us to control the timing of endocannabinoid release. In voltage-clamp recordings
367 from retrogradely labeled IT cells, we found that CCK+-evoked IPSCs underwent pronounced DSI
368 that recovered when inputs were stimulated again 30 seconds later (**Fig. 5C & F**; IPSC₁: before

369 = 158 ± 34 pA, after = 91 ± 24 pA, recovery = 149 ± 33 pA; DSI ratio = 0.57 ± 0.06 , $p = 0.004$; n
370 = 9 cells, 5 animals). We confirmed the involvement of endocannabinoid signaling by blocking
371 DSI with the CB1R inverse agonist 10 μ M AM-251 (**Fig. 5D & F**; IPSC₁: before = 189 ± 53 pA,
372 after = 168 ± 44 pA, recovery = 185 ± 55 ; DSI ratio = 0.92 ± 0.04 , $p = 0.08$; n = 7 cells, 4 animals).
373 We also confirmed this modulation was specific to CCK+ inputs, as PV+ and SOM+-evoked
374 IPSCs at IT cells had minimal DSI (**Fig. 5F & S2**; IPSC₁ DSI ratio: PV+ = 0.94 ± 0.02 , $p = 0.11$; n
375 = 7 cells, 3 animals; SOM+ = 0.87 ± 0.05 , $p = 0.02$; n = 8 cells, 3 animals; PV+ vs CCK+, $p =$
376 0.0007 ; SOM+ vs CCK+, $p = 0.008$). Surprisingly, we found minimal DSI at PT cells, indicating
377 modulation also strongly depends on postsynaptic cell type (**Fig. 5E & F**; IPSC₁ before = $208 \pm$
378 40 pA, after = 202 ± 44 pA, recovery = 205 ± 40 pA; DSI ratio = 0.95 ± 0.04 , $p = 0.22$; n = 7 cells,
379 3 animals). These findings indicate that although CCK+ interneurons broadly contact L5 pyramidal
380 cells, the connections undergo prominent DSI only at IT cells and not nearby PT cells.

381

382 **Endocannabinoid modulation depends on postsynaptic cell type**

383 The dependence of DSI on postsynaptic cell-type could have a presynaptic origin if CB1Rs are
384 expressed on a subset of CCK+ connections. To test this possibility, we next used
385 immunocytochemistry to examine CB1R+ puncta at the cell bodies of IT and PT cells. To label
386 these projection neurons, we injected retrogradely transported AAVrg-TdTomato into the cPFC
387 and AAVrg-GFP into the PAG, respectively (**Fig. 6A**). While we observed CB1R+ puncta in L5
388 around the cell bodies of both cell types (**Fig. 6A**), their density was greater at IT cells (**Fig. 6B**;
389 IT = 9.3 ± 0.5 puncta / cell, PT = 5.7 ± 0.5 puncta / cell, $p < 0.0001$; n = 4 animals, 247 IT cells,
390 207 PT cells). These results indicate that presynaptic CB1Rs are more prominent at perisomatic
391 connections onto IT cells, suggesting why these inputs are more strongly affected by DSI.

392

393 If the cell-type specificity of DSI depends on presynaptic factors, we would expect to observe
394 equivalent differences for pharmacologically evoked endocannabinoid modulation. We further
395 examined the mechanism of CB1R modulation using wash-in of the agonist WIN 55,212-1 (WIN,
396 1 μ M) followed by the inverse agonist AM-251 (10 μ M), which act directly at presynaptic CB1Rs
397 located on CCK+ axon terminals. In voltage-clamp recordings from L5 IT cells, we found that WIN
398 reduced CCK+-evoked IPSCs, which was reversed by AM-251 (**Fig. 6C**; baseline = 234 ± 53 pA,
399 WIN = 106 ± 26 pA, AM-251 = 206 ± 50 pA; baseline vs WIN, $p = 0.008$; WIN vs AM-251, $p =$
400 0.016 ; $n = 8$ cells, 5 animals). In contrast, in recordings from PT cells, we found that neither WIN
401 nor AM-251 had any effect on CCK+-evoked IPSCs, consistent with their lack of DSI (**Fig. 6D**;
402 baseline = 292 ± 55 pA, WIN = 250 ± 36 pA, AM-251 = 281 ± 37 pA; baseline vs WIN, $p = 0.81$;
403 WIN vs AM-251, $p = 0.22$; $n = 7$ cells, 4 animals). Overall, activation of CB1 receptors strongly
404 reduced CCK+-evoked IPSCs only at IT cells but not neighboring PT cells (**Fig. 6E**; WIN /
405 baseline: IT = 0.44 ± 0.05 , PT = 0.92 ± 0.09 , $p = 0.001$). Interestingly, during trains of CCK+
406 stimulation, wash-in of WIN also increased the paired-pulse ratio (PPR) at IT cells, suggesting
407 presynaptic modulation by CB1Rs (**Fig. 6F**; IPSC₂ / IPSC₁: baseline = 0.48 ± 0.03 , WIN = $0.59 \pm$
408 0.03 , AM-251 = 0.54 ± 0.02 ; baseline vs WIN, $p = 0.03$; WIN vs AM-251, $p = 0.22$; $n = 7$ cells, 4
409 animals). These findings demonstrate that differences in presynaptic endocannabinoid signaling
410 can account for the differential presence of DSI at CCK+ connections onto IT and PT cells.

411

412 **vHPC-evoked feed-forward inhibition is modulated by endocannabinoids**

413 Together, our results suggest that vHPC inputs strongly engage CCK+ interneurons that in turn
414 robustly inhibit IT cells. Based on the presence of CB1R modulation and DSI at IT cells, we
415 hypothesized that vHPC-evoked inhibition should also undergo target-specific DSI. In voltage-
416 clamp recordings from IT cells, we found that repetitive activation of vHPC inputs evoked EPSCs
417 and IPSCs at -50mV (**Fig. 7A & B**). Depolarization of IT cells (5 s to +10 mV) reduced vHPC-

418 evoked IPSCs but not EPSCs at -50 mV (**Fig. 7B & E**; before, after, recovery: IPSC₁ = 102 ± 14
419 pA, 51 ± 10 pA, 88 ± 10 pA; EPSC₁ = 248 ± 54 pA, 233 ± 48 pA, 241 ± 52 pA; DSI ratio = 0.47 ±
420 0.05, p = 0.02; DSE ratio = 0.99 ± 0.06, p = 0.30; n = 7 cells, 4 animals). These findings indicate
421 that there is no DSE at the vHPC to PFC connection, whereas there is prominent DSI onto IT
422 cells. Importantly, application of AM-251 minimized DSI, indicating that it is mediated by
423 endocannabinoids activating CB1Rs (**Fig. 7C & E**; IPSC₁: before = 136 ± 31 pA, after = 116 ± 29
424 pA, recovery = 149 ± 35 pA; DSI ratio = 0.84 ± 0.03, p = 0.02; n = 7 cells, 3 animals; DSI in ACSF
425 vs AM-251, p = 0.0006). Moreover, depolarization of PT cells had minimal effect on either vHPC-
426 evoked IPSCs or EPSCs, confirming no DSI or DSE onto this postsynaptic cell type (**Fig. 7D &**
427 **E**; IPSC₁ before = 129 ± 32 pA, after = 114 ± 27 pA, recovery = 134 ± 33 pA; DSI ratio = 0.91 ±
428 0.05, p = 0.10; DSE ratio = 0.89 ± 0.04, p = 0.01; n = 10 cells, 4 animals). These findings show
429 that DSI of vHPC-evoked inhibition only occurs at IT cells, suggesting a key role for CCK+
430 interneurons in the communication between vHPC and PFC.

431 **DISCUSSION**

432 We have explored several new features about the organization and modulation of connections
433 from vHPC to PFC (**Fig. 7F**). First, we found vHPC contacts and strongly activates CB1R-
434 expressing CCK+ interneurons in L5 of IL PFC. Second, we showed that CCK+ interneurons
435 contact nearby pyramidal cells, suggesting they participate in feed-forward inhibition. Third, we
436 found that CCK+ connections undergo CB1R-mediated modulation and DSI, which is selective
437 for IT and not PT cells. Fourth, endocannabinoids also modulate vHPC-evoked inhibition, which
438 also undergoes DSI selectively at IT cells. Together, our results reveal a central role for CCK+
439 interneurons and endocannabinoid modulation in communication between vHPC and PFC.

440

441 The PFC possesses a rich variety of GABAergic interneurons, which are known to have unique
442 roles in goal-directed behaviors (Abbas et al., 2018; Courtin et al., 2014; Kepecs and Fishell,
443 2014; Kvitsiani et al., 2013). Interestingly, the PFC has fewer PV+ interneurons and more CCK+
444 interneurons compared to other cortices (Kim et al., 2017; Whissell et al., 2015). Our results
445 indicate that CCK+ interneurons are abundant in L5 of IL PFC, which we previously showed
446 receives the strongest connections from vHPC (Liu and Carter, 2018). We found these cells are
447 distinct from PV+ and SOM+ interneurons, with different morphological and physiological
448 properties. We also found that they are enriched in presynaptic CB1Rs, as in other parts of cortex,
449 hippocampus and amygdala (Armstrong and Soltesz, 2012; Bodor et al., 2005; Vogel et al., 2016).
450 The enrichment of CCK+ interneurons across limbic areas is consistent with a role in emotional
451 behaviors (Freund and Katona, 2007; Klausberger et al., 2005). In the future, it will be interesting
452 to characterize the properties of CCK+ interneurons in other layers and subregions of PFC.

453

454 One of our key results is that vHPC inputs densely contact and strongly activate CCK+
455 interneurons in L5 of IL PFC. vHPC inputs are strong and depressing onto CCK+ interneurons, in
456 contrast to the facilitating inputs onto IT cells (Liu & Carter, 2018). Importantly, vHPC-evoked

457 firing of CCK+ interneurons occurs without activation of IT cells, indicating polysynaptic recurrent
458 activity is not required for activation of CCK+ interneurons, which are therefore likely to mediate
459 feed-forward inhibition. With higher intensity of vHPC stimulation, CCK+ interneurons may also
460 be activated by local inputs, as observed in the hippocampus (Glickfeld and Scanziani, 2006),
461 and similar to SOM+ interneurons in the cortex (Silberberg and Markram, 2007). Indeed, in the
462 hippocampus, CCK+ interneurons have been shown to participate in both feed-forward and feed-
463 back networks (Basu et al., 2013; Glickfeld and Scanziani, 2006). Our findings show that CCK+
464 interneurons have an important and underappreciated role in hippocampal evoked feed-forward
465 inhibition, and it will be important to assess whether this generalizes to other inputs to the PFC.

466

467 While we focused on CCK+ interneurons, we also confirmed that vHPC inputs engage other
468 interneurons in L5 of IL PFC. Activation of PV+ interneurons is strong, consistent with their role
469 in feed-forward inhibition in PFC and elsewhere (Anastasiades et al., 2018; Cruikshank et al.,
470 2007; Delevich et al., 2015; Gabernet et al., 2005; McGarry and Carter, 2016). Previous results
471 have indicated that vHPC engages PV+ interneurons in superficial layers of the medial PFC
472 (Marek et al., 2018). In contrast, we find particularly strong connections in L5, where our previous
473 study shows vHPC inputs are most dense (Liu and Carter, 2018). SOM+ interneuron activation is
474 weak, but increases with repetitive activity, similar to BLA inputs to superficial PFC (McGarry and
475 Carter, 2016). The engagement of SOM+ interneurons is consistent with a role in oscillations
476 linking the vHPC and PFC and involvement in working memory (Abbas et al., 2018). Interestingly,
477 the activation of SOM+ interneurons by long-range inputs also occurs in granular sensory cortex,
478 where it also builds during stimulus trains, suggesting this is a general property (Tan et al., 2008).

479

480 We found that CCK+ interneurons make strong inhibitory connections onto neighboring pyramidal
481 cells in L5 of the IL PFC. Importantly, these connections are much stronger onto PT cells, similar
482 to our previous findings for PV+ and SOM+ interneurons (Anastasiades et al., 2018). Biased

483 inhibition is thus a general property of inhibitory connections in the PFC, although strength also
484 depends on intrinsic properties (Anastasiades et al., 2018). In the hippocampus, CCK+
485 interneurons also make unique connections onto different pyramidal cell populations, although
486 these are typically defined by sublayer (Valero et al., 2015). Studying the output of CCK+
487 interneurons was enabled by using Cre-dependent viruses with Dlx enhancers (Dimidschstein et
488 al., 2016). These tools allowed us to avoid simultaneous activation of pyramidal cells, which also
489 express CCK (Taniguchi et al., 2011). In the future, this approach can be used to study how CCK+
490 interneurons contact other cells in the local network. For example, CCK+ interneurons may also
491 target other classes of interneurons, as observed in the hippocampus (Daw et al., 2009).

492

493 Another key finding of our work was that endocannabinoid modulation of CCK+ connections
494 depends on the postsynaptic target. We observe robust DSI at IT cells, but minimal DSI at nearby
495 PT cells, despite the latter receiving stronger connections. This cell-type specific DSI has rarely
496 been examined in the cortex, as most studies do not distinguish projection target. However, a
497 recent study also showed differential DSI at CCK+ connections onto projection neurons in the
498 amygdala (Vogel et al., 2016). The dependence of DSI on the postsynaptic target should be taken
499 into account in future studies of CCK+-mediated inhibition. Importantly, our experiments
500 suggested no equivalent DSI for either PV+ or SOM+ connections onto IT cells. These results
501 suggest CCK+ connections are a primary target of endocannabinoid modulation, in agreement
502 with previous studies in the cortex, hippocampus and other brain regions (Bodor et al., 2005;
503 Glickfeld et al., 2008; Gonchar et al., 2007; Katona et al., 1999; Kawaguchi and Kondo, 2002).

504

505 In principle, the selectivity of DSI at CCK+ connections onto IT cells could reflect differences in
506 postsynaptic release or presynaptic detection of endocannabinoids. Our results are consistent
507 with the latter explanation. First, our immunocytochemistry shows more CB1R puncta around IT
508 cells, suggesting these receptors are selectively localized. This result was particularly surprising

509 because we also found CCK+ interneurons make stronger connections onto PT cells. Second,
510 direct activation of CB1Rs with WIN reduces CCK-evoked IPSCs only at IT cells, with no effect at
511 neighboring PT cells. This experiment bypasses postsynaptic release of endocannabinoids,
512 suggesting a presynaptic mechanism can account for specificity of DSI. The increase in PPR is
513 also consistent with presynaptic modulation by CB1R, suggesting reduced release probability
514 (Wilson et al., 2001). This work underscores that endocannabinoid modulation is cell-type
515 specific, and it will be important to examine if similar specificity occurs in other regions of the PFC.
516

517 The ability of vHPC inputs to engage CCK+ interneurons, which in turn contact pyramidal cells,
518 implicates a key role in feed-forward inhibition. Consistent with this idea, we observed prominent
519 CB1R-mediated DSI of vHPC-evoked inhibition only at IT cells and not neighboring PT cells.
520 Because there is no change in excitation, this will selectively increase the excitation / inhibition
521 (E / I) ratio at IT cells. This could allow the vHPC to more effectively activate IT cells compared to
522 neighboring PT cells, perhaps elevating local processing within the PFC. Altered E / I balance
523 could also potentially shift PFC output to other intratelencephalic targets throughout the brain,
524 including targets in the cortex, striatum, amygdala and claustrum (Anastasiades et al., 2019).
525 Together, our findings suggest that both CCK+ interneurons and endocannabinoid modulation
526 play a fundamental role in vHPC to PFC communication. In the future, it will be important to assess
527 the contribution of this circuit to emotional control and disorders, including threat learning and
528 anxiety disorders (Peters et al., 2010; Sierra-Mercado et al., 2011; Sotres-Bayon et al., 2012).

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672 **FIGURE LEGENDS**

673

674 **Figure 1: vHPC-evoked feed-forward inhibition and CCK+ interneurons.**

675 **A)** Schematic for injections of AAV-ChR2-EYFP into vHPC and CTB-647 into cPFC.

676 **B)** *Left*, Confocal image of vHPC axons (blue) in IL PFC. Scale bar = 100 μm . *Right*, Confocal
677 image of biocytin-filled L5 IT cell in IL PFC. Scale bar = 100 μm .

678 **C)** *Left*, Average vHPC-evoked EPSCs at -65 mV (black) and IPSCs at +15 mV (grey). Blue
679 arrows = 5 pulses at 20 Hz. *Right*, Average response amplitudes as function of pulse number
680 (n = 7 cells, 3 animals).

681 **D)** Td-tomato labeling of PV+ (blue) and SOM+ (green) interneurons in PV-Cre x Ai14 and SOM-
682 Cre x Ai14 animals, respectively.

683 **E)** *Left*, Schematic for injections of viruses into PFC of CCK-Cre mouse. *Middle*, Injection of AAV-
684 DIO-GFP labels CCK+ interneurons and pyramidal cells (n = 3 animals). *Right*, Injection of
685 AAV-Dlx-Flex-GFP labels CCK+ interneurons (n = 3 animals). Scale bars = 100 μm .

686 **F)** Quantification of co-labeling of CCK+ interneurons with PV (top) and SOM (bottom) (PV
687 staining, n = 308 cells, 17 slices, 6 animals; SOM staining, n = 105 cells, 8 slices, 3 animals).

688 **G)** *Left*, Confocal image of a biocytin-filled CCK+ interneuron in L5 of IL PFC. Scale bar = 100 μm .
689 *Right*, Response to positive and negative current injections.

690 **H)** *Left*, Confocal images of GFP (green), anti-CB1R staining (purple), anti-PV staining (blue),
691 and merge. Arrow heads: white = GFP+CB1R+ co-labeling, yellow = PV+CB1R+ co-labeling.

692 *Middle*, Quantification of PV+CB1R+ and GFP+CB1R+ quanta per $10^3 \mu\text{m}^2$ (n = 9 sections, 3
693 animals). *Right*, Quantification of the ratios of CB1R+ puncta among PV+ and GFP+ puncta.

694 Each line represents counts from one slice.

695 **Figure 2: vHPC inputs differentially engage PV+, SOM+ and CCK+ interneurons.**

696 **A)** *Left*, Average vHPC-evoked EPSCs at pairs of L5 IT (grey) and CCK+ (purple) cells in IL PFC.

697 Blue arrow = light pulse. *Middle*, Summary of EPSC amplitudes. *Right*, Summary of CCK+ /

698 IT EPSC amplitude ratios (n = 9 pairs, 4 animals).

699 **(B – C)** Similar to (A) for pairs of IT and PV+ cells (n = 9 pairs, 4 animals) or pairs of IT and SOM+

700 cells (n = 9 pairs, 4 animals).

701 **D)** *Left*, vHPC-evoked EPSPs and APs recorded in current-clamp from resting membrane

702 potential at pairs of L5 IT (grey) and CCK+ (purple) cells in IL PFC, with 5 traces offset for

703 each cell. *Right*, Summary of AP probability at pairs of IT and CCK+ cells. Blue arrow =

704 3.5 mW light pulse (n = 9 pairs, 4 animals).

705 **(E – F)** Similar to (D) for pairs of IT and PV+ cells (3.5 mW light pulses, n = 8 pairs, 4 animals) or

706 pairs of IT and SOM+ cells (4.8 mW light pulses, n = 7 pairs, 3 animals).

707 * p<0.05

708 **Figure 3: vHPC inputs drive interneurons with distinct temporal dynamics.**

709 **A)** Average vHPC-evoked EPSCs at CCK+ interneurons, recorded in voltage-clamp at -65 mV
710 (5 pulses at 20 Hz). Blue arrows = light pulses (n = 6 cells, 3 animals).

711 **B)** Similar to (A) for PV+ interneurons (n = 7 cells, 3 animals).

712 **C)** Similar to (A) for SOM+ interneurons (n = 8 cells, 3 animals).

713 **D)** Average paired-pulse ratio (PPR) of vHPC-evoked EPSCs at CCK+, PV+, and SOM+
714 interneurons.

715 **E)** vHPC-evoked EPSPs and APs, recorded in current-clamp from resting membrane potential
716 at an example CCK+ interneuron in L5 of IL PFC, with 5 traces offset for the cell. Blue arrows
717 = light pulses (n = 12 cells, 5 animals).

718 **F)** Similar to (E) for PV+ interneurons (n = 6 cells, 3 animals).

719 **G)** Similar to (E) for SOM+ interneurons. Note that each SOM+ cell was studied with a nearby IT
720 cell under same recording and stimulation conditions, where the absence of AP firing at IT
721 cells indicated subthreshold of network recurrent activation (n = 6 cells, 3 animals).

722 **H)** Summary of average AP probability as a function of pulse number for CCK+, PV+, and SOM+
723 interneurons.

724 * p<0.05

725 **Figure 4: CCK+ interneurons make connections onto pyramidal cells.**

726 **A)** *Left*, Injections of AAV-Dlx-Flex-ChR2 into the PFC of CCK-Cre animals. *Right*, Light-evoked
727 firing of a ChR2-expressing CCK+ interneuron. Purple arrows = 5 light pulses at 20 Hz.

728 **B)** *Left*, Average CCK+-evoked IPSCs at L5 pyramidal cells in IL PFC. When recording at -50 mV
729 with a low Cl⁻ internal solution, only outward IPSCs were observed (black). IPSCs were
730 unchanged after wash-in of NBQX + CPP (blue) but abolished by wash-in of gabazine (red).

731 *Right*, Summary of IPSC amplitudes in the different conditions. Purple arrow = light pulse.

732 **C)** Schematic of triple injections, with CTB-647 into cPFC, red retrobeads into PAG, and AAV-
733 Dlx-Flex-ChR2 into PFC.

734 **D)** *Left*, Recording schematic of CCK+ inputs onto IT and PT cells. Middle, CCK-evoked IPSCs
735 at PT and IT cells. *Right*, Summary of IPSC amplitudes at PT and IT cells (7 cells, 4 animals).

736 * p<0.05

737 **Figure 5: Prominent DSI of CCK+ inputs onto IT cells.**

738 **A)** Injection schematic of CTB-647 into cPFC and red retrobeads (RB) into PAG, along with AAV-
739 Dlx-Flex-ChR2 into PFC of CCK-Cre mice.

740 **B)** Experimental procedure for depolarization-induced suppression of inhibition (DSI), with 30 s
741 baseline, followed by 5 s depolarization to +10 mV, and 30 s recovery, repeated every 30 s.

742 **C)** *Left*, Average CCK+-evoked IPSCs at IT cells before (black), after (red) and recovery from
743 (gray) the brief depolarization. Purple arrows = 5 light pulses at 20 Hz. *Right*, Summary of
744 amplitudes of the first IPSC, showing robust DSI (n = 9 cells, 5 animals).

745 **D)** Similar to (C) in the presence of 10 μ M AM-251, which abolished DSI (n = 7 cells, 4 animals).

746 **E)** Similar to (C) for CCK+-evoked IPSCs at PT cells, showing no DSI (n = 7 cells, 3 animals).

747 **F)** Summary of DSI ratios (IPSC after depolarization / IPSC before depolarization) across
748 experiments in (C – E).

749 * p<0.05

750 **Figure 6: Cell-type specific modulation by CB1 receptors.**

- 751 **A)** *Left*, Injection schematic of AAVrg-GFP into PFC and AAVrg-tdTomato into PAG. *Middle*,
752 Confocal image of IT cells (cyan), PT cells (purple) and CB1 receptors (yellow). Scale bar =
753 50 μm . *Right*, Magnification of region on left. Scale bar = 20 μm .
- 754 **B)** Quantification of CB1R puncta in IT and PT cells, each dot represents the average puncta
755 number per cell in a slice (n = 247 IT cells, 207 PT cells, 4 animals).
- 756 **C)** *Left*, Schematic of recordings from IT cells in L5 of IL PFC. *Middle*, Average CCK+-evoked
757 IPSCs at IT cells at baseline (black), 10 min after wash-in of 1 μM WIN 55,212-2 (red), and
758 15 minutes after additional wash-in of 10 μM AM-251 (gray). Purple arrow = light stimulation.
759 *Right*, Summary of IPSC amplitudes (n = 8 cells, 5 animals).
- 760 **D)** Similar to (C) for CCK+-evoked IPSCs at PT cells, showing lack of modulation by CB1R (n =
761 7 cells, 4 animals).
- 762 **E)** *Left*, Summary of time course of modulation at IT and PT cells, with IPSC amplitudes
763 normalized to the average response during the first 5 minutes. *Right*, Summary of normalized
764 IPSC amplitudes after WIN wash-in.
- 765 **F)** Similar to (C) for trains of CCK+ inputs onto IT cells (5 pulses at 20 Hz), showing small
766 increase in PPR after wash-in of 1 μM WIN 55,212-2 (n = 7 cells, 4 animals).

767 * p<0.05

768 **Figure 7: vHPC-evoked feed-forward inhibition at IT cells undergoes DSI.**

769 **A)** Injection schematic, showing CTB-647 in cPFC, red retrobeads (RB) in PAG, along with AAV-
770 ChR2-EYFP in vHPC.

771 **B)** *Left*, Average vHPC-evoked EPSCs and IPSCs at IT cells in L5 IL PFC, before (black),
772 immediately after depolarization (red), and after recovery (gray) (same paradigm as Figure 5).
773 Blue arrows = light stimulation. *Right*, Summary of amplitudes of first vHPC-evoked IPSCs (n
774 = 7 cells, 4 animals).

775 **C)** Similar to (B) in the presence of 10 μ M AM-251, which reduces DSI (n = 7 cells, 3 animals).

776 **D)** Similar to (B) for PT cells, showing no DSI (n = 10 cells, 4 cells).

777 **E)** Summary of DSI and DSE ratios (amplitude ratios of IPSCs or EPSCs after / before the
778 depolarizations) across the different experiments.

779 **F)** Summary schematic for vHPC-evoked feed-forward inhibition in IL PFC. vHPC inputs directly
780 contact IT over PT cells to evoke EPSCs. vHPC inputs also engage multiple interneurons to
781 evoke local inhibition. Inhibition mediated by CCK+ interneurons displays robust
782 endocannabinoid-mediated DSI, but only at IT cells, and not neighboring PT cells.

783 * p < 0.05

784 **Figure S1: Properties of CCK+ interneurons in L5 IL PFC.**

785 **A)** Co-labeling of GFP-expressing CCK+ interneurons (purple) with PV (blue) and SOM (green).

786 Immunohistochemistry of GFP and either PV (top) or SOM (bottom) in PFC slices from CCK-

787 Cre animals injected with AAV-Dlx-Flex-GFP. From left to right: GFP; PV or SOM; merged.

788 Scale bar = 100 μ m.

789 **B)** *Left*, Firing frequency (F) vs. current (I) curve for CCK+ cells. *Right*, Summary of membrane

790 resting potential (V_{rest}) and input resistance (R_{in}) of CCK+ interneurons (n = 12 cells, 4

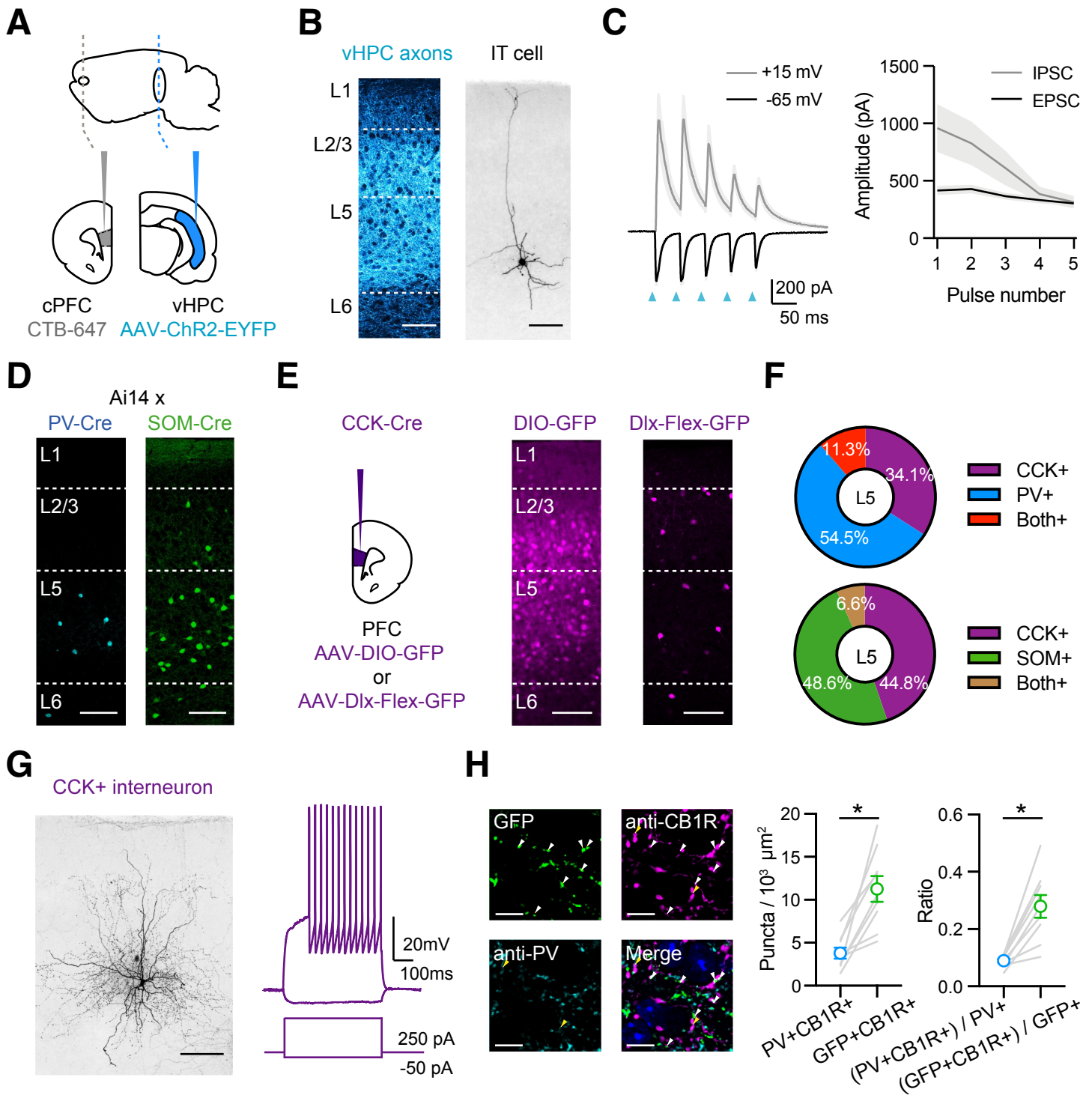
791 animals).

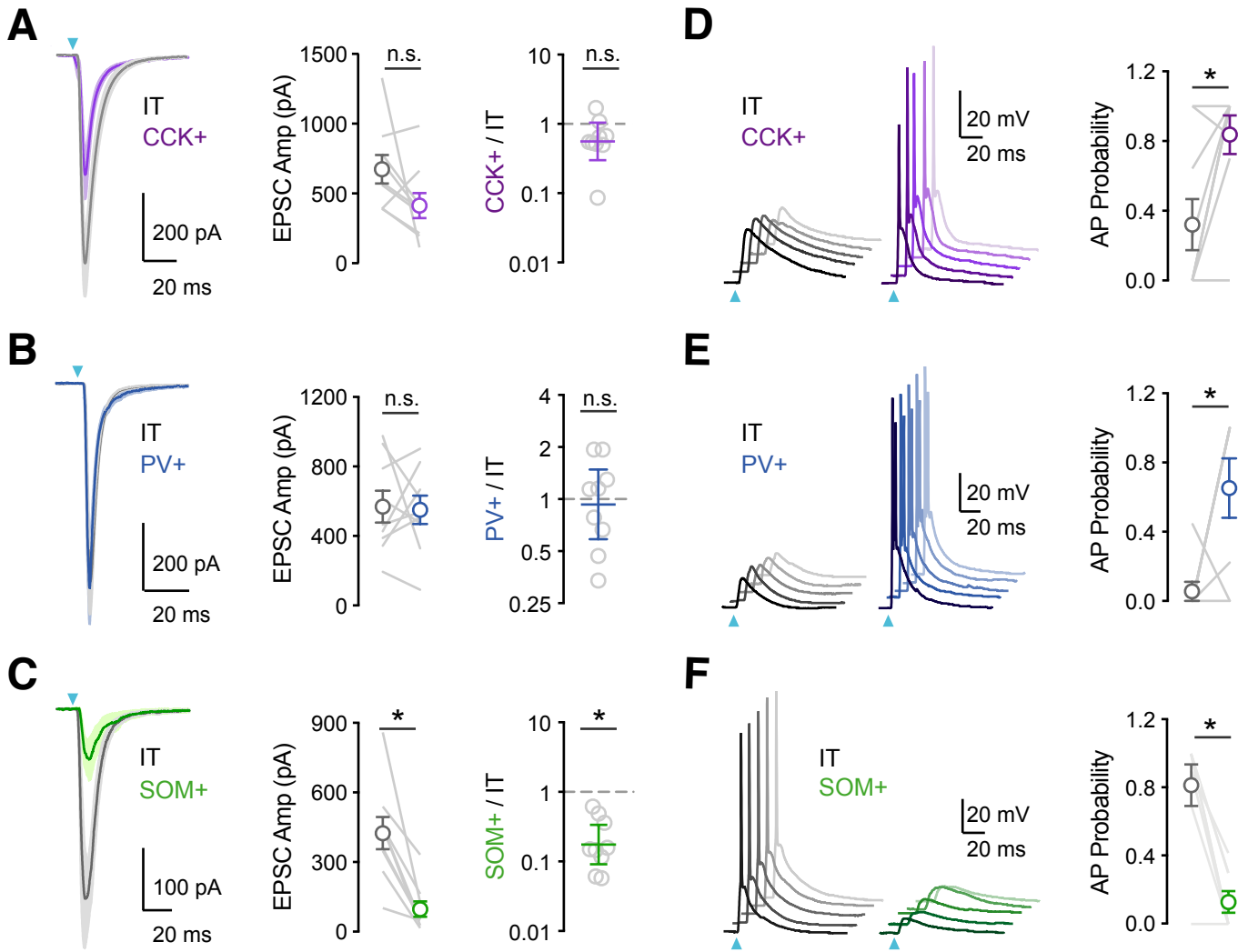
792 **Figure S2: Minimal DSI at PV+ or SOM+ interneuron connections onto IT cells.**

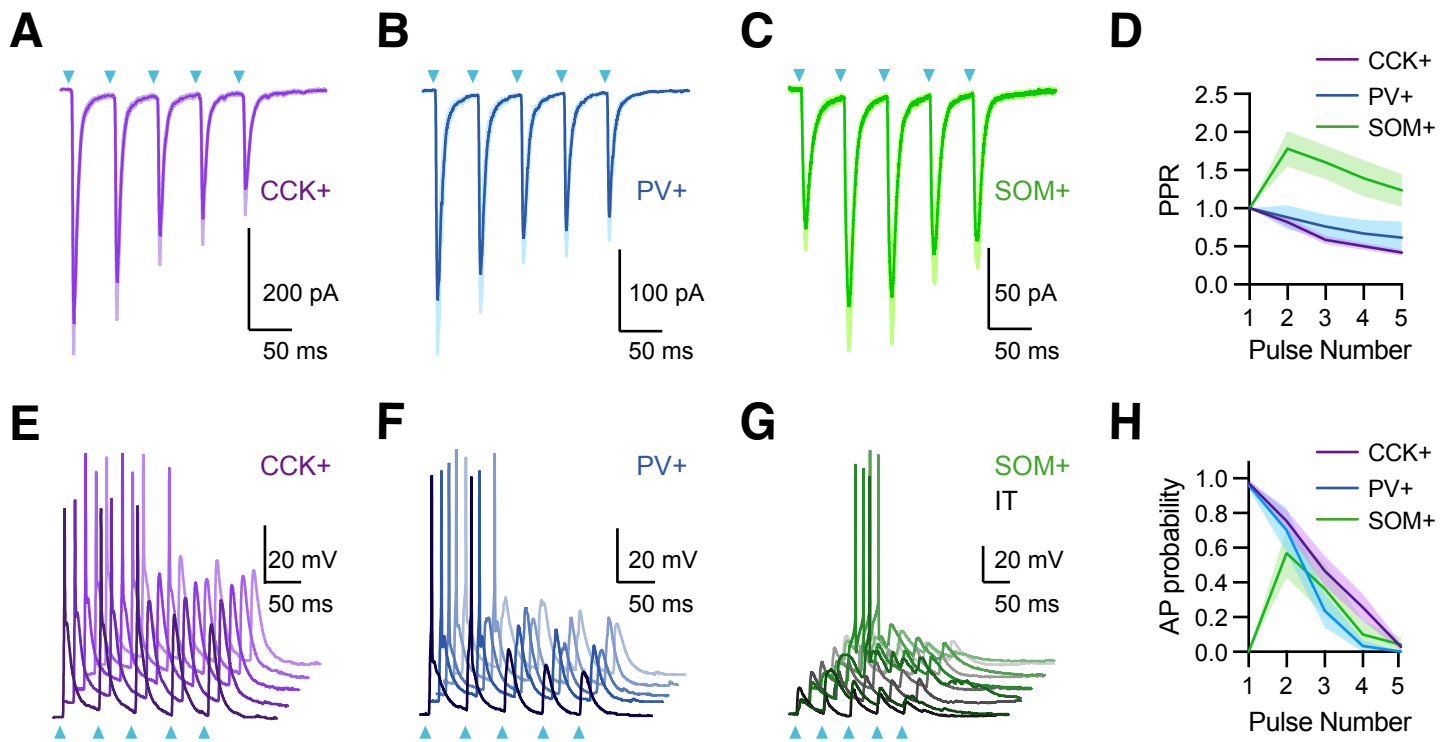
793 **A)** Injection schematics of CTB-647 into cPFC and AAV-Flex-ChR2 in PFC of PV-Cre mice.

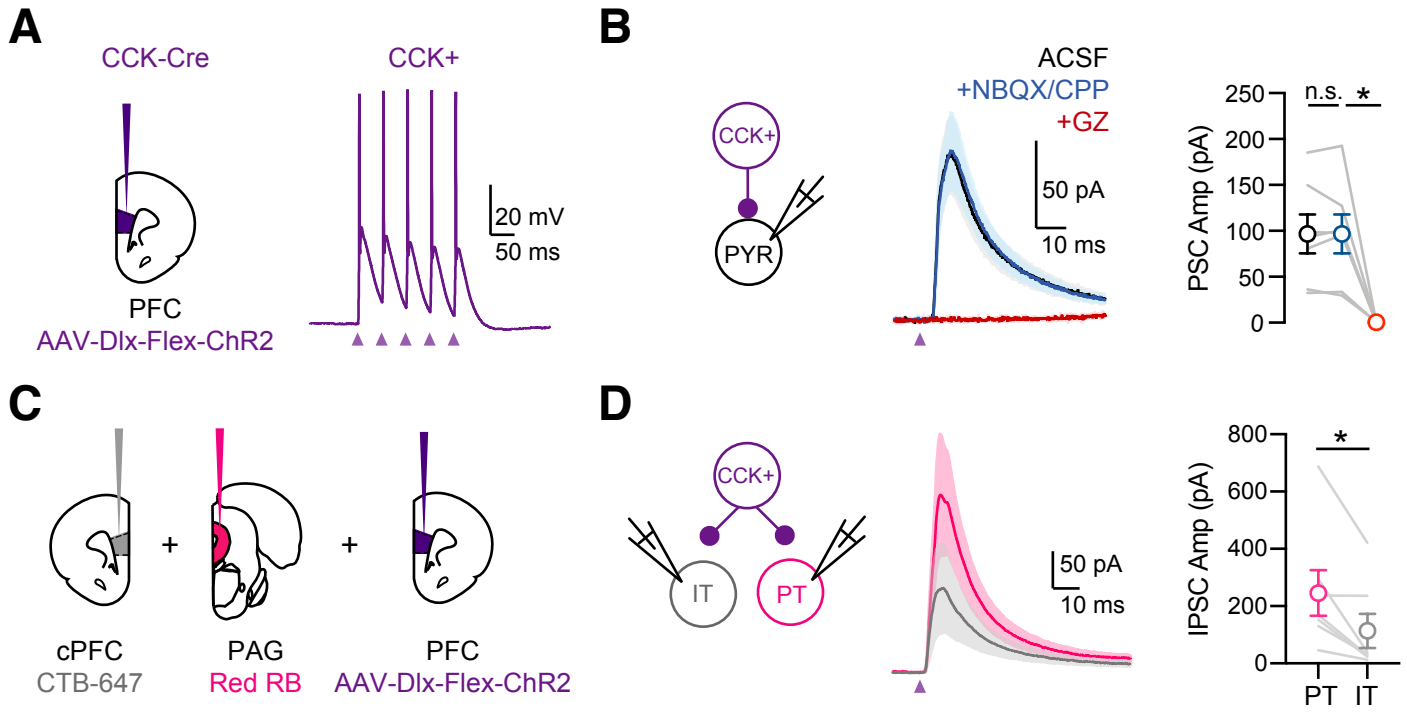
794 **B)** *Left*, Average PV+ inputs onto IT cells, evoked by 5 pulses at 20 Hz, before (black),
795 immediately after the brief depolarization (red) and after recovery (gray). Blue arrow = light
796 stimulation. *Right*, Amplitudes of the first IPSCs (n = 7 cells, 3 animals).

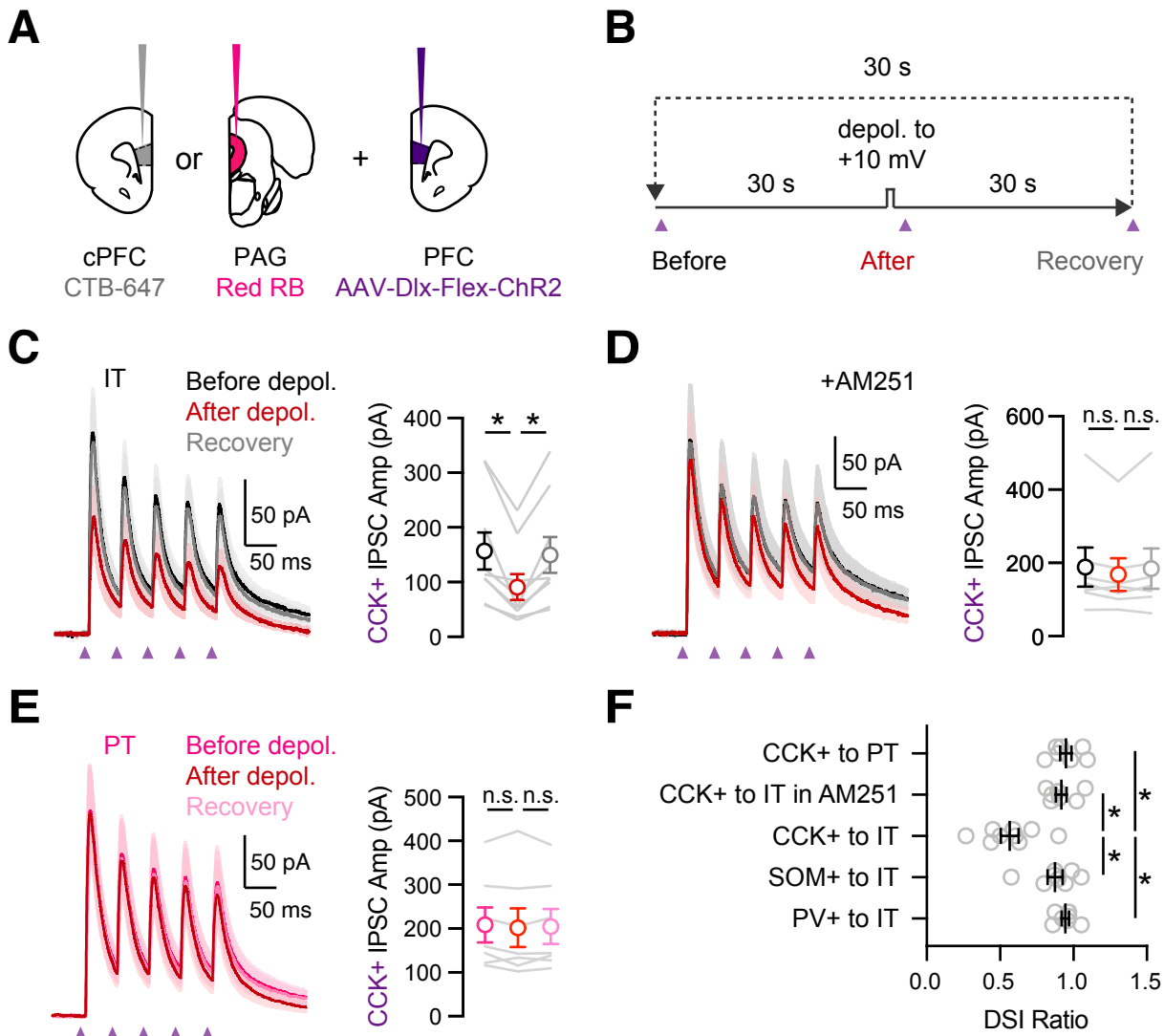
797 **C – D)** Similar to (A – B) for SOM+ inputs onto IT cells (n = 8 cells, 3 animals).

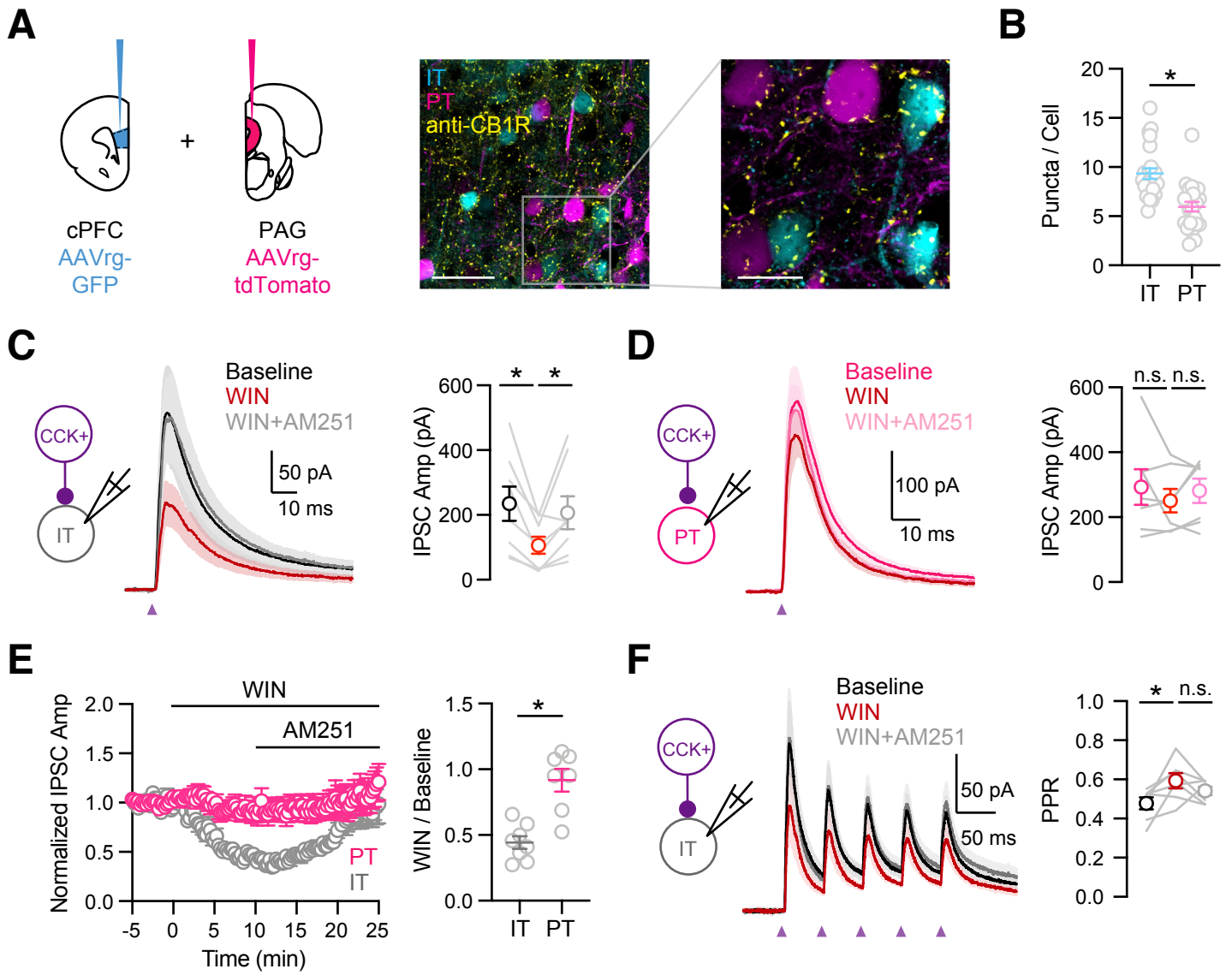


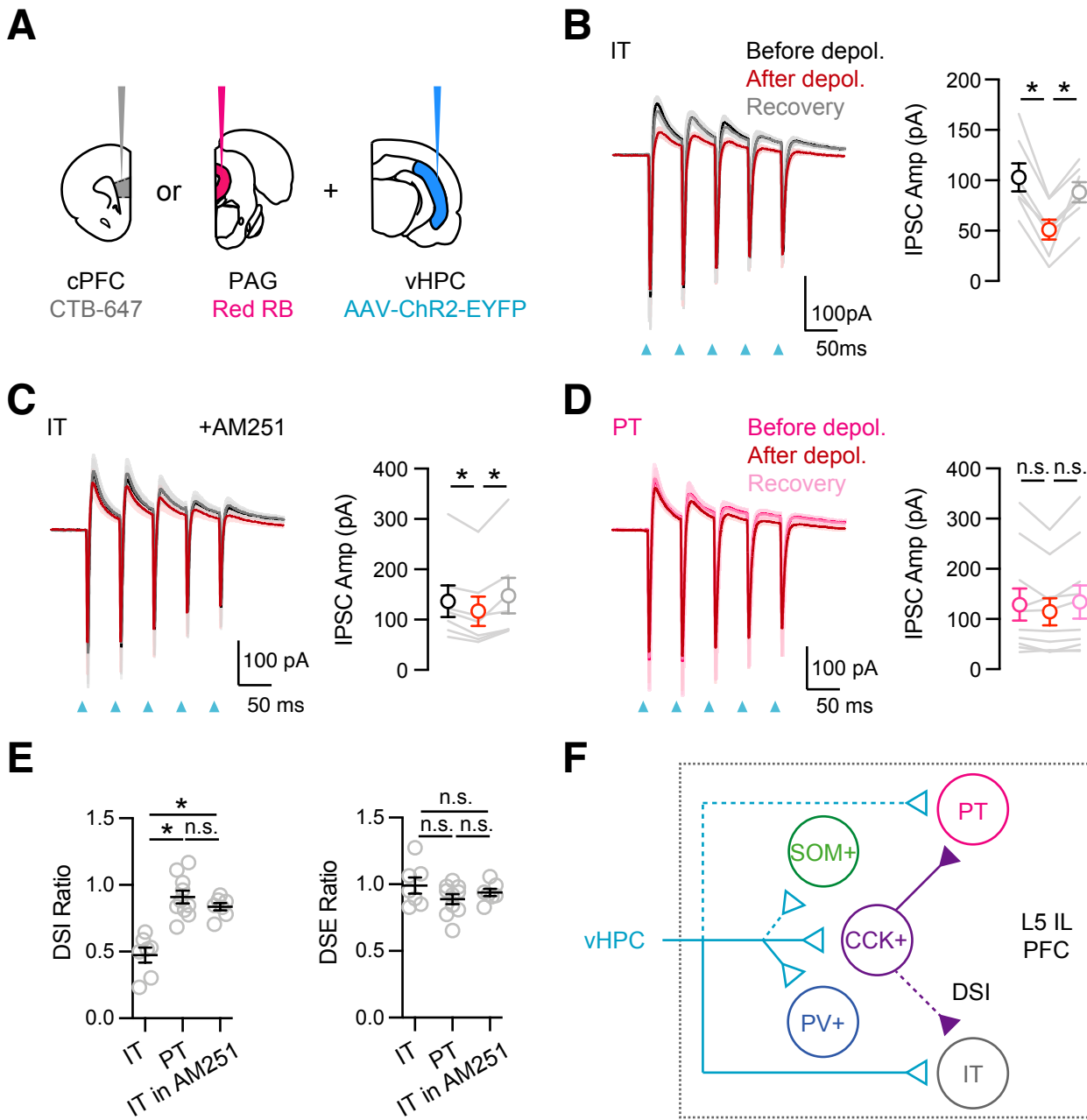






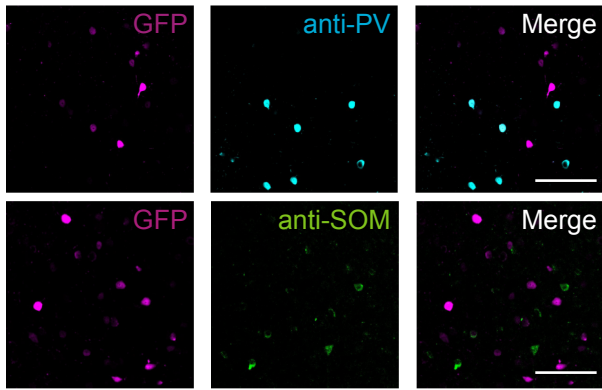






A

CCK-Cre x AAV-Dlx-Flex-GFP



B

