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1 **Top-down control of hippocampal signal-to-noise by**
2 **prefrontal long-range inhibition**

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8 **Summary**

9 The prefrontal cortex (PFC) is postulated to exert ‘top-down control’ by modulating information
10 processing throughout the brain to promote specific actions based on current goals. However, the
11 pathways mediating top-down control remain poorly understood. In particular, knowledge about
12 direct prefrontal connections that might facilitate top-down prefrontal control of information
13 processing in the hippocampus remains sparse. Here we describe novel monosynaptic long-range
14 GABAergic projections from PFC to hippocampus. These preferentially inhibit vasoactive
15 intestinal polypeptide expressing interneurons, which are known to disinhibit hippocampal
16 microcircuits. Indeed, stimulating prefrontal–hippocampal GABAergic projections increases
17 hippocampal feedforward inhibition and reduces hippocampal activity *in vivo*. The net effect of
18 these actions is to specifically enhance the signal-to-noise ratio for hippocampal representations
19 of objects. Correspondingly, stimulation of PFC-to-hippocampus GABAergic projections
20 promotes object exploration. Together, these results elucidate a novel top-down pathway in
21 which long-range GABAergic projections target disinhibitory microcircuits, thereby enhancing
22 signals and network dynamics underlying exploratory behavior.

23 **Introduction**

24 The prefrontal cortex (PFC) plays a crucial role in executive functions and the top-down control
25 of brain activity and behavior (Gazzaley and D'Esposito, 2007; Miller and Cohen, 2001; Miller,
26 2000). It is postulated that PFC bidirectionally communicates with several cortical and
27 subcortical brain regions, monitoring and gating their ongoing activity, in order to exert top-
28 down executive control over behavior. One brain region that is known to bidirectionally interact
29 with the PFC is the hippocampus (HPC), a key brain region for processing spatial information,
30 and using spatial representations to guide behavior. Accumulating evidence in humans and
31 animal models highlights an essential role of network interactions between the PFC and HPC in
32 cognitive and emotional behaviors (Eichenbaum, 2017; Jin and Maren, 2015; Preston and
33 Eichenbaum, 2013; Shin and Jadhav, 2016; Sigurdsson and Duvarci, 2016; Yu and Frank, 2015).
34 Importantly, abnormal PFC-HPC interactions are thought to contribute to cognitive and
35 emotional deficits in several neuropsychiatric disorders, including schizophrenia, depression, and
36 anxiety disorders (Cunniff et al., 2020; Godsil et al., 2013; Kupferschmidt and Gordon, 2018; Li
37 et al., 2015; Sigurdsson et al., 2010). Owing to the importance of PFC-HPC interactions in
38 normal and pathological behaviors, much work has been focused on elucidating how these
39 regions interact.

40 Functional imaging studies in humans as well as rodent studies using lesions and
41 pharmacological inactivation have shown that concurrent activity in, and communication
42 between, the PFC and HPC is essential for spatial exploratory behaviors (Bähner et al., 2015;
43 Churchwell et al., 2010; DeVito and Eichenbaum, 2010; Floresco et al., 1997; Wang and Cai,
44 2006; Yoon et al., 2008). Neural activity and network oscillations synchronize across the PFC
45 and HPC during spatial exploratory behaviors (Colgin, 2011; Jones and Wilson, 2005; O'Neill et
46 al., 2013; Spellman et al., 2015). In particular, oscillatory activity in HPC leads PFC activity
47 when rats explore spatial contexts, but this pattern of synchronization switches to PFC leading
48 when rats explore objects in their environment (Place et al., 2016) or arrive at decision points in
49 a maze (Hallock et al., 2016). Furthermore, inactivating the PFC alters the encoding of spatial
50 information in the HPC (Guise and Shapiro, 2017; Kyd and Bilkey, 2003). These findings
51 suggest that the PFC exerts top-down control over HPC activity at key behavioral timepoints, but
52 knowledge about direct anatomical projections that mediate this kind of top-down prefrontal
53 control is lacking. In fact, whereas much is known about the direct anatomical pathways from the
54 HPC-to-PFC (Hoover and Vertes, 2007; Jay and Witter, 1991), most top-down communication
55 in the PFC-to-HPC direction is thought to occur indirectly, via the thalamic nucleus reuniens
56 (NR) (Hoover and Vertes, 2012; Vertes et al., 2007; Xu and Südhof, 2013). Not only are the
57 anatomical substrates for top down control unknown, the manner in which top down control

58 operates is also unclear. I.e., does the PFC exerts top-down control by transmitting specific
59 information, e.g., representations of specific actions or goals, or alternatively, does it modulate
60 the network state, changing the nature of emergent circuit computations in downstream regions?

61 Previous studies of PFC-HPC interactions have focused on direct and indirect excitatory
62 (glutamatergic) connections between these structures. Growing evidence indicates that cortical
63 circuits also include specialized populations of long-range projecting GABAergic (LRG)
64 inhibitory neurons (Jinno et al., 2007; Melzer and Monyer, 2020; Tamamaki and Tomioka,
65 2010). In some cases, these LRG projections have been shown to control oscillatory
66 synchronization between structures (Christenson Wick et al., 2019; Francavilla et al., 2018;
67 Melzer et al., 2012), suggesting that they may be important regulators of interregional
68 communication. We recently reported that the PFC also contains specialized LRG projection
69 neurons capable of influencing behavior (Lee et al., 2014). Therefore, we hypothesized that a
70 specialized population of PFC LRG projection neurons might serve as the anatomical substrate
71 through which the PFC exerts top-down control over hippocampal information processing.

72 Here, we report a novel population of LRG neurons in the PFC that send direct inhibitory
73 projections to the dorsal hippocampus (dHPC). Notably, these prefrontal LRG projections target
74 local disinhibitory microcircuits and modulate network oscillations in dHPC. Through these
75 actions, PFC–dHPC LRG projections promote network states associated with object exploration,
76 enhance hippocampal representations of object locations, and elicit corresponding increases in
77 the time mice spend exploring objects. Together, our results show how the PFC exerts top-down
78 control over information processing in the HPC by acting through a novel circuit motif: long-
79 range GABAergic projections which inhibit disinhibitory microcircuits, thereby altering
80 emergent network dynamics and promoting specific exploratory behaviors.

81 **Results**

82 **Hippocampus projecting long-range GABAergic (LRG) neurons in the PFC**

83 To label potential PFC-to-HPC LRG projections, we used *Dlxi12b-Cre* mice, which specifically
84 express Cre recombinase in GABAergic neurons (Lee et al., 2014; Potter et al., 2009). We
85 injected an adeno-associated virus (AAV) to drive Cre-dependent expression of the fluorescent
86 reporter eYFP (AAV5-EF1 α -DIO-eYFP) in the PFC of *Dlxi12b-Cre* mice (Fig. 1A). After
87 waiting 6–8 weeks for viral transduction, we observed robust eYFP expression in the cell bodies
88 of GABAergic neurons in the PFC and also observed many axonal fibers in the CA1 and dentate
89 gyrus subfields of dHPC (Fig. 1B and Fig. S1). Importantly, no eYFP+ cell bodies were
90 observed in the HPC.

91 Next, we asked whether these PFC LRG axon terminals synapse onto neurons in the
92 dHPC. To address this, we injected AAV into the PFC of *Dlxi12b-Cre* mice to drive Cre-
93 dependent expression of Channelrhodopsin-eYFP (ChR2-eYFP) in PFC GABAergic neurons,
94 then, after waiting for expression, made recordings from acute hippocampal slices (Fig. 1C).
95 Notably, optogenetic activation of PFC LRG axonal fibers in dHPC slices elicited robust short-
96 latency postsynaptic currents (oPSCs) in dHPC neurons. These currents reversed at the GABA
97 reversal potential, were not affected by glutamatergic receptor antagonists, and were completely
98 blocked by bath application of the GABA_A receptor antagonist gabazine (10 μ M) (Fig. 1D).

99 Following the identification of PFC–dHPC LRG projections, we asked whether these
100 dHPC-projecting PFC LRG neurons have distinct electrophysiological and molecular properties,
101 and whether these neurons are located in superficial or deeper cortical layers of the PFC. To
102 address these questions, we used an intersectional strategy to selectively express ChR2-eYFP in
103 dHPC-projecting PFC LRG neurons. Specifically, we injected two viruses: a retrogradely
104 transducing canine adenovirus type-2 Cre (CAV2-Cre) into dHPC, and a Cre-dependent AAV
105 expressing ChR2-eYFP under control of the *Dlxi12b* enhancer into PFC (Lee et al., 2014) (Fig.
106 1E, F). We then made *ex-vivo* patch clamp recordings from dHPC-projecting LRG neurons in
107 PFC (identified by eYFP expression), and recorded reliable short-latency light-evoked action
108 potentials (APs) to confirm that they were ChR2-expressing (Fig. 1G). These recordings revealed
109 that the dHPC-projecting PFC LRG neuronal population is electrophysiologically diverse,
110 comprising neurons with regular spiking (9/16 neurons), irregular spiking (3/16 neurons), and
111 fast spiking (4/16 neurons) physiological properties (Fig. 1H and Table S1). dHPC-projecting
112 PFC LRG neurons were distributed across superficial and deeper layers of the prelimbic (PL)
113 portion of the PFC. By combining injection of a retrograde tracer (Alexa 594-tagged cholera
114 toxin, CTb) in dHPC with immunohistochemistry in PFC (Fig. S2A, B), we found that dHPC-
115 projecting PFC LRG neurons include parvalbumin (PV), somatostatin (SST), and vasoactive

116 intestinal polypeptide (VIP)-expressing subpopulations (Fig. S2C, D). We also observed small
117 percentages of calretinin (CR) and neuropeptide-Y (NPY) expressing PFC–dHPC LRG neurons.
118 However, none of the PFC LRG neurons in our study showed immunoreactivity for neuronal
119 nitric oxide synthase (nNOS). Taken together, these results reveal that the dHPC receives direct
120 LRG projections which originate from a heterogeneous population of GABAergic inhibitory
121 neurons located across multiple layers of the PFC.

122

123 **PFC LRG projections target hippocampal disinhibitory interneurons**

124 Next, we asked how electrophysiologically and molecularly heterogeneous PFC LRG projection
125 neurons affect circuit computations in the CA1 subregion, which is the primary output region of
126 the dHPC. Specifically, we asked whether PFC LRG projections target specific cell-types in the
127 CA1 subregion. We expressed Chr2-eYFP in PFC LRG projections and obtained *ex-vivo* patch
128 clamp recordings from excitatory pyramidal neurons and GABAergic interneurons located in
129 different topographical layers of CA1 subregion in acute hippocampal slices (Fig. 2A).
130 Interestingly, we observed robust optogenetically-evoked IPSCs in CA1 interneurons (55/70
131 connected, henceforth referred to as recipient interneurons), but not in CA1 pyramidal neurons
132 (PNs; 0/38 connected) (details of interneuron and PN classification in Methods). Notably, many
133 of the recipient CA1 interneurons were located near the border between stratum radiatum (SR)
134 and stratum lacunosum-moleculare (SLM) (Fig. 2B). Furthermore, recipient CA1 interneurons
135 comprised physiologically heterogeneous subtypes including regular spiking, irregular spiking,
136 and fast spiking interneurons (Fig. 2B and Table S2). In order to determine whether the PFC
137 LRG projections target molecularly defined interneuron subtypes in CA1, we filled a subset of
138 the recipient interneurons with biocytin and quantified the immunoreactivity for three molecular
139 markers commonly expressed in CA1 interneurons– PV, SST, and VIP. Surprisingly, we found
140 that a majority of recipient interneurons expressed VIP (7/11). By contrast, none of the recipient
141 interneurons we examined expressed PV or SST (0/10) (Fig. 2C).

142

143 **PFC LRG projections regulate excitatory input integration in CA1 microcircuit**

144 Since VIP is predominantly expressed by interneuron-selective interneurons (ISIs) which
145 produce circuit disinhibition in CA1 (Acsády et al., 1996a; 1996b; Chamberland and Topolnik,
146 2012; Turi et al., 2019), we hypothesized that PFC–dHPC LRG projections may inhibit VIP+
147 ISIs, thereby reducing disinhibition and increasing feedforward inhibition in the CA1
148 microcircuit. To test this prediction, we quantified the effect of optogenetic stimulation of PFC
149 LRG projections on excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs) elicited
150 by two major afferent input pathways: Schaffer collateral (SC) and temporoammonic (TA) inputs
151 (Fig. 2D). Specifically, during *ex-vivo* patch clamp recordings from CA1 PNs, we delivered

152 electrical stimulation to SC or TA inputs concomitant with optogenetic stimulation of Chr2+
153 PFC–dHPC LRG axon fibers. While the optogenetic stimulation of PFC–dHPC LRG axons
154 alone did not elicit discernable postsynaptic potentials in CA1 PNs, concomitant electrical and
155 optogenetic stimulation significantly increased the size of IPSPs relative to EPSPs for both SC
156 and TA inputs (Fig. 2E, F and Fig. S3A, B). This reduction in the excitation to inhibition ratio
157 (E/I ratio) for SC and TA inputs is consistent with our prediction and suggests that the PFC LRG
158 projections increase feedforward inhibition by inhibiting disinhibitory VIP+ interneurons in
159 CA1.

160 We then asked whether increased feedforward inhibition during stimulation of PFC LRG
161 projections affects the input-output transformation performed by CA1 PNs. Coincident activation
162 of SC and TA input pathways, often in a theta-burst stimulation (TBS) pattern, is known to cause
163 supralinear input summation and spiking in CA1 PNs (Ang et al., 2005; Bittner et al., 2015;
164 Malik and Johnston, 2017). This nonlinear input integration and coincidence detection in CA1
165 PNs is tightly regulated by the activity of CA1 interneurons and considered crucial for
166 hippocampal information processing (Grienberger et al., 2017; Milstein et al., 2015). To
167 determine how PFC–dHPC LRG projections modulate input integration in CA1 PNs, we
168 combined electrical TBS of SC and TA inputs with optogenetic stimulation of PFC–dHPC LRG
169 projections (20 Hz, 5 ms pulses) (Fig. 2G). Again, consistent with increased feedforward
170 inhibition, optogenetic stimulation of LRG projections reduced firing and EPSP summation
171 during TBS (Fig. 2H). Importantly, firing of CA1 PNs in response to depolarizing current
172 injections (i.e., neuronal depolarization without recruitment of microcircuit inhibition) was not
173 affected by optogenetic stimulation of these PFC LRG projections (Fig. S3C). Taken together,
174 our *ex-vivo* electrophysiological analyses show how PFC–dHPC LRG projections regulate
175 synaptic integration and input-output gain by enhancing feedforward inhibition onto CA1 PNs
176 (Fig. S3D).

177

178 **PFC–dHPC LRG projections promote object exploration**

179 Communication between PFC and HPC is implicated in many spatial and object exploration
180 behaviors (DeVito and Eichenbaum, 2010; Jin and Maren, 2015; Preston and Eichenbaum, 2013;
181 Spellman et al., 2015; Yu and Frank, 2015). Notably, both structures synchronize at theta
182 frequency with dHPC leading when rodents enter a spatial context, but the directionality
183 switches to PFC leading when animals sample an object (Place et al., 2016). This suggests an
184 important role for top-down communication from PFC to dHPC during object exploration.
185 Therefore, we quantified how PFC–dHPC LRG projections affect object exploration in freely
186 behaving mice. Optogenetic stimulation of PFC–dHPC LRG projections (20 Hz, 5 ms pulses,
187 473 nm, ~3-4 mW) dramatically increased the time *Dlx1/2-Cre* mice spent engaged in novel

188 object exploration (NOE) (Fig. 3A, B). Light delivery alone had no effect in control (Cre-
189 negative) mice. Increases in NOE occurred during both early and late portions of the testing
190 session (Fig. 3C, D), and reflected increased numbers of both short- and long-duration bouts of
191 object exploration (Fig. 3E). Optogenetic stimulation of PFC–dHPC LRG projections did not
192 affect the distance travelled in an open field, time spent on the stimulated side during a real-time
193 place preference task, or the time spent exploring a novel juvenile mouse (Fig. S4A–C). Thus,
194 activating PFC–dHPC LRG projections specifically increases NOE without nonspecifically
195 affecting movement or other exploratory behaviors.

196

197 **PFC LRG projections promote network oscillations associated with object exploration**

198 Next, we explored potential circuit mechanisms through which PFC–dHPC LRG projections
199 might impact NOE. We did this in two ways. First, we recorded local field potentials (LFPs) to
200 determine whether stimulation of PFC–dHPC LRG projections might induce network states
201 conducive to NOE (Fig. 4A). In comparison to baseline home cage (HC) exploration, NOE
202 recruited synchronized oscillations in the low-gamma (25–55 Hz) band across the PFC–dHPC
203 network. Specifically, during NOE we observed a significant increase in low-gamma power in
204 both structures as well as an increase in low-gamma phase synchrony between the PFC and
205 dHPC (Fig. 4B). While the increase in low-gamma activity was most prominent, NOE was also
206 associated with significant increases in power (but not synchrony) for high-gamma activity (both
207 structures) and theta activity (dHPC only) (Fig. S5A, B). The NOE related change in low-gamma
208 frequency oscillations is particularly notable because previous studies have shown that object
209 exploration increases low-gamma synchrony between hippocampal subfields (Trimper et al.,
210 2017). Since microcircuit interactions between local CA1 interneurons and PNs are known to
211 critically regulate gamma oscillations (Csicsvari et al., 2003; Tukker et al., 2007), we
212 hypothesized that by modulating microcircuit inhibition, PFC–dHPC LRG projections could
213 contribute to NOE-associated changes in gamma activity. To test whether PFC–dHPC LRG
214 projections might support these changes in network activity, we combined optogenetic
215 stimulation with multisite LFP recordings in *Dlx1/2b-Cre* mice expressing ChR2 in PFC–dHPC
216 LRG projections (Fig. 4C). Indeed, optogenetic stimulation of PFC LRG terminals (20 Hz, 5 ms
217 pulses, 473 nm, ~3–4 mW) in dHPC mimicked the increases in both low-gamma LFP power and
218 low-gamma phase synchrony observed during NOE (Fig. 4D and Fig. S5C, D). Thus, PFC LRG
219 projections promote a network state associated with object exploration, a behavior known to rely
220 on top-down PFC–dHPC communication.

221

222 **PFC–dHPC LRG projections reduce hippocampal activity *in vivo***

223 Having established that PFC–dHPC LRG projections promote network states associated with
224 NOE as well as NOE itself, we next studied how these projections affect NOE-associated
225 hippocampal activity at the level of single cells. For this, we expressed jGCaMP7f in dHPC CA1
226 neurons (Dana et al., 2019) and used one-photon miniaturized microscopes (miniscopes) to
227 record *in vivo* neuronal Ca^{2+} activity while mice explored novel objects. Concurrently, we
228 expressed the red-shifted excitatory opsin ChrimsonR (Klapoetke et al., 2014; Stamatakis et al.,
229 2018) in PFC–dHPC LRG projections (Fig. 5A–C and Fig. S6A, B). On day 1, mice explored a
230 novel object in the absence of optogenetic stimulation of PFC LRG projections. Across all
231 neurons, Ca^{2+} activity decreased significantly during NOE, relative to the HC epoch (Fig. 5D),
232 although a small fraction of neurons (13/55 neurons) had higher activity during NOE (Fig. S6C).
233 On day 2, we optogenetically stimulated PFC–dHPC LRG projections during both HC and NOE
234 epochs. Stimulating LRG projections during the HC epoch significantly reduced activity
235 (compared to the pre-stimulation HC period). Activity was then further reduced when the mice
236 subsequently engaged in NOE (Fig. 5D and Fig. S6C). This overall reduction in population
237 activity *in vivo* is consistent with our *ex-vivo* observation that activating PFC–dHPC LRG
238 projections tends to enhance feedforward inhibition and reduce spiking in CA1 PNs.

239

240 **PFC–dHPC LRG projections enhance the encoding of objects by hippocampal ensembles**

241 To assess whether these global changes in CA1 activity were associated with changes in how the
242 hippocampus encodes NOE-relevant information, we compared the NOE-driven changes in
243 neuronal activity on day 1 (no stimulation) vs. day 2 (LRG stimulation). As shown by the
244 seminal discovery of place cells, dHPC CA1 neurons encode information by preferentially firing
245 in specific spatial locations (Moser et al., 2008; O'Keefe, 1976; Wilson and McNaughton, 1993).
246 Therefore, we asked whether the PFC–dHPC LRG projections affect the encoding of object
247 location by individual hippocampal neurons. Specifically, for each neuron, we defined its ‘object
248 signal-to-noise ratio’ (Object_SNR) as the change in its activity within a zone surrounding the
249 object location before vs. after introducing the object (activity was z-scored relative to the mean
250 and standard deviation outside the object zone) (Fig. 6A). Based on this metric, neurons that
251 increased or decreased activity in the object zone by one standard deviation had Object_SNR of
252 1 or -1, respectively. During light stimulation, the activity of neurons decreased both within and
253 outside of the object zone; the standard deviations of neuronal activity also decreased (Fig. 6B).
254 Depending on exactly how these changes were distributed across neurons, Object_SNR values
255 could potentially increase, decrease, or remain unchanged. In fact, we observed that stimulating
256 PFC LRG projections significantly increased Object_SNR values relative to the no light
257 condition (Fig. 6B, right-most panel). Notably, light delivery alone did not affect the neuronal
258 activity or the Object_SNR in control (opsin-negative) mice (Fig. 6C). Furthermore, in opsin-

259 expressing mice, LRG stimulation did not affect an analogous ‘SNR’ calculated for a control
260 zone on the opposite side of the cage (instead of the object zone) (Fig. 6D, E). Thus, even though
261 PFC–dHPC LRG projections potentiate feedforward inhibition and reduce overall network
262 activity, their net effect on hippocampal encoding is to specifically enhance object-driven signals
263 in individual CA1 neurons.

264 **Discussion**

265 Interactions between the PFC and HPC have been implicated in numerous aspects of cognition
266 and emotion, including decisions about whether to engage in exploratory behaviors. While
267 monosynaptic excitatory projections from the ventral HPC are believed to transmit specific
268 information, e.g., the locations of goals, to the PFC (Spellman et al., 2015; Wang and Cai, 2006),
269 pathways through which the PFC exerts top-down control over the HPC, and the exact nature of
270 these top-down effects, have remained less well understood. Here, we describe a novel
271 monosynaptic projection from the PFC-to-dHPC. There are many unusual features of this
272 projection: it is GABAergic and targets hippocampal VIP+ ISIs, thus representing a ‘doubly
273 disinhibitory’ long-range motif. We show that this projection modulates microcircuit dynamics
274 in the CA1 region of dHPC, increasing feedforward inhibition, reducing spiking evoked by
275 afferent inputs, and enhancing low-gamma activity that is synchronized between the PFC and
276 dHPC. Furthermore, we show that activation of these projections reduces *in vivo* activity while
277 specifically enhancing the representations of objects in the dorsal CA1. Lastly, in accord with the
278 postulated role of PFC as a top-down controller, we found that the activation of these LRG
279 projections drives object exploration behavior in mice. Overall, our study shows that these top-
280 down prefrontal projections can dynamically control the network state and emergent circuit
281 function in the dHPC, thereby altering the signal-to-noise ratio for specific neural representations
282 and eliciting corresponding changes in behavior. This answers long-standing questions about the
283 mechanisms and nature of top-down control in the limbic system.

284

285 **Relationship to previous work**

286 Multiple lines of evidence in humans, non-human primates, and rodents have suggested that PFC
287 can exert top-down control over information processing in the HPC, particularly during
288 behaviors involving spatial and object exploration (Brincat and Miller, 2015; Eichenbaum, 2017;
289 Jin and Maren, 2015; Place et al., 2016; Preston and Eichenbaum, 2013; Shin and Jadhav, 2016;
290 Sigurdsson and Duvarci, 2016; Yu and Frank, 2015). Importantly, previous studies have shown
291 that lesion and pharmacological inactivation of PFC severely impairs spatial navigation and
292 object exploratory behaviors, and also disrupts neuronal encoding in the HPC (Churchwell et al.,
293 2010; DeVito and Eichenbaum, 2010; Floresco et al., 1997; Guise and Shapiro, 2017; Kyd and
294 Bilkey, 2003; Wang and Cai, 2006; Yoon et al., 2008). Nevertheless basic aspects of this process
295 have remained elusive. Specifically, the pathways mediating prefrontal top-down control have
296 not been identified, and it was not known whether the PFC acts by transmitting specific
297 information to the HPC vs. by modulating the network state and emergent circuit function.
298 Addressing these questions is crucially important, because interactions between the HPC and

299 PFC have been implicated in so many behaviors and disorders, and because the role of the PFC
300 in top-down control is largely taken for granted despite the paucity of knowledge about specific
301 mechanisms. Our study addresses this major gap by revealing a novel anatomical substrate
302 mediating prefrontal top-down control, and showing exactly how it regulates behavior via
303 actions on hippocampal neurons, microcircuits, network dynamics, and information processing.

304 Our anterograde tracing experiments showed that the PFC LRG projections are
305 concentrated in the dorsal HPC, relative to the intermediate and ventral parts portions of HPC.
306 By contrast, the NR, which is known to mediate indirect PFC–HPC communication,
307 preferentially innervates the intermediate and ventral HPC (Hoover and Vertes, 2012). This
308 suggests that conjunctive information transfer via the direct PFC LRG pathway and the indirect
309 PFC→NR→HPC pathway would allow PFC to orchestrate activity along the entire extent of the
310 hippocampal dorsoventral axis. Interestingly, the hippocampal dorsoventral axis is functionally
311 segregated with the dorsal HPC being crucially involved in spatial processing and the ventral
312 HPC regulating emotions, fear, and anxiety (Fanselow and Dong, 2010). Therefore, an alternate
313 possibility is that the direct PFC→dHPC LRG projections and indirect PFC→NR→ventral HPC
314 projections may mediate fundamentally distinct aspects of top-down control over cognitive vs.
315 emotional behaviors, respectively. Similar to the functional segregation along the hippocampal
316 dorsoventral axis, the PFC can also be subdivided dorsoventrally, into functionally specialized
317 subregions, e.g., anterior cingulate (ACC), prelimbic (PL), and infralimbic (IL) cortices. We
318 found PFC–dHPC LRG projections originating from PL. Notably, the ACC also sends direct
319 projections to the dHPC (Rajasethupathy et al., 2015). However, by targeting excitatory neurons
320 in the CA3 subregion of the HPC, these previously described ACC–CA3 excitatory projections
321 primarily regulate the retrieval of fear memories. While the role of ACC–CA3 projections in
322 spatial exploratory behaviors has not been investigated, it is plausible that the direct projections
323 originating from ACC and PL regions transmit parallel streams of information from the PFC to
324 alter hippocampal activity during distinct behaviors. Future work will be necessary to elucidate
325 how glutamatergic vs. GABAergic top-down projections originating from different regions of the
326 PFC, and targeting different portions of the HPC potentially interact and/or complement each
327 other.

328 Our study shows that PFC–dHPC LRG projections target inhibitory interneurons, but not
329 excitatory pyramidal neurons, within the CA1 subregion. This preferential targeting of inhibitory
330 interneurons is similar to what has been observed in previous studies of cortical LRG inputs to
331 the HPC. Specifically, the entorhinal cortex, which is the primary interface between the HPC and
332 the neocortex, sends LRG projections which target local interneurons in the HPC (Basu et al.,
333 2016; Melzer et al., 2012). However, in contrast to effect we observed, whereby PFC–dHPC
334 LRG projections increase feedforward inhibition by inhibiting VIP interneurons, entorhinal LRG

335 projections primarily act to reduce hippocampal feedforward inhibition. This raises the
336 possibility that feedforward inhibition may be a convergent pathway on which many LRG inputs
337 act to regulate hippocampal information processing. Feedforward inhibition represents an
338 attractive target, as it crucially regulates input-output gain, neuronal plasticity, and information
339 encoding in hippocampal pyramidal neurons (Grienberger et al., 2017; McKenzie, 2018).

340 VIP interneurons in the HPC are a specialized class which disinhibit other GABAergic
341 interneurons, thereby tending to promote increases in microcircuit activity. Accordingly,
342 disinhibition mediated by hippocampal VIP interneurons has been implicated in gain control,
343 memory, selective attention, and goal-directed behaviors (Cunha-Reis and Caulino-Rocha, 2020;
344 Turi et al., 2019). While we found that the CA1 interneurons which receive PFC–dHPC LRG
345 inputs are electrophysiologically heterogeneous but tend to express VIP, understanding whether
346 their axons target specific inhibitory loci within the CA1 microcircuit will help us to further
347 understand the detailed nature of their actions. This is important because VIP+ interneurons in
348 the CA1 subregion constitute electrophysiologically and morphologically diverse subtypes
349 (Acsády et al., 1996a; 1996b; Chamberland and Topolnik, 2012). Prior work has also shown that
350 specialized subpopulations of hippocampal VIP+ GABAergic neurons send long-range
351 projections which innervate different parts of the hippocampal formation, and are recruited
352 during specific oscillatory states (Francavilla et al., 2018). It is possible that PFC–dHPC LRG
353 projections target these long-range projecting VIP neurons, contributing to their state-dependent
354 patterns of activity, and helping to produce some of the changes in network dynamics we
355 observed here. Alternatively, the changes in hippocampal low-gamma oscillations we observed
356 could be result from increases in feedforward inhibition.

357

358 **Relationship to disease**

359 Many neuropsychiatric disorders – including autism, schizophrenia, depression, and anxiety
360 disorders – are proposed to involve deficits in prefrontal functions and top-down control (Gilbert
361 et al., 2008; Hare and Duman, 2020; Orellana and Slachevsky, 2013), and in particular, altered
362 connectivity and communication between PFC and HPC (Cunniff et al., 2020; Godsil et al.,
363 2013; Kupferschmidt and Gordon, 2018; Li et al., 2015; Sigurdsson et al., 2010). Abnormalities
364 in GABAergic neuron structure and function have also been heavily implicated in the
365 pathophysiology of neuropsychiatric disorders (Chattopadhyaya and Cristo, 2012; Marin, 2012;
366 Paterno et al., 2020). PFC–dHPC LRG projections obviously represent a point of convergence
367 for these different mechanisms. Thus, abnormalities in PFC–dHPC LRG projections could
368 plausibly contribute to the disruptions in network oscillations, top-down control, and PFC–dHPC
369 communication that occur in a variety of disease states.

370

371 **Conclusion**

372 In summary, our study describes a novel anatomical pathway which plays a key role in direct
373 PFC-to-HPC communication. The unique features of these projections (i.e., long-range
374 GABAergic, disinhibitory interneuron targeting) enable PFC to dynamically alter emergent
375 network activity and information processing in the HPC, and thereby exert top-down control
376 over exploratory behavior.

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382 Behavior Research Foundation (Leichtung Family investigator, R.M.). Confocal images were
383 acquired at the Nikon Imaging Center at the University of California San Francisco.

384

385 **Author contributions**

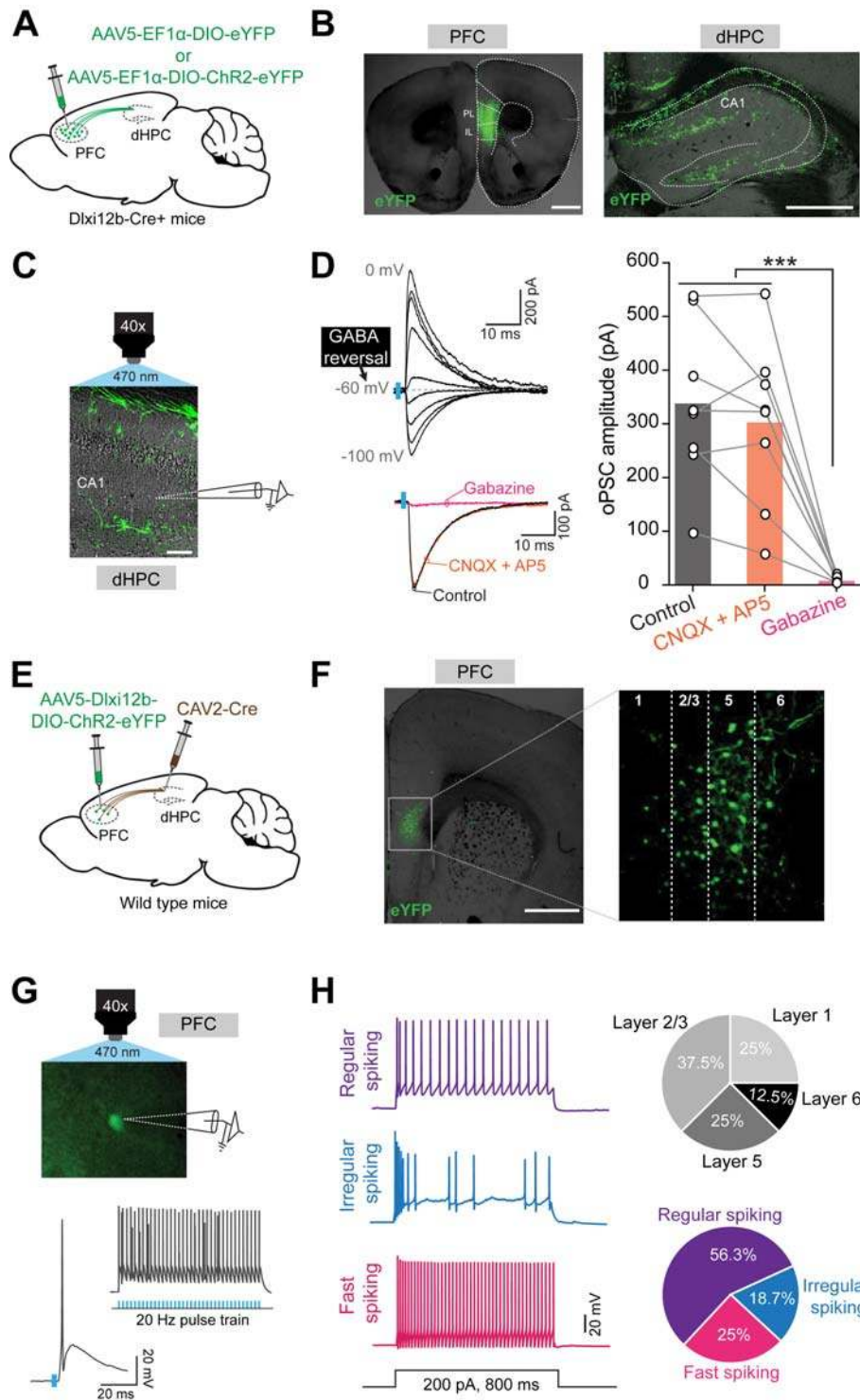
386 R.M. and V.S.S. designed the experiments and analyses. R.M. performed all experiments and
387 analyzed the data, except that R.M. and Y.L. performed immunohistochemistry. S.S. generated
388 pilot histology data for anterograde tracing experiments. R.M. and V.S.S. wrote the manuscript.

389

390 **Competing interests**

391 The authors declare no competing interests.

MAIN FIGURES AND LEGENDS



392 **Figure 1: A heterogeneous population of PFC inhibitory neurons sends direct LRG**
 393 **projections to the dHPC.**

394 (A) Schematic illustrating the anterograde tracing strategy. Cre-dependent eYFP or ChR2-eYFP
395 virus was injected into the PFC of *Dlx12b-Cre*⁺ mice.

396 (B) Representative images showing eYFP⁺ PFC GABAergic neurons (left) and eYFP⁺ axonal
397 fibers in the dHPC (right). Scale bars, 1 mm and 0.5 mm, respectively. Prelimbic cortex (PL),
398 infralimbic cortex (IL), and hippocampal CA1 regions are labeled.

399 (C) Overlaid fluorescent and DIC images of a hippocampal section showing ChR2-eYFP⁺
400 axonal fibers (green) in dorsal CA1. During *ex-vivo* patch clamp recordings from hippocampal
401 neurons, ChR2⁺ LRG axons were optogenetically activated by pulses of blue light (5 ms, 470
402 nm) delivered through the 40x objective. Scale bar, 100 μ m.

403 (D) Top left: example traces showing that optogenetically evoked postsynaptic currents (oPSCs)
404 in recipient CA1 neurons reverse at the GABA reversal potential (gray dashed line). Blue bars
405 denote light pulses. Bottom left: example oPSCs recorded from a CA1 neuron in control aCSF
406 (black), after adding CNQX + AP5 (orange), and after adding Gabazine (magenta). Right: oPSC
407 amplitudes were significantly reduced by Gabazine. Open circles represent data from individual
408 neurons (n = 8) and bars represent averages; one-way ANOVA followed by Tukey's multiple
409 comparison test, *** p < 0.001.

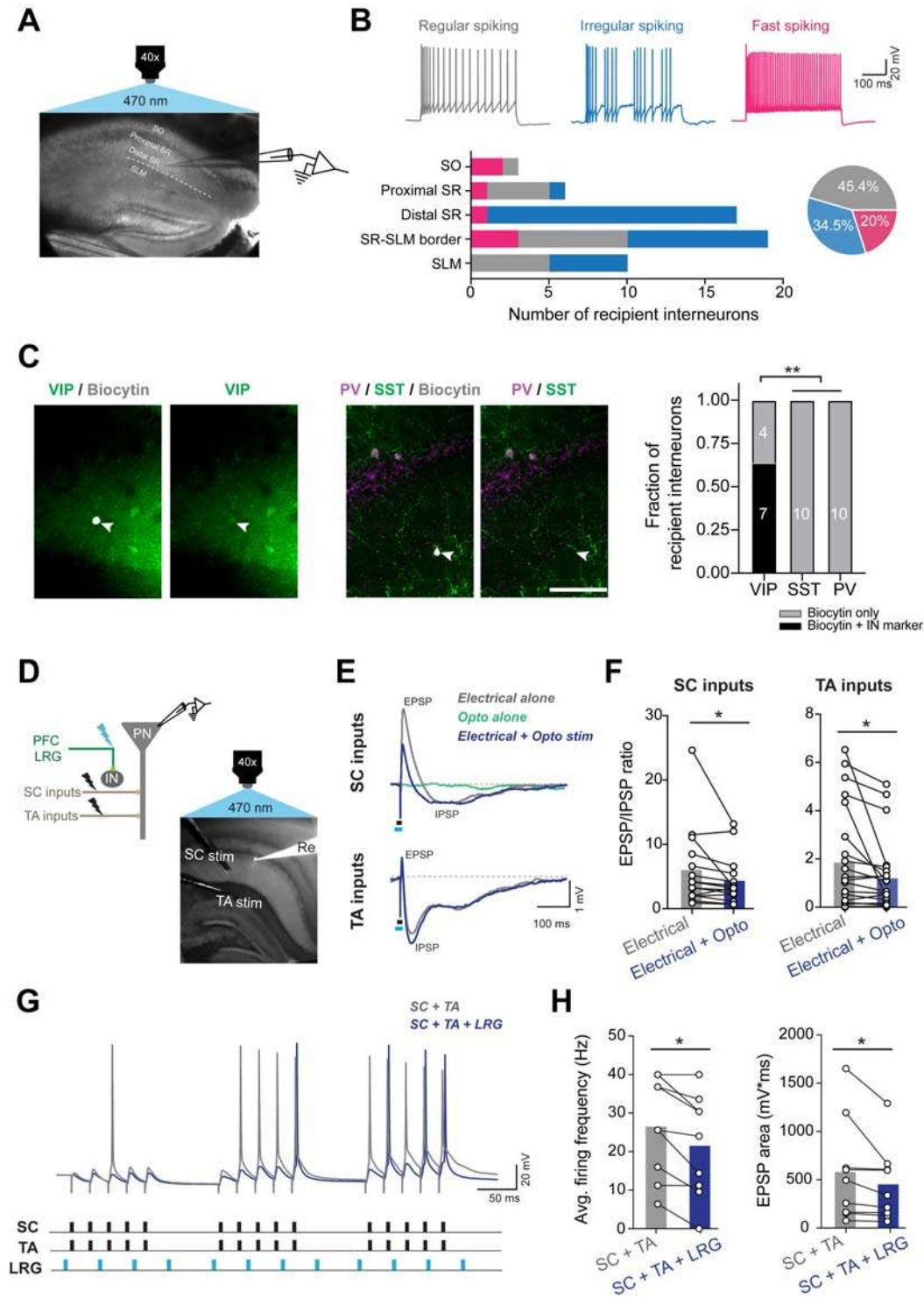
410 (E) Schematic demonstrating the intersectional strategy to target dHPC-projecting PFC LRG
411 neurons. Retrogradely transducing canine adenovirus type-2 Cre (CAV2-Cre) was injected into
412 dHPC, and a Cre-dependent AAV that drives expression of ChR2-eYFP using a GABAergic
413 neuron-specific enhancer (*Dlx12b*) was injected into PFC.

414 (F) Representative images showing ChR2-eYFP expression in dHPC projecting GABAergic
415 neurons in PFC. White dotted box in the left image corresponds to the magnified image shown in
416 right. Numbers indicate the cortical layers. Scale bar, 1 mm.

417 (G) Top: representative image showing *ex-vivo* patch clamp recording obtained from ChR2-
418 eYFP expressing PFC–dHPC LRG neuron. Bottom: example traces showing PFC–dHPC LRG
419 neuron firing elicited in response to a single light pulse (5 ms, 470 nm) or a 20 Hz train.

420 (H) Left: example voltage responses of PFC–dHPC LRG neurons to depolarizing current
421 injections. Top right: pie chart showing the laminar distribution of recorded PFC–dHPC LRG
422 neurons. Bottom right: pie chart showing the percentage of PFC–dHPC LRG neurons with
423 various physiological properties.

424 See also **Figures S1, S2** and **Table S1**.



425 **Figure 2: PFC LRG projections preferentially target interneuron-selective interneurons**
 426 **(ISIs) and increase feedforward inhibition in the CA1 microcircuit.**

427 (A) Example DIC image of a hippocampal slice showing *ex-vivo* patch clamp recording from a
 428 CA1 neuron during optogenetic stimulation of PFC LRG projections. Different layers of CA1 are

429 labeled; stratum oriens (SO), stratum radiatum (SR), and stratum lacunosum-pyramidale (SLM);
430 dashed white line represents the border between SR and SLM.

431 **(B)** Top: example voltage responses to depolarizing current injections for CA1 neurons which
432 receive input from PFC LRG projections. Regular spiking (gray), irregular spiking (blue), and
433 fast spiking properties (magenta) are observed among recipient CA1 neurons. Bottom left:
434 number of recipient interneurons with fast spiking, regular spiking and irregular spiking
435 physiology at different laminar locations in CA1 is plotted. Bottom right: pie chart showing the
436 percentage of recipient CA1 neurons with regular spiking (25/55), irregular spiking (19/55), and
437 fast spiking (11/55) properties.

438 **(C)** Left: Representative images showing staining for inhibitory neuron (IN) markers (VIP, PV
439 or SST) in recipient CA1 neurons filled with biocytin. Scale bar, 100 μm . Right: fraction of
440 recipient neurons which stained positive for VIP or (in separate sections) for PV or SST. More
441 recipient neurons stained positive for VIP vs. PV or SST (Chi-square test, $** p < 0.01$).

442 **(D)** Top left: Schematic showing the experimental configuration. Right: example hippocampal
443 image showing Alexa-594 filled recording electrode (Re) targeting a CA1 pyramidal neuron
444 (PN). Stimulating electrodes were placed in SR and SLM to stimulate Schaffer collateral (SC
445 stim) or temporoammonic (TA stim) inputs, respectively. Brief pulses of blue light (5ms, 470
446 nm) delivered through the 40x objective were used to optogenetically stimulate PFC LRG
447 projections.

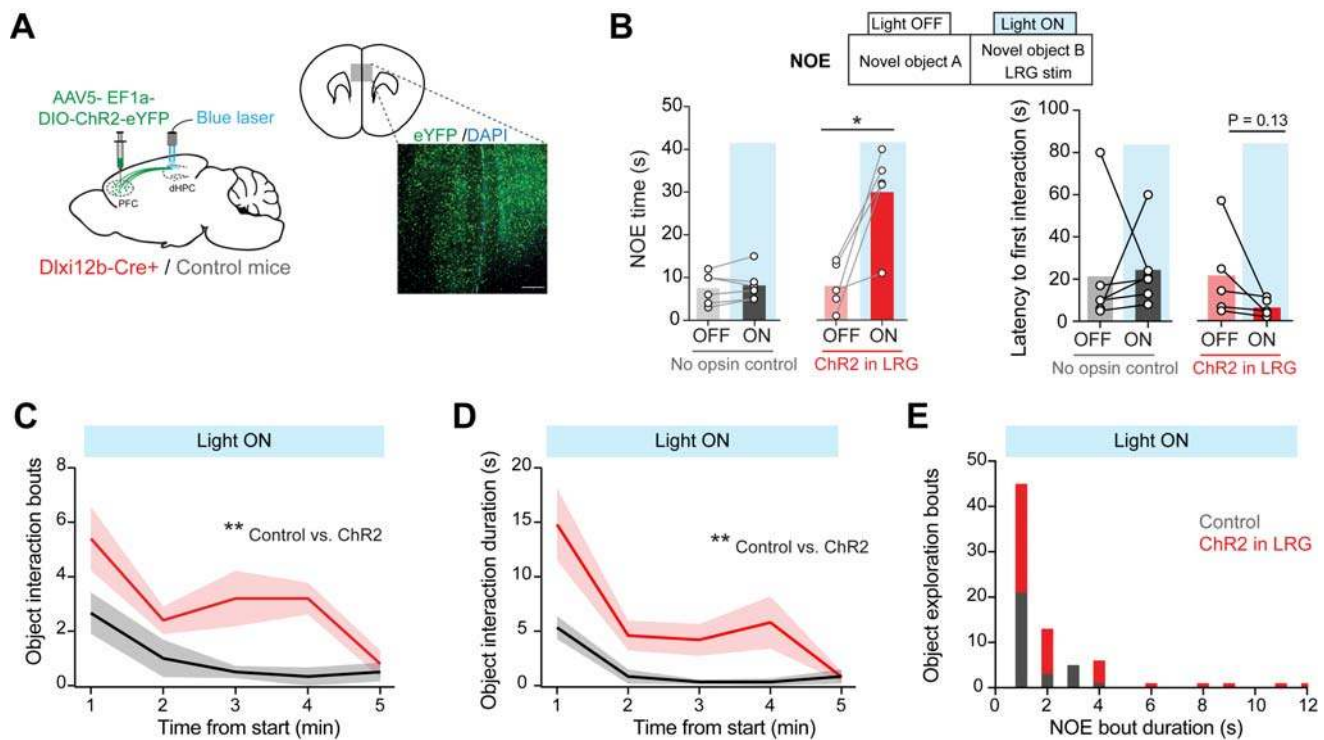
448 **(E)** Excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs) elicited by electrical
449 stimulation of SC or TA inputs (black bar) in the presence or absence of optogenetic stimulation
450 of PFC-dHPC LRG projections (cyan bar). Gray traces show responses to electrical stimulation
451 alone, blue traces show responses during combined electrical + optogenetic stimulation, and
452 green trace shows response to optogenetic stimulation alone.

453 **(F)** Right: optogenetic stimulation of PFC-dHPC LRG projections significantly decreased EPSP
454 to IPSP ratios for both SC ($n = 15$ cells) and TA ($n = 22$ cells) inputs. Lines connect values from
455 individual neurons and bars represent averages; two-way paired t-test, $** p < 0.01$, $* p < 0.05$.

456 **(G)** Example voltage responses of CA1 PN to coincident theta-burst stimulation (TBS) of SC
457 and TA inputs (black bars) combined with 20 Hz optogenetic stimulation of PFC LRG
458 projections (cyan bars). Gray trace shows voltage response to SC and TA electrical stimulation,
459 and blue trace denotes response during SC and TA electrical stimulation + optogenetic
460 stimulation of PFC LRG projections.

461 **(H)** Average firing frequency (left) and EPSP area (right) during TBS of SC and TA inputs are
462 reduced by concomitant optogenetic stimulation of PFC LRG projections. Open circles represent
463 individual neurons ($n = 9$) and bars represent averages; two-way paired t-test, $* p < 0.05$.

464 See also **Figure S3** and **Table S2**.



465 **Figure 3: Activating PFC–dHPC LRG projections increases novel object exploration.**

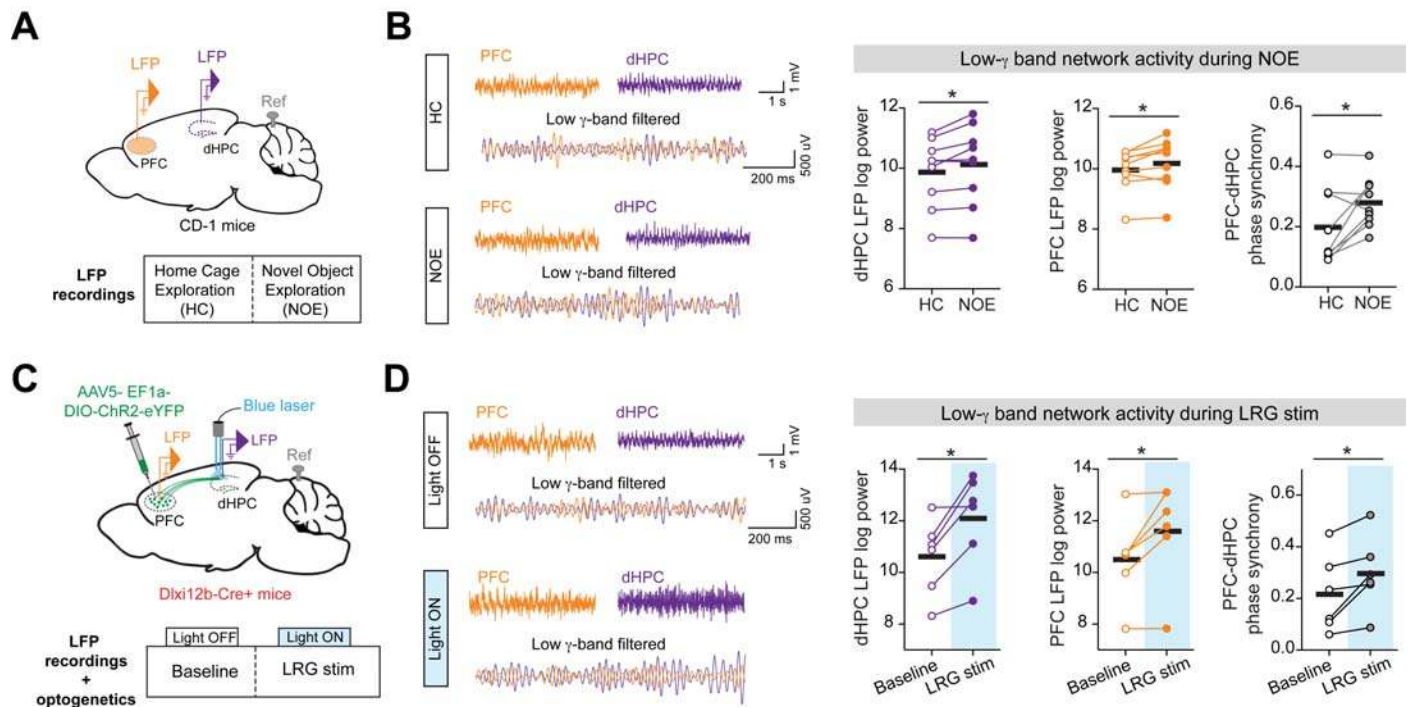
466 (A) Left: schematic illustrating the experimental design. Bilateral injections of Cre-dependent
 467 ChR2-eYFP virus into the PFC of *Dlx12b-Cre+* mice or *Cre-negative* (control) mice. Bilateral
 468 optical fibers were implanted over dHPC. Right: representative image showing ChR2-eYFP
 469 expression in PFC GABAergic neurons. Scale bar, 200µm.

470 (B) Top: novel object exploration (NOE) was measured in the presence (Light ON) or absence
 471 (Light OFF) of optogenetic stimulation (473nm, 1.5–2 mW/fiber, 5 ms pulses at 20 Hz). Bottom
 472 left: optogenetic stimulation significantly increased NOE time in ChR2+ mice (n = 5) but not in
 473 control mice (n = 6). Bottom right: Latency to first interaction with a novel object for ChR2+ and
 474 control mice is plotted. Open circles represent values from individual mice and bars indicate
 475 averages. Two-way paired t-test, * p < 0.05.

476 (C–D) Number of object interaction bouts within 1-minute bins (C), and duration of object
 477 interaction within 1-minute bins (D), over the duration of a 5-minute NOE testing session. Two-
 478 way repeated measures ANOVA, ** p < 0.01.

479 (E) Distribution of NOE bout durations during optogenetic stimulation of PFC LRG projections
 480 in control and ChR2-expressing mice.

481 See also **Figure S4**.



482 **Figure 4: Activating PFC–dHPC LRG projections increases low-gamma oscillations**
 483 **associated with NOE.**

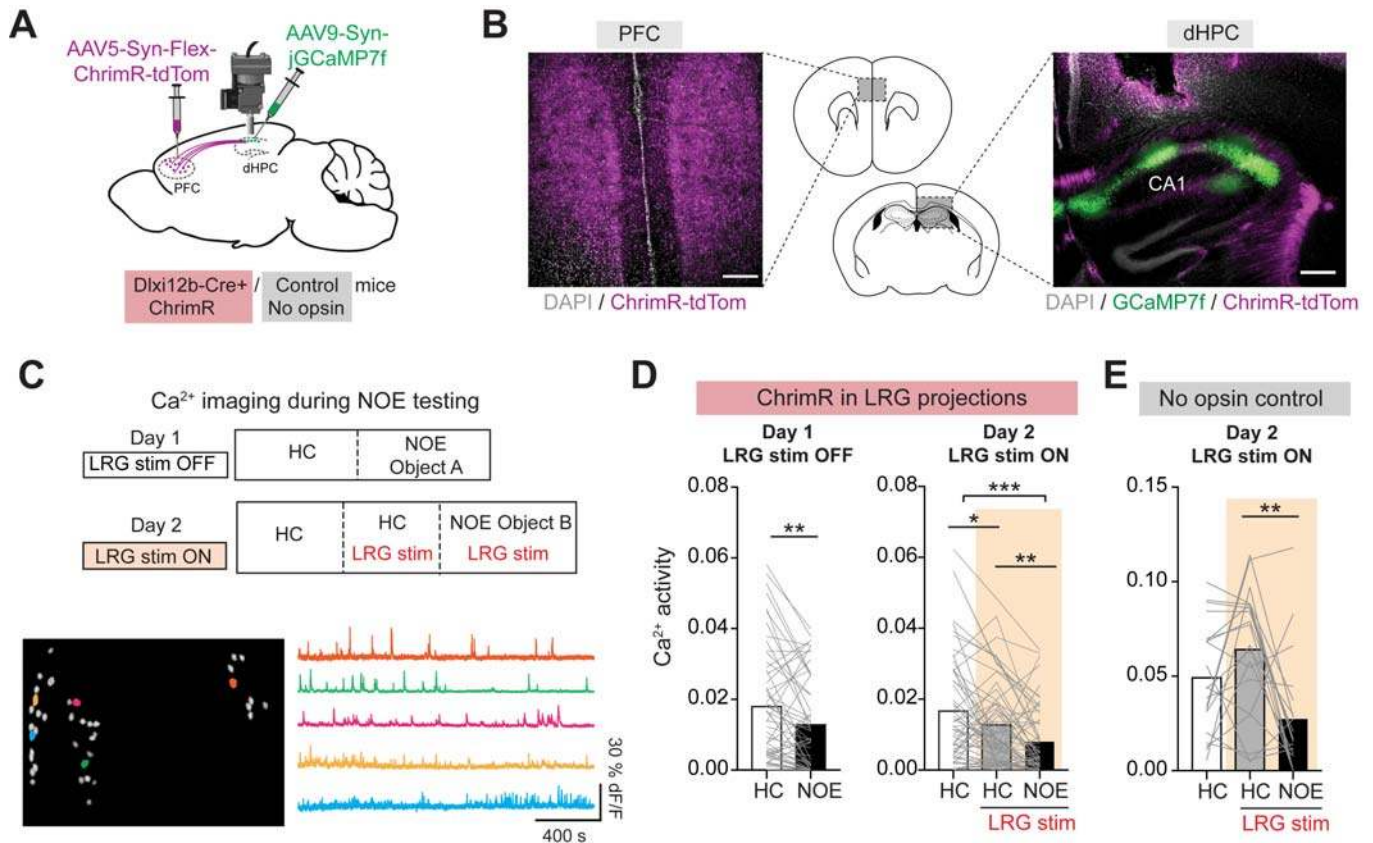
484 (A) Top: LFP electrodes were implanted in PFC and dHPC, reference electrode was implanted
 485 over the cerebellum. Bottom: LFPs recorded while mice were in their home cages (HC) were
 486 compared to LFPs recorded during NOE epochs.

487 (B) Left: example raw and low-gamma (low- γ) frequency filtered LFPs recorded during HC and
 488 NOE epochs are shown. Right: low- γ power in dHPC and PFC and low- γ phase synchrony
 489 between PFC and dHPC were all significantly higher during NOE. Open and filled circles
 490 represent data from individual mice ($n = 9$) and solid black lines represent averages. Two-way
 491 paired t-test, * $p < 0.05$.

492 (C) Top: Schematic illustrating the experimental design for combined optogenetic stimulation
 493 and LFP recordings. Cre-dependent ChR2-eYFP virus was bilaterally injected into the PFC of
 494 *Dlx12b-Cre+* mice. Bilateral optical fibers were implanted over dHPC; LFP electrodes were
 495 implanted in PFC and dHPC; reference electrode was implanted over the cerebellum. Bottom:
 496 LFPs were recorded during baseline epochs (Light OFF) or LRG stimulation epoch (Light ON;
 497 473 nm, 5 ms pulses at 20 Hz).

498 (D) Left: example raw and low- γ band filtered LFPs recorded during Light OFF and Light ON
 499 epochs. Right: low- γ power in dHPC and PFC and low- γ phase synchrony between PFC and
 500 dHPC were all significantly higher during LRG stim (Light ON) epoch. Open and filled circles
 501 represent data from individual mice ($n = 6$) and solid black lines represent averages. Two-way
 502 paired t-test, * $p < 0.05$.

503 See also **Figure S5**.



504 **Figure 5: PFC–dHPC LRG projections shape CA1 neuronal activity during object**
 505 **exploration.**

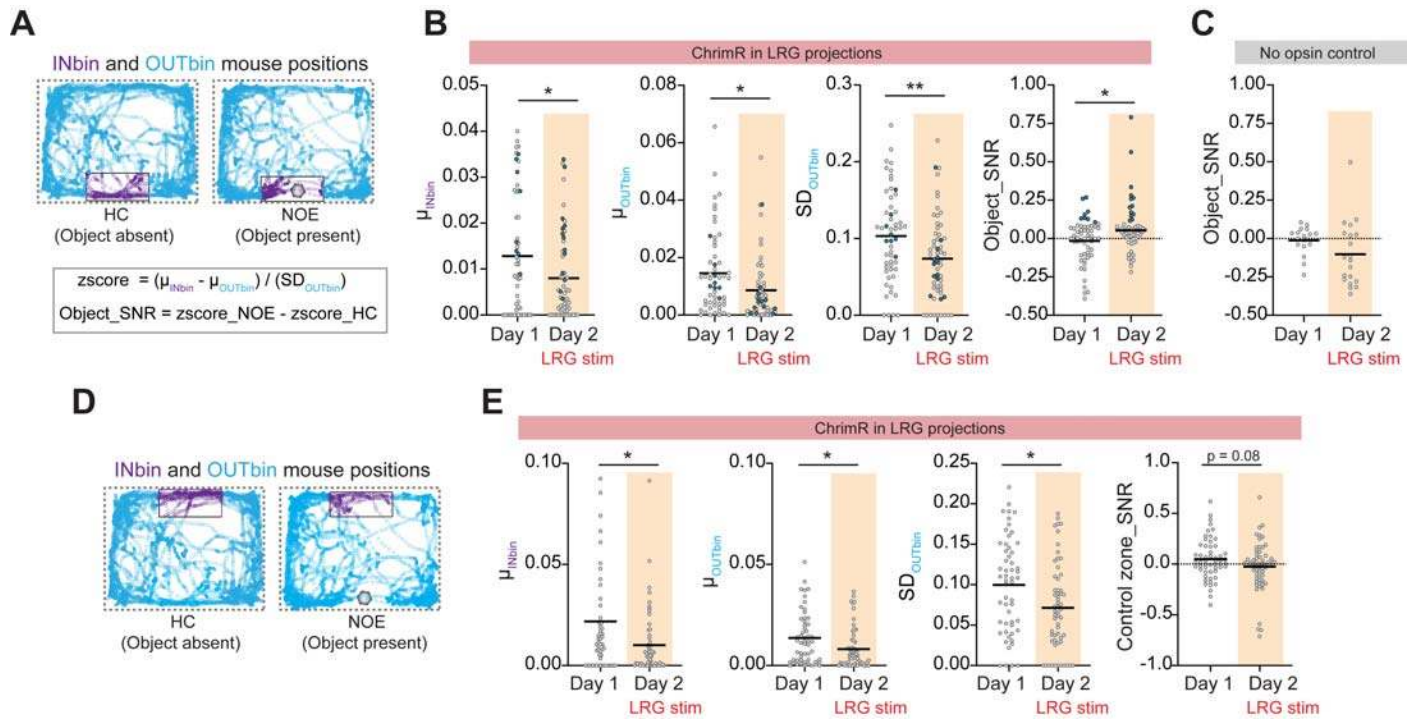
506 (A) Strategy for *in vivo* Ca²⁺ imaging and optogenetic stimulation. Cre-dependent ChrimsonR-
 507 tdTomato (ChrimR-tdTom) virus was injected into the PFC of *Dlx12b-Cre+* and *Cre-negative*
 508 (control) mice; jGCaMP7f virus was injected into dorsal CA1, and Ca²⁺ activity was imaged
 509 through an implanted GRIN lens connected to a miniscope.

510 (B) Left: DAPI stained coronal section showing ChrimR-tdTom expression in PFC GABAergic
 511 neurons. Right: DAPI stained dHPC section showing jGCaMP7f expression in CA1 neurons and
 512 ChrimR-tdTom expression in PFC–dHPC LRG axonal fibers. Scale bars, 0.5 mm.

513 (C) Top: on day 1, CA1 Ca²⁺ activity was measured during home cage (HC) and NOE epochs.
 514 On day 2, following an initial period of imaging in HC without optogenetic stimulation, Ca²⁺
 515 imaging was combined with optogenetic stimulation of PFC–dHPC LRG projections (590–650
 516 nm, ~2 mW, 5 ms pulses at 20 Hz) during HC and NOE epochs. Bottom left: regions of interest
 517 (ROIs) corresponding to neurons from a representative Ca²⁺ imaging session. Bottom right:
 518 extracted dF/F Ca²⁺ transients from example dorsal CA1 neurons. Colors of the traces on the
 519 right correspond to the colored ROIs on the left.

520 (D) Left: Ca²⁺ activity in CA1 neurons was significantly reduced during NOE. Each gray line
 521 represents a single neuron and bars represent the mean (n = 55 neurons from 2 mice); two-way

522 paired t-test. Right: During the HC epoch on day 2, optogenetic stimulation of PFC–dHPC LRG
523 projections reduced CA1 Ca²⁺ activity. On Day 2, activity was then further reduced during NOE
524 (n = 59 neurons, 2 mice). One-way ANOVA followed by Tukey’s multiple comparison test; ***
525 p < 0.001, ** p < 0.01, * p < 0.05.
526 (E) Same as D for control (opsin-negative) mice. Light delivery alone did not affect Ca²⁺
527 activity. One-way ANOVA followed by Tukey’s multiple comparison test; ** p < 0.01.
528 See also **Figure S6**.



529 **Figure 6: PFC–dHPC LRG projections increase neuronal signal-to-noise ratio for**
 530 **representation of object location in CA1.**

531 (A) Top: Frame-by-frame mouse positions during HC (left) and NOE (right) epochs are plotted.
 532 Example data from day 2 recording session is shown. Blue circles denote frames where mouse
 533 was outside the object zone (‘OUTbin’). Purple circles denote frames where mouse was in the
 534 object zone (‘INbin’). Gray shaded hexagon indicates the location of the novel object during
 535 NOE epoch and rectangle with black solid lines denotes the object zone. Bottom: z-scored Ca^{2+}
 536 activity was calculated as the difference between the mean Ca^{2+} activity when mouse was within
 537 (μ_{INbin}) vs. outside (μ_{OUTbin}) the object zone, divided by the standard deviation of activity outside
 538 the object zone (SD_{OUTbin}). The difference in z-scored Ca^{2+} activity between HC to NOE epochs
 539 was used to compute Object_SNR.

540 (B) Optogenetic stimulation of PFC–dHPC LRG projections significantly reduced μ_{INbin} , μ_{OUTbin} ,
 541 and SD_{OUTbin} . Object_SNR was increased on day 2 compared to day 1 in opsin-expressing mice.
 542 Empty gray circles represent from individual neurons and horizontal black lines show means.
 543 Filled blue circles indicate neurons exceeding an arbitrary threshold for Object_SNR (>0.1) to
 544 illustrate μ_{INbin} , μ_{OUTbin} , and SD_{OUTbin} for these high-SNR neurons. Two-way unpaired t-test; ** p
 545 < 0.01, * p < 0.05.

546 (C) Object_SNR in control (opsin negative) mice was not affected by light delivery.

547 (D) Frame-by-frame mouse positions during HC (left) and NOE (right) epochs are plotted.
 548 Example data from day 2 recording session is shown. Blue circles denote frames where mouse
 549 was away from a control zone (‘OUTbin’) that was the mirror image of the object zone, but on

550 the opposite side of the cage. Purple circles denote frames where mouse was in the control zone
551 ('INbin'). Gray shaded hexagon denotes the novel object location in NOE epoch and rectangle
552 with black solid lines denotes the control zone.
553 (E) For the control zone, optogenetic stimulation of PFC–dHPC LRG projections significantly
554 reduced μ_{INbin} , μ_{OUTbin} , and $\text{SD}_{\text{OUTbin}}$ (similar to the object zone). However, SNR computed for
555 the control zone was not affected by optogenetic stimulation of LRG projections (in contrast to
556 the object zone). Empty gray circles represent values from individual neurons and black lines
557 represent mean values. Two-way unpaired t-test; * $p < 0.05$.

558 **METHODS**

559 **Animals**

560 All animal care procedures and experiments were conducted in accordance with the National
561 Institutes of Health guidelines and approved by the Administrative Panels on Laboratory Animal
562 Care at the University of California, San Francisco. Mice were housed in a temperature-
563 controlled environment (22–24 °C) with ad libitum access to food and water. Mice were reared
564 in normal lighting conditions (12-h light/dark cycle). Adult mice from the following lines were
565 used: *Dlxi12b-Cre* (Potter et al., 2009) and wild-type CD-1.

566

567 **Virus and retrograde tracer injections**

568 Mice were anesthetized with isoflurane and placed on a stereotaxic frame (David Kopf
569 Instruments). An incision was made to expose the skull, and bregma and lambda were used as
570 references to align the skull. Body temperature was maintained using a heating pad. Virus was
571 injected (at the rate of 100 nl/min) with a microinjection syringe (Nanofil 10 µl with 35 gauge
572 needle, World Precision Instruments) connected to a microsyringe pump (World Precision
573 Instruments, UMP3 UltraMicroPump). Coordinates for injections into PFC were (in mm, relative
574 to Bregma) 1.8 anterior-posterior (AP), ±0.3 mediolateral (ML), -2.4 dorsoventral (DV); and
575 coordinates for injections into dHPC were -1.35 AP, ±0.65 ML, -1.5 DV.

576 For anterograde tracing, Chr2 assisted circuit-mapping and optogenetic stimulation
577 experiments, either AAV5-EF1α-DIO-eYFP virus or AAV5-EF1α-DIO-ChR2-eYFP virus (UNC
578 Vector core, 650 nl) was injected into PFC of *Dlxi12b-Cre+* mice and *Cre-negative* mice. For
579 intersectional labeling of dHPC projecting PFC LRG neurons, CAV2-Cre virus (del Rio et al.,
580 2019; Hnasko et al., 2006) (650 nl) was injected in the dHPC and AAV5-Dlxi12b-BG-DIO-
581 ChR2-eYFP virus (Lee et al., 2014) (650 nl) was injected in the PFC of CD-1 mice. For
582 retrograde labeling of dHPC projecting PFC LRG neurons, Alexa Flour 594 Cholera toxin beta
583 subunit conjugate (CTb-594, Invitrogen; 0.5% w/v, 400–500 nl) was injected in dHPC of CD-1
584 mice. After virus or tracer injection, the microinjector needle was left in place for 5–6 min before
585 being removed from the brain. Mice were sutured (if receiving viral/tracer injection only) and
586 were allowed to recover on a heated pad until ambulatory.

587

588 **Optic fiber implantation**

589 Following bilateral AAV5-EF1α-DIO-ChR2-eYFP virus injection in PFC, dual fiber-optic
590 cannulas (Doric lenses; 200/240 mm, 0.22NA) were implanted in dHPC (-1.35 AP, ±0.65 ML, -
591 1.4 DV). During these surgeries, the skull was scored with a scalpel to improve implant
592 adhesion. We waited at least 7 weeks after surgery to allow time for viral expression.

593

594 **Optogenetic stimulation of PFC–dHPC LRG projections**

595 A 473 nm blue laser (OEM Laser Systems, Inc.) was coupled to the dual fiber-optic cannula
596 (implanted in dHPC) through a dual fiber-optic patch cord (Doric Lenses, Inc.), and was
597 controlled via a function generator (Agilent 33500B Series Waveform Generator). Laser power
598 was adjusted such that the final light power was 3–4 mW total, summed across both fibers, and
599 averaged over 20 Hz light pulses (5 ms duration).

600

601 **Behavioral assays**

602 After sufficient time for surgical recovery and viral expression, mice were handled and
603 habituated for multiple days (3–5 days). Briefly, mice were first habituated to the behavioral
604 testing room for 30 min prior to handling each day. For 2–3 days before starting testing, mice
605 were habituated to the cable tethers in their home cage for 15 min. The experimenter was blinded
606 to experimental groups during behavioral testing and scoring. A USB webcam (Logitech)
607 connected to a computer running ANY-maze (Stoelting Co.) was used to record behavior
608 movies. The position of mice was tracked using the built-in tracking in ANY-maze software. In
609 some experiments, mouse positions were tracked using trained neural networks in DeepLabCut
610 open-source software package (Mathis et al., 2018).

611

612 *Novel object exploration*

613 For measuring novel object exploration, one previously unexplored object was placed in the
614 home cage of an experimental mouse for 5 min. A blinded observer manually scored the
615 following parameters: exploration time, bouts of exploration, and latency to the first exploration.
616 Objects used in our study were usually lego toys, dice, small plumbing connectors, and falcon
617 tube caps. For experimental mice with dual-fiber optic implants, two object interaction tests were
618 performed over two days: day 1 testing was performed without light stimulation, and day 2
619 testing was done during optogenetic stimulation of PFC–dHPC LRG projections. *Cre-negative*
620 mice (no opsin control) with dual-fiber optic implants underwent similar behavior testing
621 procedures.

622

623 *Social interaction test*

624 For social interaction test, a novel juvenile (3–4 week old) mouse of the same sex was introduced
625 in the home cage of an experimental mouse for 5 min. A blinded observer manually scored the
626 time (in seconds) the experimental mouse spent with its nose in direct contact with the novel
627 juvenile intruder. For all experimental mice, two social interaction tests were performed over two
628 days: day 1 testing was performed without light stimulation, and day 2 testing was done during

629 optogenetic stimulation of PFC LRG projections.

630

631 *Open field exploration test*

632 Mice were placed in the center of a 50 x 50 cm open-field arena and were allowed to freely
633 explore for 12 min. The testing time was divided into four (3 min) epochs. PFC–dHPC LRG
634 projections were optogenetically stimulated during the 2nd and 4th epochs. Distance traveled
635 during no stimulation (light OFF) and during optogenetic stimulation (light ON) epochs was
636 quantified using the ANY-maze tracking software.

637

638 *Real-time place preference (RTPP) test*

639 Real-time place preference (RTPP) testing protocol consisted of three 20 min sessions conducted
640 over 3 days. An apparatus with two identical chambers was used for RTPP testing. On day 1,
641 mice were habituated to the apparatus for 15 min. On day 2, mice were placed into one randomly
642 chosen chamber and the time spent in the two chambers was recorded. On day 3, one of the
643 chambers was randomly assigned as the stimulated chamber. When mice entered this chamber,
644 they received 20 Hz laser pulses (473 nm, 3–4 mW, 5 ms). The ratio of the time spent in the
645 simulated chamber vs. the non-stimulated chamber was used as the preference index. The sides
646 of the stimulated chambers were counterbalanced across all mice.

647

648 **LFP recordings: surgery and analysis**

649 *Surgery*

650 Mice were anesthetized with isoflurane and placed on a stereotactic frame. After cleaning, the
651 skull was scored with a scalpel to improve implant adhesion. For LFP recordings from wild-type
652 CD-1 mice, tungsten electrodes (Microprobes) were inserted into the PFC (1.8 AP, -0.3 ML, -2.4
653 DV) and dHPC (-1.35 AP, -0.65 ML, -1.5 DV). For multisite LFP recordings combined with
654 optogenetics, one LFP electrode was implanted after AAV5-EF1 α -DIO-ChR2-eYFP virus
655 injection into PFC of *Dlxi12b-Cre+* mice. A custom-made optrode (optical fiber + electrode)
656 was implanted in dHPC to stimulate ChR2+ PFC–dHPC LRG axon terminals during LFP
657 recordings. To fabricate optrodes, a tungsten LFP recording electrode was affixed to one of the
658 fibers of the dual-fiber optic cannula such that the tip of the electrode protruded 200–300 μ m
659 beyond the end the optic fiber (Lee et al., 2019). Reference and ground screws were implanted
660 above the cerebellum. Electrodes and screws were cemented to the skull with Metabond (Parkell)
661 and connected to a headstage for multi-channel recordings (Pinnacle). Following surgery, mice
662 were monitored postoperatively, given analgesics, and individually housed.

663

664 *Recording and analysis*

665 LFP data were acquired at 2 KHz and band-pass filtered from 0.5-150Hz. Electrode placements
666 were histologically confirmed. Analysis of LFP data was done using custom MATLAB
667 (Mathworks) scripts. Briefly, signals were imported into MATLAB and LFP log power (for both
668 channels) was calculated using the power spectral density output from the spectrogram function.
669 For phase-synchrony and amplitude covariance analysis, LFPs were FIR-filtered for different
670 frequency bands, then Hilbert transformed to yield the instantaneous amplitudes and phases. The
671 following frequency bands were compared: theta band (4–12 Hz), beta band (15–25 Hz), low-
672 gamma band (25–55 Hz), and high-gamma band (65–85 Hz).

673 To detect nonzero phase interdependencies (phase synchrony) between LFP signals
674 recorded at PFC and dHPC electrodes, we estimated the weighted Phase Lag Index (wPLI)
675 (Vinck et al., 2011) using the imaginary component of the cross-spectrum (S_{xy}) (Equations 1.1
676 and 1.2). A_x and A_y are instantaneous amplitudes; and Φ_x and Φ_y are instantaneous phases for
677 PFC and dHPC signals, respectively.

$$678 \quad S_{xy} = A_x A_y e^{i(\phi_x - \phi_y)} \quad (1.1)$$

$$679 \quad wPLI = \frac{|\sum |imag(S_{xy})| sgn(S_{xy})|}{\sum |imag(S_{xy})|} \quad (1.2)$$

681
682 The amplitude covariation between PFC and dHPC was calculated as the maximum normalized
683 cross-correlation (xcorr function in MATLAB) of the instantaneous band-pass filtered
684 amplitudes of LFP signals at each electrode. Coherence between LFP signals was computed
685 using the mscohere function in MATLAB. Log power, wPLI, amplitude covariation, and
686 coherence were calculated over short time intervals (at least 3 sec in duration), i.e., the intervals
687 during which a mouse was actively exploring an object or matched intervals during baseline
688 periods when the mouse was in its home cage.

689 ***Ex-vivo* slice physiology**

690 *Slice preparation*

691 Adult mice were anesthetized with an intraperitoneal injection of euthasol and transcardially
692 perfused with an ice-cold cutting solution containing (in mM) 210 sucrose, 2.5 KCl, 1.25
693 NaH₂PO₄, 25 NaHCO₃, 0.5 CaCl₂, 7 MgCl₂, 7 dextrose (bubbled with 95% O₂–5% CO₂, pH
694 ~7.4). Mice were decapitated and the brains were removed. For acute prefrontal sections: two
695 parallel cuts were made along the coronal plane at the rostral and caudal ends of the brains;
696 brains were mounted on the flat surface created at the caudal end; three coronal slices (250 μm
697 thick) were obtained using a vibrating blade microtome (VT1200S, Leica Microsystems Inc.).
698 Dorsal hippocampal (dHPC) slices were obtained using a blocking technique described
699

700 previously (Malik et al., 2015). Briefly, dHPC slices were obtained by making a blocking cut at a
701 45° angle from the coronal plane starting at the posterior end of the forebrain. A second blocking
702 cut was made at 45° relative to the coronal plane, but starting from approximately one-third of
703 the total length of the forebrain (from the most anterior point). Brains were mounted on the flat
704 surface created by the first blocking cut. Approximately, 3 dorsal slices were obtained from each
705 hemisphere.

706 Slices were allowed to recover at 34°C for 30 min followed by 30 min recovery at
707 room temperature in a holding solution containing (in mM) 125 NaCl, 2.5 KCl, 1.25 NaH₂PO₄,
708 25 NaHCO₃, 2 CaCl₂, 2 MgCl₂, 12.5 dextrose, 1.3 ascorbic acid, 3 sodium pyruvate.

709 710 *Ex-vivo patch clamp recordings*

711 Somatic whole-cell current-clamp and voltage-clamp recordings were obtained as previously
712 described (Malik and Johnston, 2017; Malik et al., 2019). Briefly, submerged slices were
713 perfused in heated (32–34°C) artificial cerebrospinal fluid (aCSF) containing (in mM): 125
714 NaCl, 3 KCl, 1.25 NaH₂ PO₄, 25 NaHCO₃, 2 CaCl₂, 1 MgCl₂, 12.5 dextrose (bubbled with 95%
715 O₂/5% CO₂, pH ~7.4). Neurons were visualized using DIC optics (and eYFP fluorescence in a
716 few experiments) fitted with a 40x water-immersion objective (BX51WI, Olympus microscope).
717 During recordings from prefrontal slices, dHPC projecting PFC LRG neurons in all cortical
718 layers were identified by eYFP expression. During recordings from hippocampal slices, CA1
719 pyramidal neurons (PNs) and CA1 local inhibitory neurons (INs) were identified using laminar
720 location (under DIC optics) and intrinsic properties of the recorded neurons.

721 Patch electrodes (2–3 MΩ) were pulled from borosilicate capillary glass of external
722 diameter 1 mm (Sutter Instruments) using a Flaming/Brown micropipette puller (model P-2000,
723 Sutter Instruments). For current-clamp recordings, electrodes were filled with an internal
724 solution containing the following (in mM): 134 K-gluconate, 6 KCl, 10 HEPES, 4 NaCl, 7 K₂-
725 phosphocreatine, 0.3 Na-GTP, and 4 Mg-ATP (pH ~7.3 adjusted with KOH). Biocytin (Vector
726 Laboratories) was included (0.1–0.2%) for subsequent histological processing of recipient CA1
727 neurons. For voltage-clamp recordings, the internal solution contained the following (in mM):
728 130 Cs-methanesulfonate, 10 CsCl, 10 HEPES, 4 NaCl, 7 phosphocreatine, 0.3 Na-GTP, 4 Mg-
729 ATP, and 2 QX314-Br (pH ~7.3 adjusted with CsOH). In a few recordings, 15 μm AlexaFluor-
730 594 (Invitrogen) was also added to the internal solution. Electrophysiology data were recorded
731 using Multiclamp 700B amplifier (Molecular Devices). Voltages have not been corrected for
732 measured liquid junction potential (~8 mV). Data collection was started 5–8 min after
733 successful transition to the whole-cell configuration. Series resistance and pipette capacitance
734 were appropriately compensated before each recording. Series resistance was usually 10–
735 20 MΩ, and experiments were terminated if series resistances exceeded 25 MΩ.

736

737 *Data analysis*

738 *Ex-vivo* electrophysiology data were analyzed using custom routines written in IGOR Pro
739 (Wavemetrics). Resting membrane potential (RMP) was measured in current-clamp mode
740 immediately after reaching whole-cell configuration. Input resistance (R_{in}) was calculated as the
741 slope of the linear fit of the voltage-current plot generated from a family of hyperpolarizing and
742 depolarizing current injections (-50 to +20 pA, steps of 10 pA). Firing output was calculated as
743 the number of action potentials (APs) fired in response to 800 ms long depolarizing current
744 injections (25–500 pA). Firing frequency was calculated as the number of APs fired per second.
745 Firing traces in response to 50 pA current above the rheobase were used for analysis of single AP
746 properties – AP threshold, maximum dV/dt (rate of rise of AP), AP amplitude, AP half-width,
747 and fast afterhyperpolarization (fAHP) amplitude. AP threshold was defined as the voltage at
748 which the value of third derivative of voltage with time is maximum. Action potential amplitude
749 was measured from threshold to peak, and the half-width was measured at half this distance. Fast
750 afterhyperpolarization was measured from the threshold to the negative voltage peak after the
751 AP. Index of spike-frequency accommodation (SFA) was calculated as the ratio of the last inter-
752 spike interval to the first inter-spike interval.

753 Recorded inhibitory neurons (INs) in PFC and dorsal CA1 were classified as fast spiking,
754 regular spiking or irregular spiking based on electrophysiological properties. Specifically, INs
755 were classified as fast spiking if they met 3 out of the 4 following criteria: AP half-width was <
756 0.5 ms, firing frequency > 50 Hz, fAHP amplitude > 14 mV, and SFA index < 2. Irregular
757 spiking INs were initially visually identified based on their high variability in inter-spike interval
758 and burst-like intermittent spiking properties. This classification was confirmed using a firing
759 frequency threshold (<50 Hz) and/or a SFA index threshold (>2). Dorsal CA1 neurons were
760 classified as pyramidal neurons if they satisfied the following criteria: cell body located in
761 stratum pyramidale, AP half-width > 1 ms, fAHP amplitude < 5 mV, and maximum firing
762 frequency < 20 Hz.

763 To measure optogenetically evoked spiking in ChR2-eYFP+ PFC INs and to measure
764 optogenetically evoked postsynaptic currents (oPSCs) in CA1 neurons, ChR2 was stimulated
765 using 5 ms long light pulses (maximum light power, 4 mW/mm²) generated by a Lambda DG-
766 4 high-speed optical switch with a 300 W Xenon lamp (Sutter Instruments) and an excitation
767 filter centered around 470 nm. Light pulses were delivered to the slice through a 40x objective
768 (Olympus). To measure the reversal potential of oPSCs, the holding potentials were
769 systematically varied from -100 to +20 mV in 10 mV steps. The drugs applied were 6-cyano-7-
770 nitroquinoxaline-2,3-dione disodium salt hydrate (CNQX), 2-(3-carboxypropyl)-3-amino-6-(4-
771 methoxyphenyl)-pyridazinium bromide (Gabazine), and d-2-amino-5-phosphonopentanoic acid

772 (D-AP5) (Tocris). Drugs were prepared as concentrated stock solutions and were diluted in
773 ACSF on the day of the experiment.

774 To measure afferent input mediated feedforward excitation and inhibition in CA1 PNs,
775 bipolar stimulating electrodes (Microprobes) were placed at stratum radiatum (SR) and stratum
776 lacunosum-moleculare (SLM) to stimulate Schaffer collateral (SC) and temporo ammonic (TA)
777 inputs, respectively. The protocol for theta-burst stimulation (TBS) consisted of bursts with five
778 electrical stimulations (40 Hz) repeated at 5 Hz. To measure the effect of PFC LRG inputs on
779 firing output and EPSP summation during TBS protocol, train of 470 nm light pulses (20 Hz, 5
780 ms) was delivered through the 40x objective. Firing frequency during TBS was calculated as the
781 average number of APs fired per burst, and summation was estimated as the area of the last
782 EPSP in the TBS train.

783

784 ***In vivo* Ca²⁺ imaging**

785 *Surgery*

786 Mice underwent two stereotactic surgeries. Cre-dependent AAV5-Syn-FLEX-ChrimsonR-
787 tdTomato virus (Addgene) was injected in PFC (1.8 AP, ±0.3 ML, -2.4 DV) of *Dlx1/2b-Cre+*
788 and *Cre-negative* mice. Following this, 500–550 nl of AAV9-Syn-jGCaMP7f-WPRE virus
789 (diluted 1:2; Addgene) was injected in dorsal CA1 to express synapsin-driven calcium sensor
790 jGCaMP7f (injection coordinates: -1.4 AP, +0.8 ML, -1.5 DV). After 3–4 weeks of viral
791 expression, cortex overlying dorsal CA1 was slowly aspirated and a 1 mm diameter x 4 mm long
792 integrated GRIN lens (Inscopix) was slowly advanced above the dorsal CA1 and cemented in
793 place with Metabond dental cement. Mice were allowed to recover for at least 3 weeks before
794 starting behavior and imaging experiments.

795

796 *Combined Ca²⁺ imaging and optogenetics*

797 Imaging data were collected using a miniaturized one-photon microscope (nVoke2; Inscopix
798 Inc.). GCaMP7f signals (Ca²⁺ activity) were detected using 435–460 nm excitation LED (0.1–0.2
799 mW), and optogenetic stimulation of ChrimR expressing axons was performed using a second
800 excitation LED centered around 590–650 nm (5 ms pulses at 20 Hz, 1–2 mW light power). Ca²⁺
801 movies were acquired at 20 frames per second, spatially downsampled (4x), and were stored for
802 offline data processing.

803 Mice were placed into a large housing cage (48 x 35 cm) for 2–3 days for 20 min where
804 they habituated to the scope. After habituation, mice underwent a two-day behavioral testing
805 protocol for recording NOE related Ca²⁺ activity in CA1 neurons. On day 1, mice were allowed
806 to explore the large home cage for 15 min (HC epoch). Following this, mice were allowed to
807 explore a novel object introduced in the cage for 15 min (NOE epoch). On day 2, mice were

808 allowed to explore the home cage for 15 min (HC epoch) followed by optogenetic stimulation
809 during home cage exploration for 10 min (HC + LRG stim epoch). Mice were then allowed to
810 explore a novel object combined with optogenetic stimulation of PFC–dHPC LRG projections
811 (NOE + LRG stim epoch). The behavior of mice during different epochs was recorded using
812 ANY-maze software, and input TTL pulses from ANY-maze to nVoke2 acquisition software
813 were used to synchronize Ca^{2+} imaging and mouse behavior movies.

814

815 *Data analysis*

816 Ca^{2+} imaging movies were preprocessed using Inscopix Data Processing Software (IDPS;
817 Inscopix, Inc.). The video frames were spatially filtered (band-pass) with cut-offs set to 0.005
818 pixel^{-1} (low) and 0.5 pixel^{-1} (high) followed by frame-by-frame motion correction for removing
819 movement artifacts associated with respiration and head-unrestrained behavior. The mean image
820 over the imaging session was computed, and the dF/F was computed using this mean image. The
821 resultant preprocessed movies were then exported into MATLAB, and cell segmentation was
822 performed using an open-source calcium imaging software (CIAPKG) (Corder et al., 2019).
823 Specifically, we used a Principal Component Analysis/Independent Component Analysis
824 (PCA/ICA) approach to detect and extract ROIs (presumed neurons) per field of view (Mukamel
825 et al., 2009). For each movie, the extracted output neurons were then manually sorted to remove
826 overlapping neurons, neurons with low SNR, and neurons with aberrant shapes.

827 Accepted neurons and their Ca^{2+} activity traces were exported to MATLAB for further
828 analysis using custom scripts (as previously described in (Frost et al., 2020)). Briefly, we
829 calculated the standard deviation (σ) of the Ca^{2+} movie and used this to perform threshold-based
830 event detection on the traces by first detecting increases in dF/F exceeding 2σ (over one second).
831 Subsequently, we detected events that exceeded 10σ for over two seconds and had a total area
832 under the curve higher than 150σ . The peak of the event was estimated as the local maximum of
833 the entire event. For an extracted output neuron, active frames were marked as the period from
834 the beginning of an event until the Ca^{2+} signal decreased 30% from the peak of the event (up to a
835 maximum of 2 seconds).

836

837 *Procedure for measuring object-related changes in Ca^{2+} activity*

838 Frame-by-frame x-y positions of the head of a mouse in the testing cage were detected using
839 DeepLabCut. A small rectangular area surrounding the object location in the testing cage was
840 marked as the object zone. Time points (frames acquired at 30 Hz and resampled at 20 Hz, using
841 resample function in MATLAB) when the mouse's head was inside the object zone were
842 classified as INbin and the remaining frames were classified as OUTbin. We then recorded the
843 frame-by-frame Ca^{2+} activity of neurons corresponding to the INbin and OUTbin position

844 frames. For all extracted neurons, the mean activity for INbin (μ_{INbin}) and OUTbin (μ_{OUTbin})
845 frames were calculated. We also calculated the standard deviation (SD_{OUTbin}) of neuronal activity
846 in OUTbin frames. The z-scored activity of each neuron was estimated using equation 2.1. The
847 object signal-to-noise ratio (Object SNR) was calculated using the z-scored activity during HC
848 and NOE epochs (Equation 2.2).

$$849 \quad zscore = \frac{\mu_{INbin} - \mu_{OUTbin}}{SD_{OUTbin}} \quad (2.1)$$

$$850 \quad Object\ SNR = zscore_{NOE} - Zscore_{HC} \quad (2.2)$$

851

852 **Histological processing**

853 *Assessment of virus expression and anterograde tracing of LRG projections*

854 Animals were transcardially perfused with PBS, and then with 4% paraformaldehyde (PFA). The
855 brains were post-fixed for at least one day in PFA solution. Coronal sections (50–75 μ m thick)
856 were obtained using a vibratome. Sections that included the injection sites, electrode
857 implantation sites and lens implantation sites were mounted on slides and cover-slipped using a
858 glycerol-base, aqueous mounting medium (Vectashield Plus Antifade Mounting Medium, Vector
859 labs). Sections were first scanned using an upright wide-field fluorescence microscope.
860 Following this, confocal images were taken with 10x and 20x objectives on an Andor Borealis
861 CSU-W1 spinning disk confocal mounted on a Nikon Ti Microscope (UCSF Nikon Imaging
862 Center, NIH S10 Shared Instrumentation grant 1S10OD017993-01A1) and captured with an
863 Andor Zyla sCMOS camera and Micro-Manager software (Open Imaging).

864

865 *Inhibitory neuron marker expression in recipient CA1 neurons*

866 Slices containing biocytin-filled cells were fixed overnight in a buffered solution containing 4%
867 PFA. Slices were rinsed in PBS, then blocked and permeabilized in PBS with 5% Donkey
868 Serum, 0.3% Triton X-100 and 1% BSA. Slices were immuno-stained overnight with one or two
869 primary antibodies: rabbit anti-PV (Swant; diluted 1:200), rat anti-SST (Millipore, diluted
870 1:200), or rabbit anti-VIP (Immunostar, diluted 1:200). Slices were washed 6 x 10min in PBS
871 containing 0.3% Triton X-100. Slices were incubated with donkey anti-rabbit Alexa-488, donkey
872 anti-rat Alexa 594 secondary antibody (1:800, Thermo Fisher), and Streptavidin-647 (1:300,
873 Thermo Fisher) overnight at 4°C. After washing 6 x 10min in PBS with 0.3% Triton X-100,
874 slices were mounted with an aqueous mounting medium. Confocal mages were obtained as
875 described above.

876

877 *Inhibitory neuron (IN) marker expression in CTb tagged PFC LRG neurons*

878 5-7 days after CTb injection, mice were transcardially perfused with PBS followed by 4% PFA

879 solution, and brains were post-fixed for at least one day. Coronal sections (75 μ m) were obtained
880 using a vibratome, and immunohistochemistry was performed (as described above). The following
881 primary antibodies were used to stain for IN markers: rabbit anti-PV (Swant; diluted 1:200); rat
882 anti-SST (Millipore, diluted 1:200); rabbit anti-VIP (Immunostar, diluted 1:200); rabbit anti-
883 NPY (Immunostar, diluted 1:500); rabbit anti-calretinin (Immunostar, diluted 1:500); rabbit anti-
884 nNOS (Life technologies, diluted 1:500), and goat anti-CTb (List, diluted 1:500). The following
885 secondary antibodies were used: donkey anti-rabbit Alexa 488; donkey anti-rat Alexa 488; and
886 donkey anti-goat Alexa 594. For each IN marker, confocal images collected from mounted
887 sections were used to manually count the number of CTb+ and IN marker+ PFC neurons (ImageJ
888 software).

889

890 **Statistical analysis**

891 Detailed statistical analyses were performed using MATLAB and Graphpad Prism. Comparisons
892 of means were performed using paired or unpaired two-tailed Student's t test, one-way ANOVA
893 or two-way repeated measures ANOVA with Tukey post hoc test unless otherwise stated. For
894 non-parametric data sets, we used a Chi-square test to determine significance. Sample sizes and
895 statistical tests and parameters are listed in the figure legends. Data are reported as mean \pm
896 S.E.M. unless otherwise stated.

897

898 **Data availability**

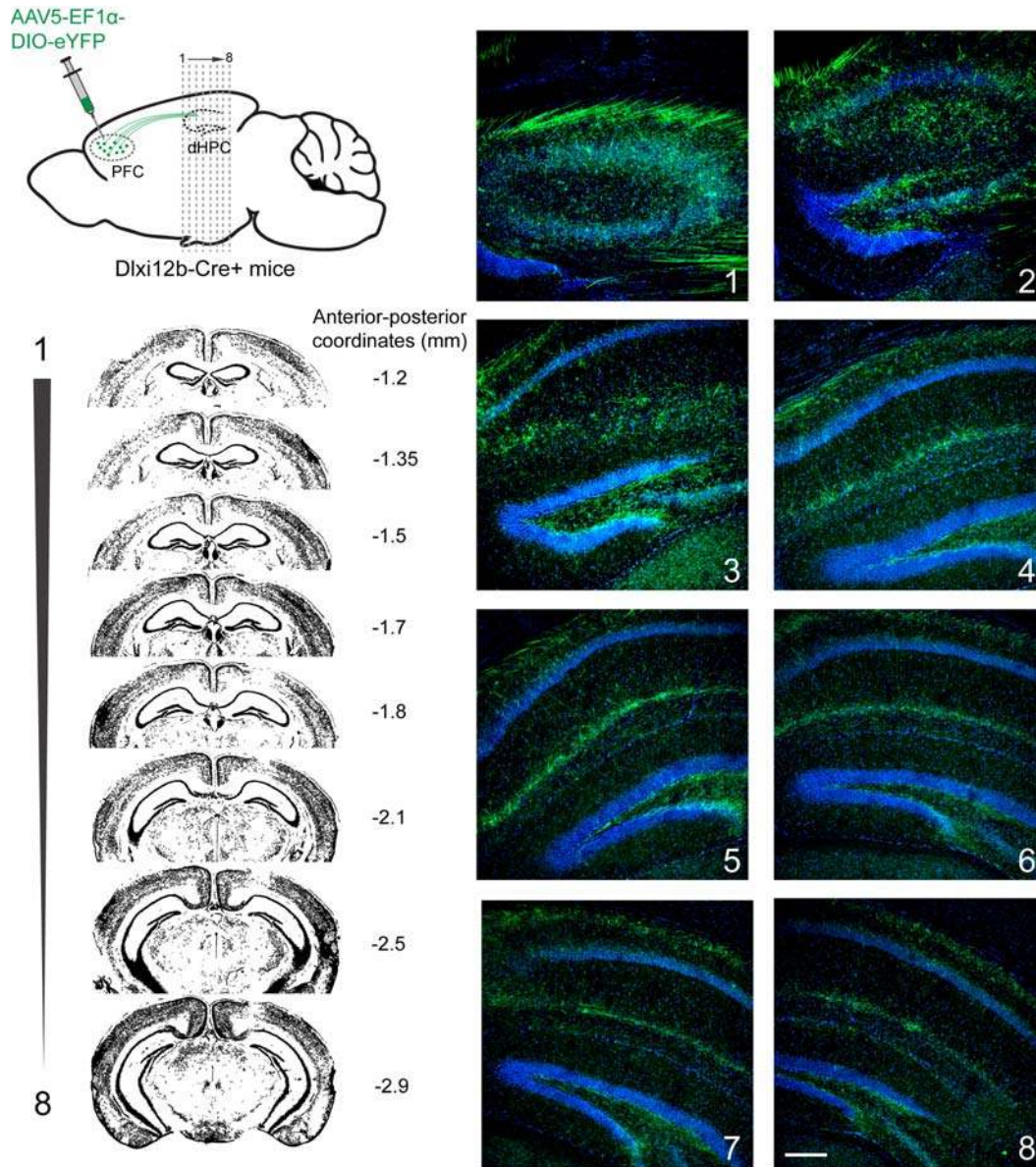
899 Data supporting the findings of this study are available from the corresponding author upon
900 reasonable request.

901

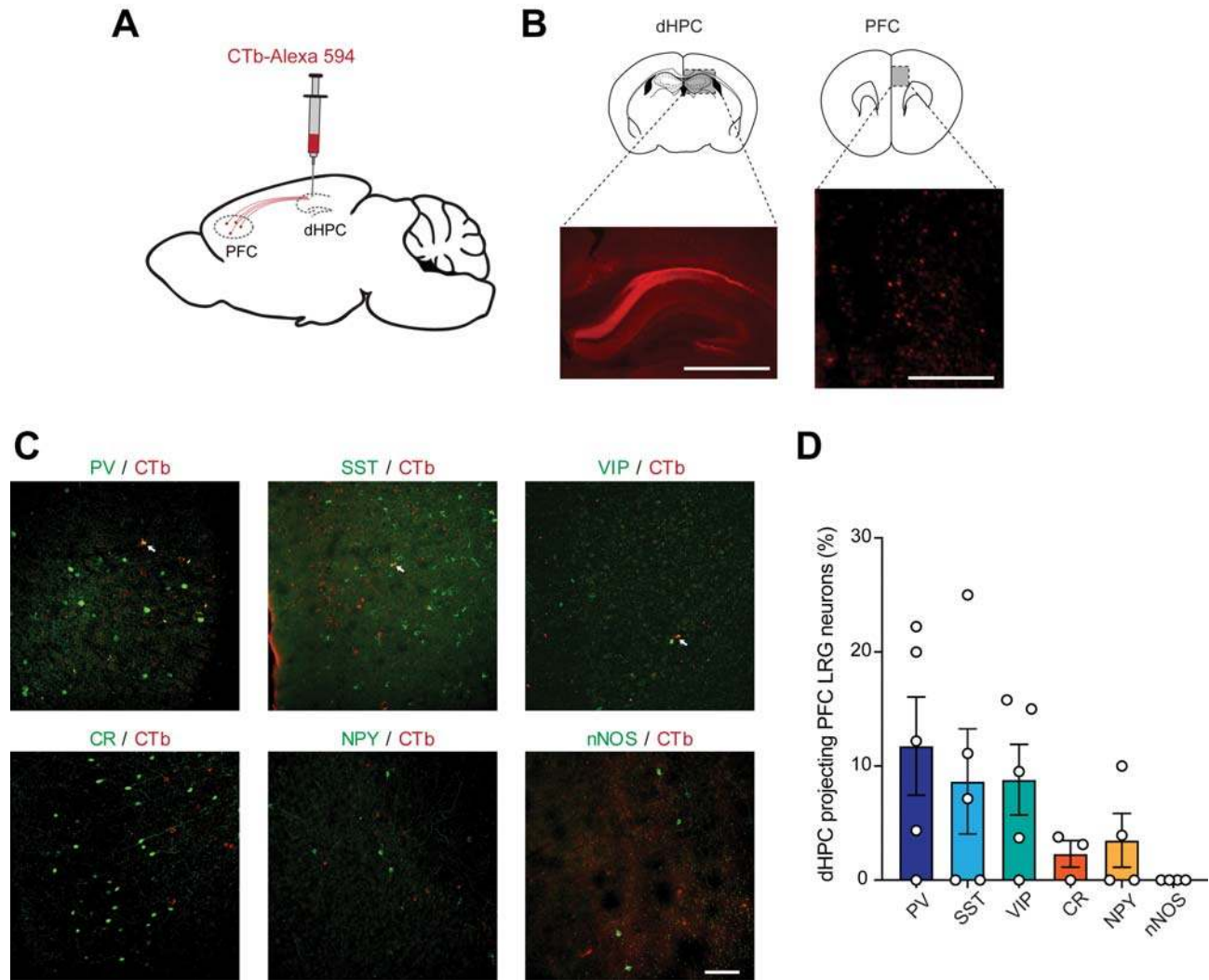
902 **Code availability**

903 Custom code used in this study is available from the corresponding author upon reasonable
904 request.

SUPPLEMENTAL INFORMATION: FIGURES AND LEGENDS



905 **Figure S1, related to Figure 1: Anterograde tracing of HPC projecting PFC LRG neurons.**
906 Left: Schematic illustrating the anterograde tracing strategy. Microinjection of Cre-dependent
907 eYFP virus into the PFC of *Dlx12b-Cre+* mice. Coronal slices of the hippocampus were
908 obtained at increasing anterior-posterior (AP) distance from Bregma. Right: DAPI stained
909 hippocampal sections showing eYFP+ PFC LRG axon terminals (green). Numbers on the right
910 indicate the anterior-posterior (AP) coordinates w.r.t. bregma. Scale bar, 200 μ m.



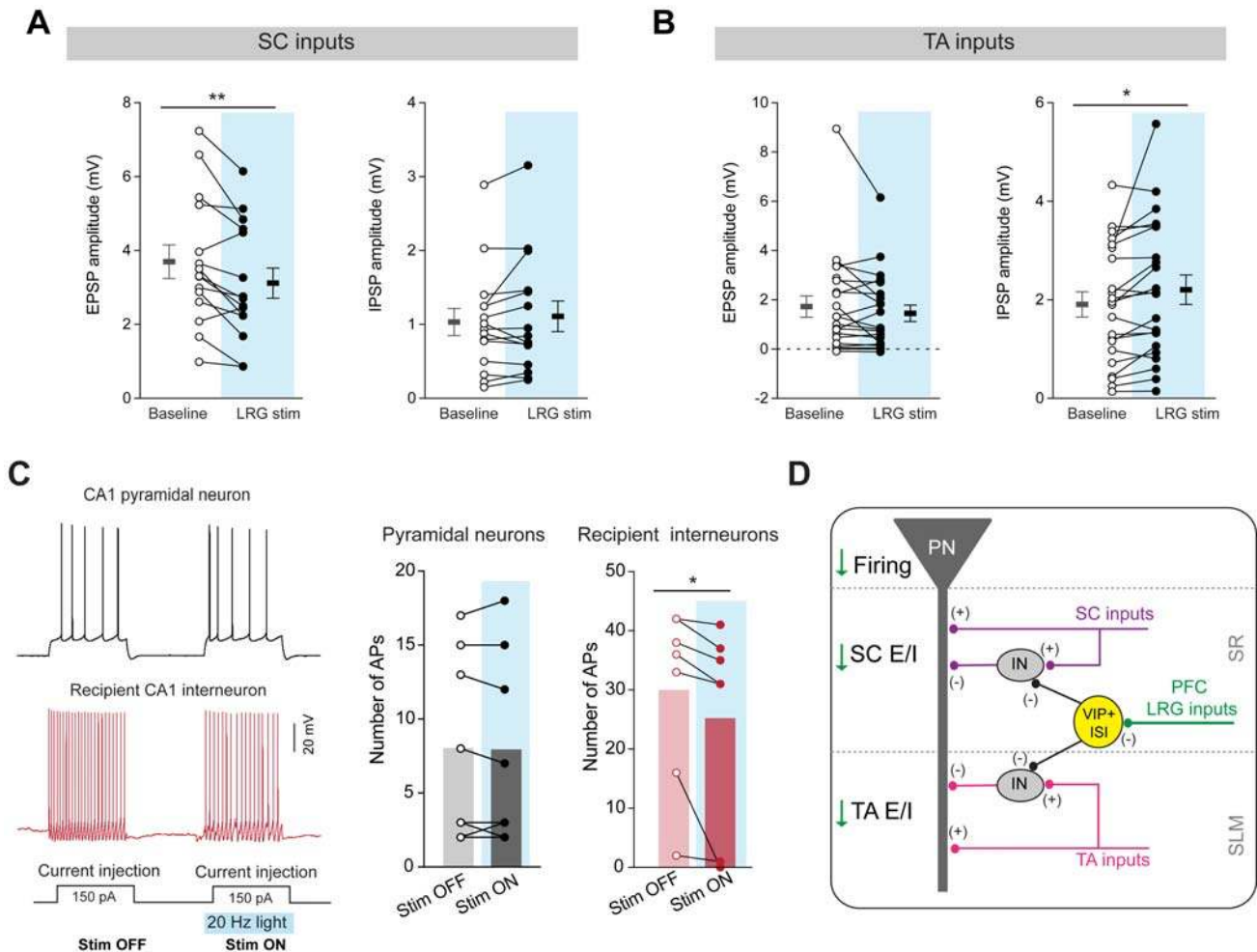
911 **Figure S2, related to Figure 1: Inhibitory neuron marker expression in dHPC projecting**
912 **PFC LRG neurons.**

913 (A) Schematic illustrating the experimental design: Retrograde tracer, Alexa 594 conjugated
914 Cholera Toxin (CTb), was injected into dHPC.

915 (B) Representative images showing Alexa 594-CTb injection site in dHPC section (left) and
916 CTb+ neurons in PFC (right). Note: there is a high density of labeled axons in the corpus
917 callosum. Scale bar, 1mm (left) and 250 μ m (right).

918 (C) Example images showing co-labeling of inhibitory neuron markers (green) in CTb+ dHPC-
919 projecting PFC LRG neurons. Parvalbumin (PV), Somatostatin (SST), Vasoactive intestinal
920 polypeptide (VIP), Calretinin (CR), Neuropeptide Y (NPY), neuronal nitric oxide synthase
921 (nNOS). Scale bar, 100 μ m.

922 (D) Percentage CTb+ dHPC projecting PFC LRG neurons co-expressing various inhibitory
923 neuron markers (mean \pm SEM). Empty circles represent values from individual sections.



924 **Figure S3, related to Figure 2: Dorsal HPC projecting PFC LRG neurons increase afferent**
 925 **input mediated feedforward inhibition in the CA1 microcircuit.**

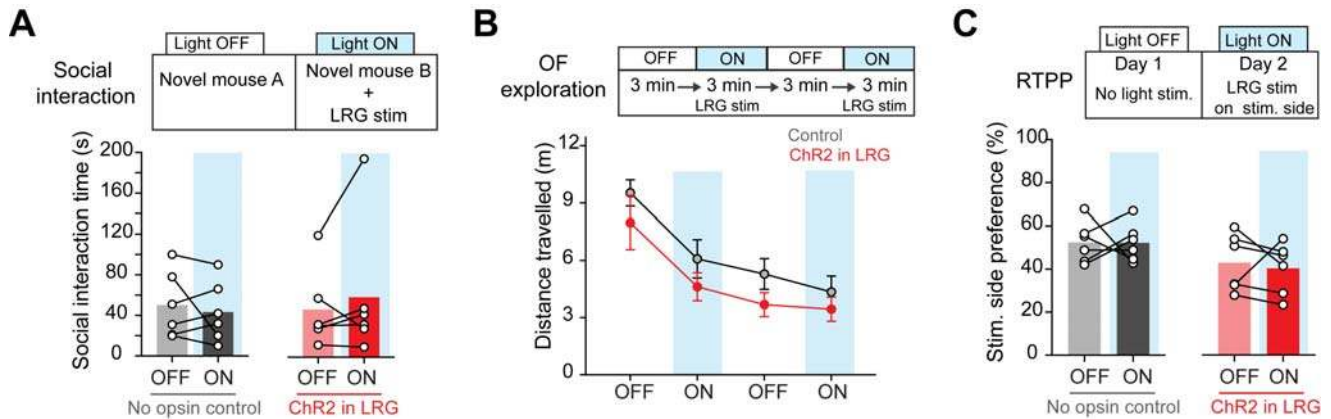
926 (A) Amplitudes of SC (Schaffer collateral) pathway mediated EPSPs (left) and IPSPs (right)
 927 recorded in CA1 pyramidal neurons with (LRG stim) and without (Baseline) concomitant
 928 optogenetic stimulation of PFC LRG projections. Open and filled circles represent individual
 929 neurons (n = 15), horizontal lines represent averages (\pm SEM). Paired two-way t-test, ** p <
 930 0.01.

931 (B) Same as A for TA (temporoammonic) input pathway mediated EPSPs and IPSPs (n = 22).
 932 Paired two-way t-test, * p < 0.05.

933 (C) Left: example voltage traces showing firing in response to depolarizing current injections
 934 with (Stim ON) and without (Stim OFF) concomitant optogenetic stimulation of PFC-dHPC
 935 LRG projections (470 nm, 5 ms pulses at 20 Hz; blue bar). Black trace is an example voltage
 936 response in CA1 pyramidal neuron. Red trace shows example voltage response in recipient CA1
 937 interneuron. Right: firing output in the absence of optogenetic stimulation (Stim OFF) and during

938 concomitant optogenetic stimulation (Stim ON) in CA1 pyramidal neurons (n = 10) and recipient
939 CA1 interneurons (n = 7). Open and filled circles represent individual neurons, bars represent
940 the average values. Two-way paired t-test; * p < 0.05.

941 (E) Schematic illustrating the effects of PFC–dHPC LRG projection activation on microcircuit
942 computation in dorsal CA1. PFC LRG projections inhibit VIP+ disinhibitory interneurons, and
943 thereby increase SC and TA pathway mediated feedforward inhibition onto CA1 pyramidal
944 neurons. The reduction in excitation-inhibition ratio at the afferent inputs reduces the afferent
945 input mediated firing of CA1 pyramidal neurons.

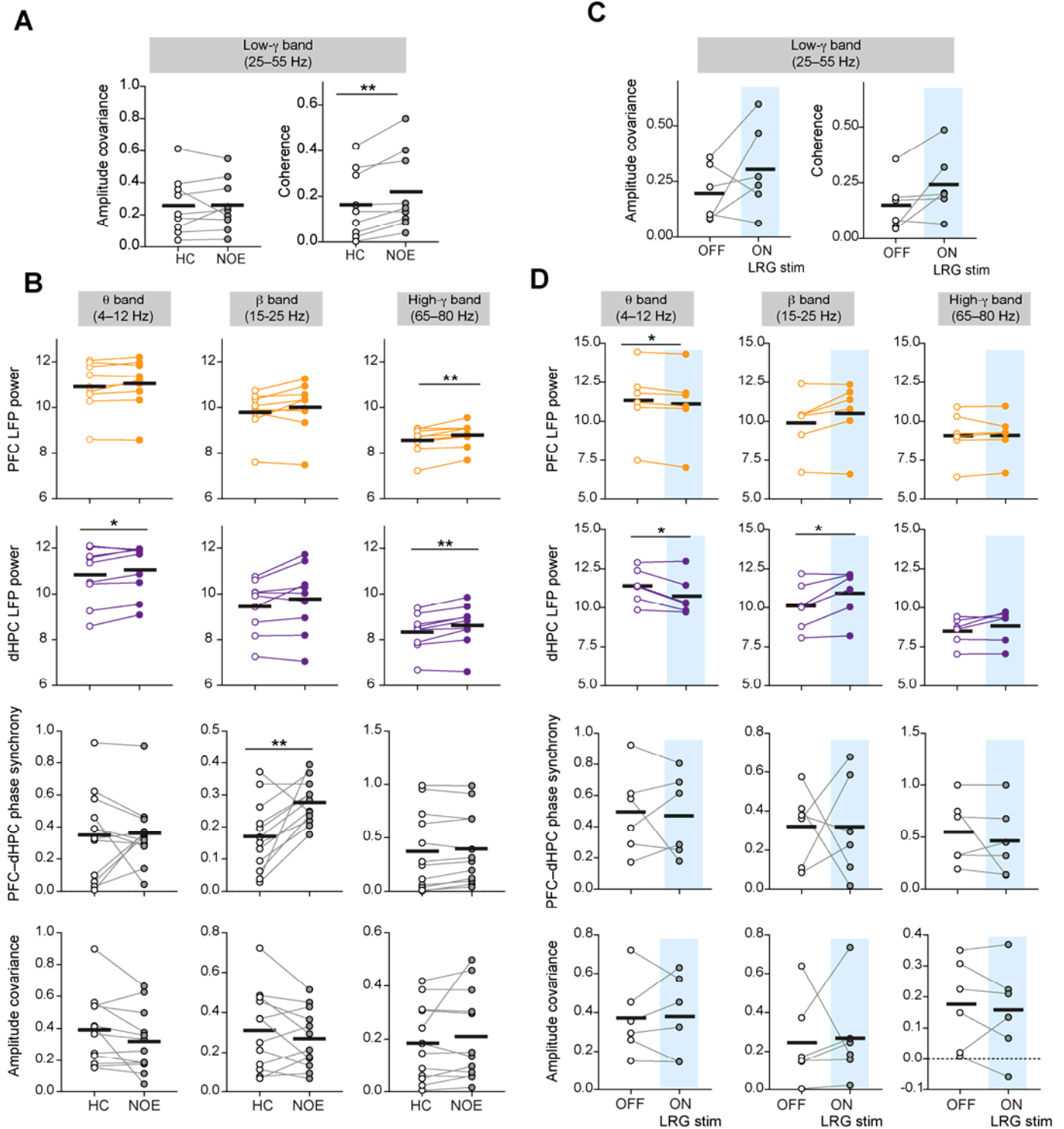


946 **Figure S4 related to Figure 3: Optogenetic stimulation of PFC-dHPC LRG projections**
 947 **during social and exploratory behaviors.**

948 (A) Top: mice were tested for social interaction behavior with and without optogenetic
 949 stimulation. Bottom: Time spent interacting with a novel juvenile mouse is plotted for control
 950 and ChR2-expressing mice during light OFF and light ON periods. Open circles represent data
 951 from individual mice and bars represent the average values.

952 (B) Top: During open-field (OF) exploration, PFC-dHPC LRG projections were optogenetically
 953 stimulated during the second and fourth 3-min epochs of the testing session. Bottom: Average (\pm
 954 SEM) distance travelled during OF exploration during light ON and light OFF epochs is plotted
 955 for control and ChR2-expressing mice.

956 (C) Top: experimental design for real-time place preference (RTPP) test. Bottom: Preference for
 957 the stimulated chamber during light OFF and light ON conditions (expressed as a % of the total
 958 time) is plotted for control and ChR2-expressing mice. Open circles represent data from
 959 individual mice and bars represent the average values.



960 **Figure S5 related to Figure 4: Effect of optogenetic stimulation of PFC-dHPC LRG**
 961 **projections and NOE on oscillatory activity in dHPC and PFC.**

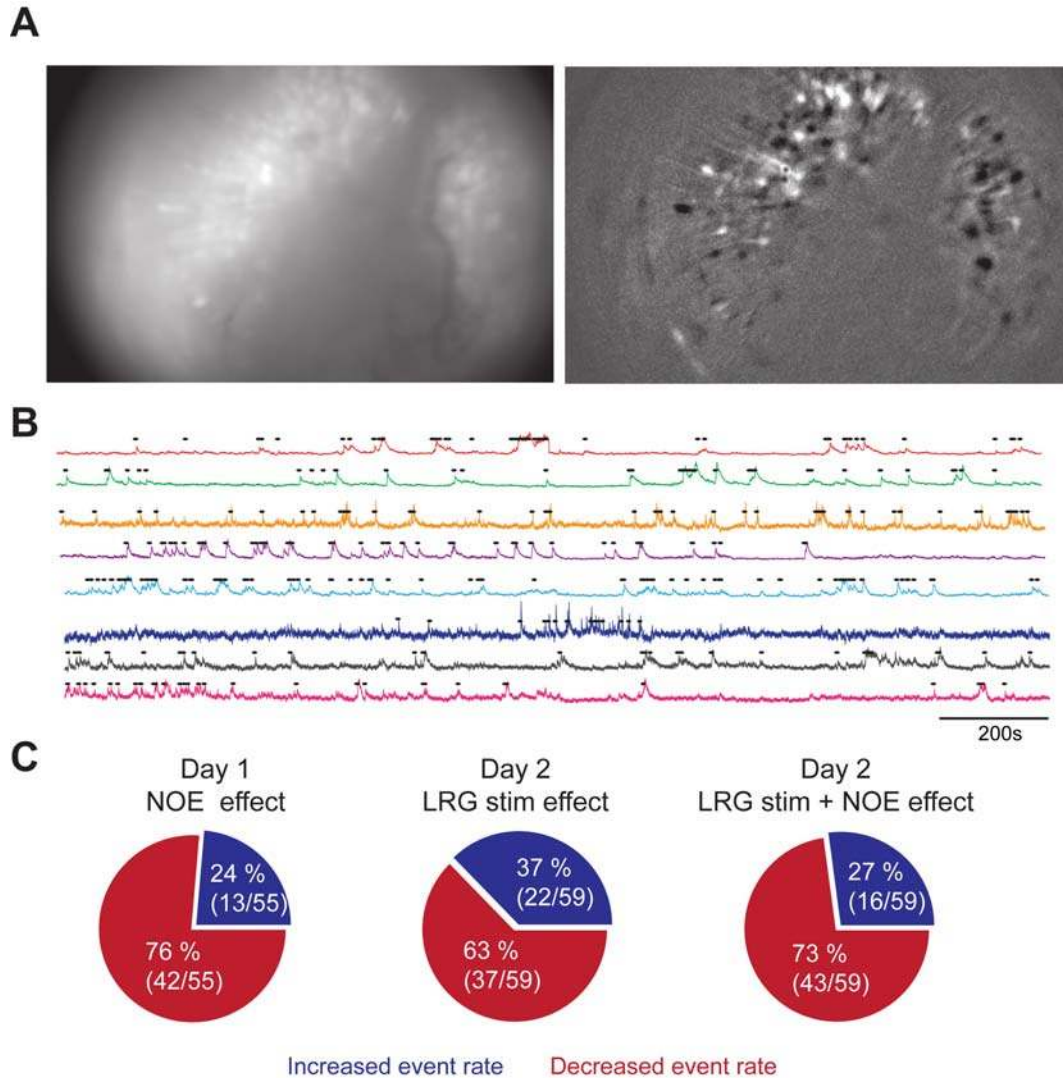
962 (A) Low-gamma (low- γ) amplitude covariance and coherence between PFC and dHPC LFPs
 963 recorded during home cage exploration (HC) and novel object exploration (NOE) epochs. Open

964 and filled circles represent data from individual mice ($n = 9$) and solid black lines represent
965 average values. Two-way paired t-test, $** p < 0.01$.

966 **(B)** PFC and dHPC LFP log power, PFC-dHPC phase synchrony, and PFC-dHPC amplitude
967 covariance for the theta (θ), beta (β) and high gamma (high- γ) frequency bands during HC and
968 NOE epochs are plotted. Open and filled circles represent data from individual mice ($n = 9$) and
969 solid black lines represent average values. Two-way paired t-test; $* p < 0.05$, $**p < 0.01$.

970 **(C)** Low- γ amplitude covariance and coherence between PFC and dHPC LFPs recorded during
971 Light OFF and Light ON epochs (optogenetic stimulation; 473 nm, 5 ms pulses at 20 Hz; blue
972 bars). Open and filled circles represent data from individual mice ($n = 6$) and solid black lines
973 represent average values.

974 **(D)** PFC and dHPC LFP log power, PFC-dHPC phase synchrony, and PFC-dHPC amplitude
975 covariance for the theta (θ), beta (β) and high-gamma (high- γ) frequency bands during Light
976 OFF and Light ON epochs are plotted. Open and filled circles represent data from individual
977 mice ($n = 6$) and solid black lines represent average values. Two-way paired t-test; $* p < 0.05$.



978 **Figure S6, related to Figure 5: *In vivo* Ca²⁺ activity of dorsal CA1 neurons during**
979 **optogenetic stimulation of PFC LRG projections.**

980 (A) Left: Representative raw epifluorescence image showing jRCaMP7f expression in the dorsal
981 CA1 imaged using a miniaturized microscope in a freely behaving mouse. Right: Transformed
982 image showing relative change in fluorescence (dF/F).

983 (B) Representative Ca²⁺ transients (colored lines) recorded from dorsal CA1 neurons. Black dots
984 above the traces represent the detected active Ca²⁺ events.

985 (C) Pie charts showing the fraction of CA1 neurons that decrease or increase activity during
986 NOE on day 1 testing, during LRG stimulation on day 2 testing, or during NOE in the presence
987 of LRG stimulation on day 2 testing.

SUPPLEMENTAL INFORMATION: TABLES

988 **Table S1, related to Figure 1: Electrophysiological heterogeneity of dHPC projecting PFC**
 989 **LRG neurons.**

990 Mean (\pm SEM) values for resting membrane properties, action-potential (AP) properties, and
 991 firing properties of dHPC projecting PFC LRG neurons classified as regular spiking, fast
 992 spiking, and irregular spiking (Fig. 1H). AHP, afterhyperpolarization; SFA, spike frequency
 993 accommodation.

Electrophysiological property	Regular spiking n = 9	Fast spiking n = 4	Irregular spiking n = 3
Resting membrane potential (mV)	-71 ± 1.1	-65.5 ± 2.7	-67.6 ± 1.6
Input Resistance (M Ω)	256.2 ± 25.4	171 ± 47.2	383.3 ± 75.3
Membrane time constant (ms)	15.1 ± 2.3	11.3 ± 2.1	20.9 ± 4.6
Sag ratio	1.01 ± 0.07	1.03 ± 0.02	1.05 ± 0.05
Rebound slope (mV)	-0.04 ± 0.01	-0.04 ± 0.02	-0.4 ± 0.41
Rheobase (pA)	113.8 ± 18.6	162.5 ± 54.5	50 ± 25
AP amplitude (mV)	75.3 ± 4.4	86.5 ± 4	93 ± 2.2
AP rate of rise (mV/s)	308.2 ± 32.2	468.4 ± 67.4	316.8 ± 51.9
AP threshold (mV)	-41.6 ± 1	-50.6 ± 1.7	-47.7 ± 1.2
AP half-width (ms)	0.74 ± 0.07	0.37 ± 0.04	0.84 ± 0.19
Fast AHP (mV)	13.7 ± 0.3	19.1 ± 0.4	13.5 ± 1.2
SFA index	2.5 ± 0.2	1.5 ± 0.1	5.4 ± 0.5
Max. firing frequency (Hz)	46 ± 4.8	117.9 ± 11.5	16.8 ± 1.53

994 **Table S2, related to Figure 2: Electrophysiological properties of dHPC interneurons receiving**
 995 **direct PFC LRG inputs.**

996 Mean (\pm SEM) values for resting membrane properties, action-potential (AP) properties, firing
 997 properties, and optogenetically evoked postsynaptic current (oPSC) amplitudes recorded from recipient
 998 CA1 interneurons classified as regular spiking, fast spiking, and irregular spiking (Fig. 2B).

999 AHP, afterhyperpolarization; SFA, spike frequency accommodation.

Electrophysiological property	Regular spiking n = 25	Fast spiking n = 11	Irregular spiking n = 19
Resting membrane potential (mV)	-65 \pm 2.1	-68.3 \pm 1.3	-66.8 \pm 1.2
Input Resistance (M Ω)	218.2 \pm 18.2	248.2 \pm 27.2	235.4 \pm 21.9
Rheobase (pA)	105 \pm 15.3	77.2 \pm 30.2	100 \pm 15.6
AP amplitude (mV)	84.2 \pm 2.7	83.1 \pm 2.8	85.5 \pm 2.07
AP rate of rise (mV/s)	386.9 \pm 20.9	376.5 \pm 25.9	358 \pm 17.1
AP threshold (mV)	-46.1 \pm 0.8	-47.5 \pm 1.4	-46.9 \pm 1
AP half-width (ms)	0.58 \pm 0.03	0.43 \pm 0.03	0.54 \pm 0.02
Fast AHP (mV)	10.1 \pm 1	16.3 \pm 1.35	13.5 \pm 0.9
SFA index	3.5 \pm 0.3	2.2 \pm 0.26	5.9 \pm 0.7
Max. firing frequency (Hz)	53.7 \pm 2.44	127.5 \pm 8.1	50.4 \pm 8.5
oPSC amplitude (pA)	324.3 \pm 53.2	193.4 \pm 47.3	172.3 \pm 25.3

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