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# Inhibitory interneurons with differential plasticities at their connections tune excitatory–inhibitory balance in the spinal nociceptive system

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1 **Title:**

2 **Inhibitory interneurons with differential plasticities at their connections**

3 **tune excitatory/inhibitory balance in the spinal nociceptive system**

4

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## 22 **Abstract**

23 Networks of the dorsal-horn of the spinal-cord process nociceptive information from the  
24 periphery. In these networks, the excitation/inhibition balance is critical to shape this nociceptive  
25 information and to gate it to the brain where it is interpreted as pain. Our aim was to define  
26 whether short-term plasticity of inhibitory connections could tune this inhibition/excitation  
27 balance by differentially controlling excitatory and inhibitory microcircuits. To this end, we used  
28 spinal-cord slices from adult mice expressing enhanced green fluorescent protein (eGFP) under  
29 the GAD65 promoter and recorded from both eGFP+ (putative inhibitory) and eGFP- (putative  
30 excitatory) neurons of lamina II while stimulating single presynaptic GABAergic interneurons at  
31 various frequencies. Our results indicate that GABAergic neurons of lamina II simultaneously  
32 contact eGFP- and eGFP+ neurons, but these connections display very different frequency-  
33 dependent short-term plasticities. Connections onto eGFP- interneurons displayed limited  
34 frequency-dependent changes, and strong time-dependent summation of inhibitory synaptic  
35 currents that was however subjected to a tonic activity-dependent inhibition involving A1  
36 adenosine receptors. In contrast, GABAergic connections onto eGFP+ interneurons expressed  
37 pronounced frequency-dependent depression, thus favoring disinhibition at these synapses by a  
38 mechanism involving the activation of GABA<sub>B</sub> autoreceptors at low frequency. Interestingly, the  
39 balance favors inhibition at frequencies associated with intense pain whether it favors excitation  
40 at frequencies associated with low pain. Therefore, these target- and frequency-specific  
41 plasticities allow to tune the balance between inhibition and disinhibition while processing  
42 frequency-coded information from primary afferents. These short-term plasticities and their  
43 modulation by A1 and GABA<sub>B</sub> receptors might represent an interesting target in pain-alleviating  
44 strategies.

## 45 **Introduction**

46 Nociceptive information conveyed from the periphery by primary afferents is integrated in the  
47 dorsal-horn of the spinal-cord before being forwarded to the brain where it can lead to pain  
48 perception. Among sensory neurons, C- and A $\delta$ -primary afferents conveying nociceptive  
49 information mostly project to networks within dorsal-horn superficial layers (laminae I-III). In  
50 lamina II (LII), primary afferents synapse with local excitatory or inhibitory interneurons [24; 30;  
51 33; 34]. In turn, LII inhibitory interneurons contact either excitatory or inhibitory interneurons  
52 [24; 33; 34; 44; 54]. Inhibitory interneurons in these networks play a critical role in the processing  
53 of nociceptive information, and alteration of inhibition is associated with  
54 physiological/pathological pain states [23; 39; 48; 50]. LII interneurons are inhibited by both  
55 GABAergic and glycinergic connections but local inhibitory connections between LII neurons are  
56 mostly GABAergic [27; 33].

57 Sensory neurons projecting to these networks encode the intensity of adequate stimuli using an  
58 action-potential frequency code [1; 2; 7; 51]. In other regions of the central nervous system, the  
59 processing of such frequency-coded information is known to involve frequency-selective  
60 synapses [13; 26; 29]. These synapses display short-term plasticity (STP) processes changing  
61 dynamically their efficacy over time as a function of the delay between presynaptic action-  
62 potentials [6; 37]. Therefore, networks in the spinal nociceptive system are also expected to  
63 display short-term plasticity in order to process frequency-coded sensory information.  
64 Surprisingly, this question has been little addressed experimentally, and has mostly used simple  
65 paired-pulse stimulation protocols [8; 20; 24; 43; 53] and very rarely protocols using trains of  
66 stimulations [9; 20; 27; 53].

67 Data on the impacts of stimulation trains in information processing in the dorsal-horn are also  
68 pending to fully understand the mechanisms engaged by the rapidly-expanding stimulation  
69 approaches used in pain alleviation such as spinal-cord stimulation [17; 22]. Together with other  
70 targets, this approach has indeed been suggested to activate inhibitory LII interneurons [11].

71 The objective of the present study was to examine short-term plasticities of GABAergic  
72 connections onto LII neurons, and to determine whether such plasticities were different when  
73 the synapses involved an excitatory or an inhibitory postsynaptic (target) neuron. To this end, we  
74 prepared acute slices from mice expressing enhanced green fluorescent protein (eGFP) under the  
75 control of the GAD65 promoter [10]. We recorded from either eGFP-expressing (eGFP+) or eGFP  
76 negative (eGFP-) neurons, and also performed simultaneous recordings of eGFP+ and eGFP-  
77 neurons while stimulating single presynaptic GABAergic neurons.

78 Our results indicate that GABAergic connections onto eGFP+ neurons of LII display different  
79 short-term plasticities than those onto eGFP- neurons, favoring inhibition at frequencies  
80 occurring during intense nociceptive stimulation and favoring excitation at frequencies occurring  
81 during nociceptive stimulation of low intensity.

82

## 83 **Methods**

### 84 **Animals**

85 For all experiments, we used male heterozygous C57BL/6 BAC transgenic mice eGFP under the  
86 control of the GAD65 promoter obtained from Ferenc Erdelyi and Gabor Szabo (Institute of  
87 Experimental Medicine, Budapest) [10]. In these mice, ~80% of LII eGFP+ neurons are GABA-  
88 immunopositive and ~60% of GABA-immunopositive neurons are eGFP+ [10]. These mice were  
89 interbred at the local animal facility, The Chronobiotron (agreement number: A67-2018-38). The

90 animals were housed at room temperature (22-25°C) with a 12hr light/dark cycle with free access  
91 to food and water. All procedures used were in accordance with laws for laboratory animal  
92 welfare and approved by the local ethical committee of the University of Strasbourg (CREMEAS;  
93 agreement number: APAFIS#8138-2016121008385362 v3).

94

### 95 **Slicing procedure**

96 Adult transgenic mice (5-9 weeks) were anaesthetized with urethane (1.9 g.kg<sup>-1</sup>). Under deep  
97 anesthesia, intracardiac perfusion was performed with oxygenated ice-cold (~4°C) sucrose  
98 artificial cerebrospinal fluid (sACSF) bubbled with carbogen (95% O<sub>2</sub>, 5% CO<sub>2</sub>) containing (in mM):  
99 248 sucrose, 26 NaHCO<sub>3</sub>, 11 glucose, 2 KCl, 2 CaCl<sub>2</sub>, 1.3 MgSO<sub>4</sub>, 1.25 KH<sub>2</sub>PO<sub>4</sub>, 2.5 kynurenic acid.  
100 The lumbar part (L3-L5) of the spinal cord was removed by laminectomy and 400 μM thick  
101 transverse slices were cut with a vibrating microtome (Leica, VT1200S). Slices were kept until  
102 recording at room temperature (~25°C) in oxygenated ACSF containing in mM: 126 NaCl, 2.5 KCl,  
103 2 CaCl<sub>2</sub>, 2 MgCl<sub>2</sub>, 26 NaHCO<sub>3</sub>, 1.25 NaH<sub>2</sub>PO<sub>4</sub>, 10 glucose.

104

### 105 **Patch-clamp recordings**

106 After recovery (~1 h), slices were transferred to the recording chamber, maintained at 32 ± 1°C  
107 and continuously perfused by oxygenated ACSF at 3-4 ml/min. In order to isolate GABAergic  
108 IPSCs, glycinergic and glutamatergic ionotropic transmissions were blocked by adding in ACSF 1  
109 μM strychnine and 10 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) respectively. Whole-cell  
110 patch recordings were made from inhibitory (eGFP+) and putative excitatory (eGFP-) LII neurons.  
111 Recording and extracellular stimulation electrodes (4-7 MΩ) were pulled from borosilicate glass  
112 capillaries (1.2 mm inner diameter, 1.69 mm outer diameter, Warner Instruments, Harvard

113 Apparatus) using a P1000 electrode puller (Sutter Instruments). Recording electrodes were filled  
114 with, in mM: 140 KCl, 2 MgCl, 10 HEPES, 2 MgATP; pH 7.3. In experiments using increased  
115 stimulation amplitudes, 1 mM QX314 was added to this intrapipette solution to prevent spiking  
116 of the recorded neuron. Junction potentials were not corrected. Whole-cell patch clamp  
117 recordings (in current-clamp and voltage-clamp recording modes) were made using a Multiclamp  
118 700A amplifier (Molecular Devices). Signals were low-pass filtered at 5 kHz, sampled at 20 kHz,  
119 digitized using a BNC-2110 data acquisition card (National Instruments) and acquired with the  
120 Strathclyde electrophysiology software (WinWCP, John Dempster, University of Strathclyde,  
121 Glasgow, UK).

122

### 123 **Experimental design**

124 Our recordings started in the current-clamp mode. Holding current was adjusted in order to keep  
125 the recorded neuron at a membrane potential of -60 mV. In this mode, firing patterns were  
126 determined in response to 1 s-long depolarizing current injections through the recording  
127 electrode (20-80 pA in 20 pA steps). For some experiments in the current-clamp mode, we  
128 simulated excitatory synaptic potentials (EPSPs) by injecting EPSCs current traces. These EPSCs  
129 current traces were constructed by averaging excitatory postsynaptic currents evoked by local  
130 electrical extracellular stimulation (EPSCs) recorded in LII neurons (same protocol as for eIPSCs  
131 described below) with 10  $\mu$ M bicuculline and 1  $\mu$ M strychnine. In these conditions, average  
132 eEPSCs was of  $88.9 \pm 10.6$  pA with a rise time of  $2.1 \pm 0.3$  ms and a decay time constant of  $5.7 \pm$   
133  $0.9$  ms ( $n = 4$  neurons). To simulate the convergence of excitatory inputs as suggested by Grudt  
134 & Perl (2002) who recorded C-fibers mediated EPSCs in islet cells of above 400 pA, we injected  
135 the current corresponding to 4 times the average eEPSC we measured.

136 After this initial phase, neurons were recorded in the voltage-clamp mode at a holding potential  
137 of -60 mV. Monosynaptic IPSCs were evoked by local extracellular electrical stimulation. This  
138 stimulation was performed by applying current steps (0.25 ms; 0.10-0.40 mA; average  $0.21 \pm 0.01$   
139 mA) via a patch-pipette filled with ACSF. This stimulation electrode was placed at a distance of  
140 20-150  $\mu\text{m}$  from the cell body of the recorded neuron. For each recorded neuron, the lowest  
141 amplitude of stimulation evoking inhibitory postsynaptic currents was determined. This  
142 amplitude was increased by 0.05 mA to evoke inhibitory postsynaptic currents (eIPSCs) for each  
143 stimulation applied. Synaptic contacts were identified as monosynaptic unitary connections  
144 when the following criteria were satisfied: (1) all-or-none appearance of eIPSCs, (2) absence of  
145 increase in eIPSC amplitude when minimal stimulation amplitude was increased by 0.05 mA, (3)  
146 disappearance of eIPSCs when stimulation polarity was inverted, and (4) constant latency of the  
147 eIPSCs.

148 The same criteria were applied for simultaneous recordings of pairs of eGFP- and eGFP+ neurons  
149 connected by the same presynaptic neuron.

150 For all neurons, paired-pulse stimulations were applied with an inter-stimulation interval (ISI) of  
151 200 ms repeated every 10 s. In a subset of neurons, paired-pulse stimulations with multiple ISIs  
152 were applied (ISIs of 20, 50, 75, 100, 200, 300 ms) and pairs of stimulation were separated by 10  
153 s. Trains of 11 stimulations repeated 10 times were used in most experiments. Unless otherwise  
154 stated, trains applied at 5 Hz were repeated every 20 s whereas trains applied at 50 Hz were  
155 applied every 60 s.

156

157 **Data quantification and analysis**



158 Action-potential firing patterns were analyzed offline using Clampfit 10 (Molecular Devices, USA).  
159 Neurons firing multiple spikes during the whole duration of the depolarizing current step and  
160 displaying a constant interval between each spike were classified as Tonic-firing type (tonic) (Fig.  
161 1A); neurons with a burst of spikes at the beginning of depolarizing current step and showing a  
162 decrease in inter-spike interval duration during the step as well as a progressive reduction of  
163 spike amplitudes were classified as Initial bursting type (IB) (Fig. 1A); neurons fulfilling neither of  
164 these criteria were classified as “Other type” of firing pattern.

165 eIPSCs were also analyzed offline using Clampfit 10. Synaptic inhibitory transmission was  
166 quantified by measuring the amplitude of individual eIPSCs. To take into account baseline  
167 changes due to eIPSCs summation, the amplitude immediately before stimulation was subtracted  
168 from the peak amplitude of each eIPSC. Unless otherwise stated, stimulation protocols were  
169 applied ten times in each neuron and the within-cell average across trials was calculated by  
170 averaging eIPSCs amplitudes from these ten repetitions. When protocols consisted of trains of  
171 stimulations, amplitudes of eIPSCs of the same rank in the train were averaged. These within-cell  
172 average across ten trials were used for statistical analysis (Fig. S1).

173 Averaging amplitudes of raw eIPSCs gives greater weight to connections in which eIPSCs display  
174 larger amplitudes. Therefore, in addition to analyzing raw eIPSCs amplitudes, we calculated  
175 normalized eIPSCs amplitudes. These were calculated by normalizing for each neuron the  
176 averaged eIPSCs amplitudes to the averaged amplitude of the first eIPSCs in the train. This  
177 allowed to analyze relative changes in amplitude during trains regardless of the initial raw  
178 amplitude values of eIPSCs.

179 Both raw and normalized eIPSCs amplitudes were used for statistical analysis.

180 For paired-pulse stimulation experiments, the paired-pulse ratio (PPR) was calculated as the  
181 amplitude of the second eIPSC divided by the amplitude of the first eIPSC.

182 The weighted decay time constant ( $\tau_w$ ) was calculated as described in Labrakakis et al. [28] from  
183 biexponential fits using equation 1.

184 Equation 1.  $\tau_w = (\tau_f \cdot A_f + \tau_s \cdot A_s) / (A_f + A_s)$

185 where  $\tau_f$  and  $\tau_s$  are the fast and slow decay time constants, respectively and  $A_f$  and  $A_s$  the  
186 corresponding amplitudes used as weighting factors.

187 For experiments with GABA<sub>B</sub> and A1 antagonists, trains of stimulations were repeated before,  
188 during and after perfusion of the antagonists. Ten trains were applied in control conditions and  
189 the antagonist was subsequently applied for 10 minutes. The effect of antagonists was measured  
190 after at least 3 minutes of perfusion. For each neuron, amplitudes of eIPSCs of the same rank in  
191 the two conditions (i.e. control vs. antagonist) were averaged. Results are expressed as mean  $\pm$   
192 SEM.

193 For some experiments, we recorded miniature IPSCs. These synaptic events were detected using  
194 WinEDR (V3.8.6, Strathclyde Electrophysiology Software, John Dempster, University of  
195 Strathclyde, Glasgow, UK) with an amplitude threshold detection algorithm and were visually  
196 inspected for validity. Peak amplitude of miniature IPSCs was determined using WinWCP (version  
197 5.4.5). Events were detected during at least 200 s.

198

## 199 **Statistics**

200 Statistical analysis were performed using averaged eIPSCs amplitudes (i.e. within-cell average  
201 across trials) of each neurons (Fig. S1). These were calculated for each neuron by averaging

202 amplitudes of eIPSCs of the same rank (2 ranks for paired-pulse experiments, 11 ranks for trains  
203 of stimulations). The number of neurons is given as the n-value in the result section.

204 Student's *t*-test was used to compare amplitudes of isolated eIPSCs, decay time constants and  
205 mIPSCs after having tested for data normality (Kolmogorov-Smirnov test,  $p > 0.05$ ).

206 For paired-pulse protocols, the proportions of neurons displaying a facilitation ( $PPR > 1.1$ ), a  
207 depression ( $PPR < 0.9$ ) or no change in PPR ( $0.9-1.1$ ) were analyzed with a log-linear analysis  
208 performed on the 3-way contingency table of the proportion of facilitation, ISI and eGFP+/eGFP-  
209 condition using Statistica 13 (StatSoft, USA). The  $\chi^2$  values and p-values given in the text  
210 correspond to those of marginal associations between the two parameters examined. To  
211 compare amplitudes of the two first eIPSCs during stimulation trains protocols, Wilcoxon Signed  
212 Rank Test for Paired Data was used since data were not always normally distributed.

213 Non-linear regression analysis [38] was used to analyze PPR values as a function of ISIs and  
214 changes in eIPSCs amplitudes during stimulation train protocols. Individual data of all neurons  
215 were always used for curve fitting and average values were used for illustration (Fig. S1).

216 PPR values as a function of ISIs for eGFP+ and eGFP- neurons were fitted with the equation 2.

217 Equation 2.  $Y = A1 + ((A1 - A2) * \text{EXP}(-X/A3))$

218 where A1 corresponded to Y value for ISI=0; A2 corresponded to the asymptotic value of the fit;  
219 and A3 corresponded to the exponential decay constant of PPR value as a function of ISI.

220 Parameters A1, A2 and A3 were determined by nonlinear curve fitting using KyPlot 6.0 (KyensLab,  
221 Tokyo, Japan). A global fit on the same data was also performed using the same equation. This  
222 global fit was compared to the sum of fits from separated data obtained from eGFP+ and from  
223 eGFP- neurons.

224 Non-linear curve fitting was used to analyze and compare changes in eIPSCs amplitudes during  
225 stimulation train protocols. This analysis was performed using averaged eIPSCs amplitudes of  
226 each neurons.

227 Amplitudes of eIPSCs as a function of their respective rank in the train were fitted using the  
228 equation 3.

229 Equation 3.  $Y=A1+A2*(1-EXP(-X/A3))$

230 where A1 corresponded to Y value of the 1<sup>st</sup> eIPSCs of the train, A2 corresponded to the change  
231 in amplitude for the curve asymptote, A3 corresponded to the constant of the exponential  
232 function.

233 A1 and A2 are in pA for fits of raw eIPSCs amplitudes and are dimensionless for fits of normalized  
234 eIPSCs amplitudes. A3 unit is the rank of stimulation in the train.

235 To define whether STP was expressed during trains of stimulations, i.e. whether significant  
236 changes in amplitude occurred during the train, fits with Equation 3 were compared with linear  
237 fits with slope values forced to 0. When fits with Equation 3 provided statistically significant  
238 improvements with respect to the linear fit with slope value forced to 0, the connections were  
239 considered as displaying a significant STP during the corresponding protocol [38].

240 To compare two different conditions (e.g. eGFP- vs. eGFP+ or presence/absence of antagonists)  
241 data from each conditions were fitted with Equation 3, either individually (sum of two functions)  
242 or pooled (one single function). When the sum of two individual fits provided statistically  
243 significant improvements with respect to the fit of pooled data, the two conditions were  
244 considered as having distinct effects [38]. Fits of pooled data are illustrated (in black) when the  
245 two conditions were not different whereas individual fits are illustrated (in color) when the two

246 conditions were significantly different. For illustration purpose, these fits are illustrated together  
247 with the averages of “cross-trial averages”.

248 In the result section, p-value of model comparisons are given as well as the corresponding  
249 number of neurons (n). The significance level used for all analysis was set at 0.05. The goodness-  
250 of-fit was also compared by calculating for both conditions Bayesian Information Criterion (BIC),  
251 Akaike Information Criterion (AIC), and the corresponding sample-size bias-corrected values  
252 (AICc) [47]. A model was considered as better if model comparison p-value was < 0.05 and if the  
253 model provided a reduction in AICc. The only case where the p-value and AICc are in conflict (p <  
254 0.05 and increase in AICc for the effect of DPCPX on eGFP+ neurons) is mentioned in the result  
255 section. Details of all models (A1, A2, A3, degree of freedom, Residual sum of squares, AIC, AICc,  
256 BIC) as well as details of model comparison (F, P, differences in AIC, AICc and BIC) are given in  
257 Supplementary tables 1-7.

258

### 259 **Drug application / Pharmacology**

260 All pharmacological agents were bath-applied. 1  $\mu$ M strychnine and 10  $\mu$ M CNQX were present  
261 in all our electrophysiological experiments to isolate GABAergic eIPSCs. CGP55845 ((2S)-3-[[[(1S)-  
262 1-(3,4-Dichlorophenyl)ethyl]amino-2hydroxypropyl]](phenylmethyl)phosphinic acid  
263 hydrochloride; 10  $\mu$ M, Tocris) and DPCPX (8-Cyclopentyl-1,3-dipropylxanthine solid; 10  $\mu$ M,  
264 Sigma) were used to block GABA<sub>B</sub> receptors and Adenosine A1 receptors, respectively. In a subset  
265 of experiments, eEPSCs were recorded in presence of strychnine (1  $\mu$ M) and bicuculline (10  $\mu$ M,  
266 sigma). Miniature IPSCs were recorded in the presence of tetrodotoxin (TTX) (0.5 $\mu$ M, Latoxan).  
267 CNQX and CGP55845 were dissolved in DMSO, DPCPX was dissolved in ethanol and strychnine in  
268 water; all were prepared as x10000 concentrated stock solutions. For experiments with these

269 antagonists, analysis of changes in normalized eIPSCs amplitudes allowed to detect modulations  
270 engaged during the train (phasic modulation). All substances were diluted to their final  
271 concentration in ACSF at the beginning of each experiment.

272

## 273 **Results**

274

### 275 **Firing properties of eGFP+ and eGFP- neurons**

276 LII neurons responded to sustained depolarizing current injections with various patterns of  
277 action-potential firing [19; 24; 34]. Two main firing patterns dominated (Fig. 1AB, S2): To type (40  
278 %, 51/128) and IB type (34 %, 43/128). Other firing patterns were less frequently observed  
279 (altogether 27 %, 34/128). Most of eGFP- neurons displayed an IB firing pattern (46 %, 28/61),  
280 and a lower proportion of Tonic (36 %, 11/61) and other firing patterns (18 %, 22/61). Most of  
281 eGFP+ neurons displayed a Tonic firing pattern (60 %, 40/67), and a lower proportion of IB (22%,  
282 15/67) and other firing patterns (18 %, 12/67). The proportions of IB, Tonic and other firing  
283 patterns in eGFP- and eGFP+ neurons were significantly different (Fisher's exact test,  $P = 6.3 \cdot 10^{-6}$ ,  
284 Fig. 1B). The average firing frequency of both eGFP- IB neurons and eGFP+ Tonic neurons was  
285 above 50 Hz during the first 200 ms of 80 pA current steps ( $95.1 \pm 11.9$  Hz and  $52.4 \pm 5.7$  Hz,  
286 respectively, Fig. 1C). When EPSPs were simulated by injection of EPSCs at 50 Hz during 200 ms,  
287 the proportion of EPSPs triggering an action-potential was of  $79.1 \pm 13.0$  % in eGFP- neurons ( $n$   
288 = 7) and of  $100.0 \pm 13.0$  % in eGFP+ neurons which occasionally discharge more than one action  
289 potential per simulated EPSP ( $n = 4$ ; Fig. 1DE). These data indicate that neurons of LII are able to  
290 discharge action-potentials at several tens of hertz, at least during few hundreds of milliseconds.

291

## 292 **GABAergic synaptic transmission in LII**

293 Average amplitude of pharmacologically-isolated eIPSCs (Fig. 2A) was not significantly different  
294 when recorded in eGFP- neurons ( $-60.5 \pm 5.1$  pA;  $n = 54$ ) or in eGFP+ neurons ( $-55.6 \pm 4.4$  pA;  $n =$   
295 49); unpaired Student's *t*-test,  $t = -0.709$ ,  $df = 101$ ,  $P = 0.48$ . eIPSCs were evoked at similar  
296 stimulation amplitude intensity in eGFP- neurons ( $0.21 \pm 0.01$  mA;  $n = 53$ ) or in eGFP+  
297 ( $0.21 \pm 0.01$  mA;  $n = 45$ ), unpaired Student's *t*-test,  $t = 0.176$ ,  $df = 96$ ,  $P = 0.86$ .

298 The weighted decay time constant ( $\tau_w$ ) measured for isolated eIPSCs (Fig. 2B) was not  
299 significantly different when recorded in eGFP- neurons ( $32.8 \pm 2.8$  ms;  $n = 29$ ) or in eGFP+ neurons  
300 ( $31.1 \pm 4.5$  ms,  $n = 18$ ); unpaired Student's *t*-test,  $t = -0.340$ ,  $df = 45$ ,  $P = 0.736$ .

301 In the same preparation, amplitude of miniature IPSCs (mIPSCs, Fig. 2C) was not significantly  
302 different when recorded in eGFP- neurons ( $-16.7 \pm 0.9$  pA;  $n = 9$ ) or in eGFP+ neurons ( $-17.8 \pm 1.2$   
303 pA;  $n = 22$ ) unpaired Student's *t*-test,  $t = 0.526$ ,  $df = 29$ ,  $P = 0.603$ . Therefore, eIPSCs amplitude  
304 represented on average 3 times the amplitude of mIPSCs.

305

## 306 **Postsynaptic target-specific paired-pulse plasticity at GABAergic connections in LII**

307 Short-term synaptic plasticity was first examined using paired-pulse stimulation protocols with  
308 various ISIs.

309 Inhibitory connections onto eGFP+ or eGFP- neurons can either display paired-pulse facilitation  
310 ( $PPR > 1.1$ ), paired-pulse depression ( $PPR < 0.9$ ) or no change in paired-pulse ratio ( $PPR: 0.9-1.1$ ).

311 Proportions of neurons in each category were significantly different in eGFP- and eGFP+ neurons  
312 (Fig. 2D;  $\chi^2 = 11.97$ ;  $P = 0.003$ ), with a larger proportion of paired-pulse facilitation in eGFP-  
313 neurons.

314 Values of PPR at various ISIs were significantly different in eGFP+ and eGFP- neurons (non-linear  
315 curve fitting,  $P = 7.2 \cdot 10^{-3}$ , Table S1, Fig. 2E). In eGFP+ neurons, average PPR values were close to  
316 1 at all tested ISIs whereas in eGFP- neurons, average PPR values were above 1.1 for ISIs below  
317 100 ms and progressively decreased at longer ISIs.

318 These data indicate that GABAergic connections onto eGFP- neurons preferentially displayed  
319 facilitation at short ISIs, and that GABAergic connections onto eGFP+ neurons preferentially  
320 displayed depression at long ISIs. Although PPR revealed differences between connections onto  
321 eGFP+ and eGFP- neurons, incoming information from the periphery is usually under the form of  
322 trains of action-potentials rather than isolated pairs of impulses. In the following experiments we  
323 therefore examined the effect of trains of stimulations.

324

### 325 **Short-term plasticity during 5 Hz activation of GABAergic connections in LII.**

326 We examined the short-term synaptic plasticity expressed by GABAergic connections onto LII  
327 neurons during their repeated activation at low frequency. We applied trains of 11 stimulations  
328 at 5 Hz (Fig. 3A). These trains were repeated 10 times at an interval of 20 s. For these  
329 experiments, 54 eGFP- and 49 eGFP+ neurons were recorded (averages from individual neurons,  
330 Fig. S4A-D, Table S2).

331 Evolution of eIPSCs amplitudes during 5 Hz trains remained similar in the first five and the last  
332 five trains for both eGFP- and eGFP+ neurons, indicating that no train-to-train plasticity was  
333 engaged under these experimental conditions ( $P = 0.889$ , eGFP-;  $P = 0.312$ , eGFP+, Table S2,  
334 Fig. 3). Moreover,  $eIPSC2/eIPSC1$  remained unchanged in the first five and the last five trains for  
335 connections on both eGFP- and eGFP+ neurons ( $P_{eGFP-} = 0.678$ ,  $P_{eGFP+} = 0.223$ , Wilcoxon Signed



336 Rank Test for Paired Data, Fig. S6). Therefore, for each neuron, amplitudes of eIPSCs of the same  
337 rank in all 10 trains were averaged.

338 Average amplitude of the first eIPSCs of 5 Hz trains was not significantly different when recorded  
339 in eGFP- or eGFP+ neurons ( $P = 0.349$ ). However, evolution of normalized eIPSCs amplitudes  
340 during 5 Hz trains was significantly different in eGFP- or eGFP+ neurons ( $P = 1.94 \cdot 10^{-14}$ , Table S2,  
341 Fig. 3B). Such significant difference between eGFP- and eGFP+ neurons was also observed in  
342 experiments where stimulation amplitudes were increased from 0.20 mA to 0.45 mA (11 eGFP-  
343 neurons and 11 eGFP+ neurons,  $P_{\text{normalized}} = 8.70 \cdot 10^{-15}$ , Table S3, Fig. S5) indicating that changes  
344 in stimulation efficacy during trains of stimulations were unlikely to influence our results.

345 In eGFP- neurons, amplitudes of normalized eIPSCs did not significantly change (Fig. 3B, S3A)  
346 indicating that no plasticity was expressed during the train ( $P_{\text{normalized}} = 0.451$ , Table S2).  
347 Contrasting with these data, the amplitude of normalized eIPSCs recorded from in eGFP+ neurons  
348 significantly decreased during the train ( $P_{\text{normalized}} = 2.04 \cdot 10^{-6}$ , Fig. 3B) decreasing by 20% at the  
349 asymptote (see parameters of the curve fitting Table S2). Similar data were obtained for raw  
350 eIPSCs amplitudes (Fig. S3A, Table S2).

351 These data indicated that on average, GABAergic connections onto eGFP- neurons displayed no  
352 plasticity during 5 Hz trains, whereas connections onto eGFP+ displayed strong depression.

353

#### 354 **Short-term plasticity during 50 Hz activation of GABAergic connections in LII.**

355 We examined the short-term synaptic plasticity expressed by GABAergic connections onto LII  
356 neurons during their repetitive activation at high frequency ( $n = 27$  eGFP- neurons and  $n = 19$   
357 eGFP+ neurons). We applied trains of 11 stimulations at 50 Hz (Fig. 4A). These trains were  
358 repeated 10 times (averages from individual neurons Fig. S1E-H). In a first set of experiments,

359 trains were repeated with long inter-train intervals of 60 s in order to limit the development of  
360 train-to-train plasticities. In these conditions, evolution of eIPSCs amplitudes during 50 Hz trains  
361 remained similar in the first five and the last five trains for both eGFP- and eGFP+ neurons ( $P_{\text{eGFP-}}$   
362 = 0.278;  $P_{\text{eGFP+}} = 0.391$ , Fig. 4). This indicates, that no train-to-train plasticity was engaged under  
363 these experimental conditions allowing to average the traces of the 10 trains for each neurons.  
364 Nevertheless, whereas the plasticity remained similar in the first five and the last five trains,  
365 eIPSC2/eIPSC1 significantly decreased for connections onto eGFP- neurons but not onto eGFP+  
366 neurons ( $P_{\text{eGFP-}} = 0.048$ ,  $P_{\text{eGFP+}} = 0.098$ , Wilcoxon Signed Rank Test for Paired Data, Fig. S6)  
367 suggesting that the facilitation observed in eGFP- neurons during paired-pulse stimulation at  
368 short intervals (Fig. 2E) is replaced by other types of plasticities during repeated trains of  
369 stimulation at 50 Hz.

370 Evolution of eIPSCs amplitudes during 50 Hz trains was significantly different in eGFP- or eGFP+  
371 neurons for both normalized amplitudes ( $P = 1.88 \cdot 10^{-9}$ , Table S4, Fig. 4B) and raw amplitudes ( $P$   
372 =  $3.73 \cdot 10^{-8}$ , Table S4, Fig. S3C). In eGFP- neurons, amplitudes of normalized eIPSCs did not  
373 significantly change indicating that no plasticity was expressed during the train ( $P_{\text{normalized}} = 0.647$ ,  
374 Table S1.4). By contrast, the amplitude of normalized eIPSCs recorded in eGFP+ neurons  
375 significantly decreased during the train ( $P_{\text{normalized}} = 9.03 \cdot 10^{-6}$ , Table S4, Fig. 4B), decreasing by 28  
376 % at the asymptote (see parameters of the curve fitting, Table S4). Similar data were obtained  
377 for raw eIPSCs amplitudes (Fig. S3C, Table S4).

378 These data indicated that on average, GABAergic connections onto eGFP- neurons displayed no  
379 plasticity during 50 Hz trains whereas connections onto eGFP+ strongly depressed.

380

381 **Comparison of plasticities at 5 Hz and 50 Hz**

382 The evolution of normalized eIPSCs amplitudes during 5 Hz and 50 Hz trains was significantly  
383 different for both eGFP- neurons ( $P = 5.23 \cdot 10^{-3}$ , Table S5, Fig. 3B, 4B) and eGFP+ neurons ( $P =$   
384  $2.76 \cdot 10^{-10}$ , Table S5, Fig. 3B, 4B) indicating that the characteristics of the effects depended on the  
385 frequency (see parameters of the curve fitting, Table S5). In eGFP+ neurons, the decrease of  
386 normalized eIPSCs during 5 Hz and 50 Hz differed in both the magnitude and the kinetic of the  
387 depression. The decrease of normalized amplitudes at the asymptote was of 20 % with 5 Hz  
388 protocols whereas it was of 28 % with 50 Hz protocols. In these neurons, 90 % of asymptotic  
389 values were reached at the 11<sup>th</sup> stimulation with 5 Hz protocols and before the 5<sup>th</sup> stimulation  
390 with 50 Hz protocols.

391 These data indicate that plasticities expressed by GABAergic connections depended on both the  
392 neurochemical identity of the postsynaptic target neuron and the frequency of electrical activity  
393 of the presynaptic GABAergic neuron.

394

#### 395 **Summation of eIPSCs during trains at 50 Hz**

396 During 50 Hz trains, the eIPSCs had not fully returned to baseline at the onset of the following  
397 stimulation, a situation that resulted in their summation. We therefore reanalyzed our data  
398 measuring summed eIPSCs amplitudes from the basal holding current of each train (Fig. 4C, S3E;  
399 statistics Table S4). Evolution of summed eIPSCs amplitudes during 50 Hz trains was significantly  
400 different in eGFP- or eGFP+ neurons for both normalized amplitudes ( $P = 4.90 \cdot 10^{-7}$ , Fig. 4C) and  
401 raw amplitudes ( $P = 4.12 \cdot 10^{-8}$ , Fig. S3E).

402 Amplitudes of summated eIPSCs significantly increased during 50 Hz trains in eGFP- neurons  
403 ( $P_{\text{normalized}} = 1.15 \cdot 10^{-5}$ , Fig. 3E;  $P_{\text{raw}} = 3.98 \cdot 10^{-10}$ , Fig. S3E). At the asymptote, the increase in raw  
404 amplitude was of 123.33 pA and represented an increase by 2.29 folds of the normalized

405 amplitudes. Importantly, only 86.5 % of the asymptotic value was reached at the end of the train  
406 (i.e. 11<sup>th</sup> eIPSC) suggesting that further summation was still possible with longer lasting trains. In  
407 eGFP+ neurons, no significant increase was detected for normalized summed eIPSCs ( $P_{\text{normalized}} =$   
408 0.076, Fig. 4C) but raw summated eIPSCs significantly increased during 50 Hz trains ( $P_{\text{raw}} =$   
409  $0.67 \cdot 10^{-3}$ , Fig. S3E). In these neurons, the increase in raw summed amplitude was of 44.47 pA and  
410 95 % of the asymptotic value was reached before the 6<sup>th</sup> eIPSC indicating that maximal  
411 summation is reached with few eIPSCs.

412 This difference between eGFP- and eGFP+ neurons in eIPSCs-summation during 50 Hz trains was  
413 not caused by differences in deactivation kinetics of isolated eIPSCs since these were similar in  
414 eGFP- and eGFP+ neurons (see above).

415 These data indicated that GABAergic connections onto eGFP- and eGFP+ neurons displayed  
416 different characteristics of short-term plasticities under high frequency activity: connections onto  
417 eGFP+ neurons showed strong depression and limited summation whereas connections onto  
418 eGFP- neurons showed no depression and large summation.

419

#### 420 **Distinct short-term plasticity in simultaneously recorded eGFP- and eGFP+ neurons.**

421 We next defined whether connections from a given presynaptic neuron displayed distinct short-  
422 term plasticities when the postsynaptic neuron was eGFP- or eGFP+. To this end, we performed  
423 simultaneous recordings of eGFP- and eGFP+ neurons and searched for a presynaptic neuron  
424 connecting the recorded ones. For each pair of neuron, we subtracted the normalized eIPSCs  
425 amplitudes recorded on eGFP+ neurons from those recorded on eGFP- neurons (Fig. 5).

426 For both 5 Hz and 50 Hz trains, the relative eIPSCs amplitude difference was significantly larger  
427 than zero ( $P_{5\text{Hz}} = 1.26 \cdot 10^{-5}$ ,  $n = 6$  pairs;  $P_{50\text{Hz}} = 6.21 \cdot 10^{-6}$ ,  $n = 4$  pairs, Table S7), indicating that

428 eIPSCs recorded in eGFP+ neurons depressed relatively to eIPSCs simultaneously recorded in  
429 eGFP- neurons.

430 These data indicated that synapses involving the same presynaptic neuron expressed  
431 postsynaptic-target specific plasticities.

432

### 433 **Target-specific short-term plasticity involving GABA<sub>B</sub> receptors**

434 We next assessed whether the short-term plasticity of GABAergic connections onto LII neurons  
435 may involve GABA<sub>B</sub> receptors activated by synaptically released GABA (Fig. 6, S7, statistics Table  
436 S8). The protocols of 10 trains of stimulations used in the previous experiments were applied  
437 twice, i.e. before and during bath application of 10 μM CGP55845, a GABA<sub>B</sub> receptors antagonist.  
438 First, trains at 5 Hz were repeated every 20 s whereas trains at 50 Hz were repeated every 60 s.  
439 When evolution of raw eIPSCs amplitudes was considered, no significant effects of CGP55845  
440 were detected during trains at 5 and 50 Hz in neither eGFP- nor eGFP+ neurons ( $P_{\text{eGFP}^-, 5 \text{ Hz}} = 0.892$ ,  
441  $n = 6$ ;  $P_{\text{eGFP}^-, 50 \text{ Hz}} = 0.571$ ,  $n = 12$ ;  $P_{\text{eGFP}^+, 5 \text{ Hz}} = 0.881$ ,  $n = 8$ ;  $P_{\text{eGFP}^+, 50 \text{ Hz}} = 0.448$ ; Fig. S7B, E, H, K, N),  
442 indicating that GABA<sub>B</sub> receptors were not tonically controlling these connections under these  
443 conditions.

444 Since GABA<sub>B</sub> receptors may be activated in a phasic manner by the GABA released during the  
445 train of activity, we also analyzed effects of CGP55845 on eIPSCs amplitudes normalized to the  
446 firsts eIPSCs of the control trains (Fig. 6, S7, Table S8). Normalized eIPSCs recorded in eGFP-  
447 neurons were not modified in presence of CGP55845 during both trains at 5 Hz and at 50 Hz  
448 ( $P_{\text{eGFP}^-, 5 \text{ Hz}} = 0.900$ ,  $n = 6$ ;  $P_{\text{eGFP}^-, 50 \text{ Hz}} = 0.232$ ,  $n = 12$ , Fig. 6A, B). Interestingly, in presence of  
449 CGP55845, amplitudes of normalized eIPSCs recorded in eGFP+ neurons during trains at 5 Hz  
450 were significantly increased by 38 % with respect to control trains ( $P = P_{\text{eGFP}^+, 5 \text{ Hz}} = 1.11 \cdot 10^{-10}$ ,  $n =$

451 8, Fig. 6C). A weak effect of CGP55845 was observed during trains at 50 Hz ( $P_{eGFP+, 50\text{ Hz}} = 6.05 \cdot 10^{-3}$ , n = 6, Fig. 6D).

453 Since trains at 5 Hz were repeated every 20 s and trains at 50 Hz every 60 s, the longer delay for  
454 50 Hz trains may be involved in the lack of CGP55845 effect at this frequency.

455 Nevertheless, CGP55845 had no effect when trains at 50 Hz were repeated every 20 s ( $P = 0.330$ ;  
456 n = 7, Fig. 6E) indicating that the effect of CGP55845 was not depending on the delay between  
457 trains.

458 Importantly, during trains at 50 Hz repeated every 20s, control eIPSCs amplitudes were larger  
459 than during protocols repeated every 60 s ( $P = 2.75 \cdot 10^{-9}$ ), and similar to amplitudes recorded in  
460 presence of CGP55845 during trains at 5 Hz repeated every 20 s ( $P = 0.380$ ). This suggested that  
461 facilitating mechanisms engaged at 50 Hz might have overcome an inhibition by GABA<sub>B</sub> receptors  
462 (compare amplitudes Fig. 6D and 6E).

463 These results indicated that under our experimental conditions, GABA<sub>B</sub> receptors are involved in  
464 a phasic modulation of GABAergic connections between LII neurons. This involvement of GABA<sub>B</sub>  
465 receptors is target-specific, it only occurs at synapses between two GABAergic neurons and at  
466 low stimulation frequency (i.e. at 5 Hz).

467

#### 468 **Target-specific and frequency dependent tonic inhibition involving adenosine A1 receptors**

469 Since synaptic vesicles contain ATP which can be rapidly hydrolyzed into adenosine by  
470 extracellular ectonucleotidases [12], we checked whether the short-term plasticity of GABAergic  
471 connections onto LII neurons might involve the activation of adenosine A1 receptors (Fig. 7, S8,  
472 statistics Table S8). Protocols of 10 trains of stimulations as described previously were applied  
473 twice: before and during bath application of 10  $\mu\text{M}$  DPCPX, an antagonist of A1 receptors.

474 In eGFP- neurons (Fig. 7A-C), DPCPX significantly increased raw eIPSCs amplitudes during trains  
475 at 5 Hz repeated every 20 s ( $P_{\text{eGFP-, 5 Hz}} = 6.28 \cdot 10^{-16}$ ,  $n = 9$ , Fig. 7A) and trains at 50 Hz repeated  
476 every 20 s ( $P_{\text{eGFP-, 50 Hz}} = 2.40 \cdot 10^{-3}$ ,  $n = 9$ , Fig. 7C) but not during trains at 50 Hz repeated every 60  
477 s ( $P_{\text{eGFP-, 50 Hz}} = 0.119$ ,  $n = 7$ , Fig. 7B). In these neurons, no effect of DPCPX was detected on eIPSCs  
478 amplitudes normalized to the firsts eIPSCs of the control trains (Fig. S8), neither at 5 Hz every 20  
479 s ( $P_{\text{eGFP-, 5 Hz}} = 0.777$ ,  $n = 9$ , Fig. S8C), nor at 50 Hz every 20 s ( $P_{\text{eGFP-, 50 Hz}} = 0.050$ ,  $n = 9$ , Fig. S8I),  
480 nor at 50 Hz every 60 s ( $P_{\text{eGFP-, 50 Hz}} = 0.600$ ,  $n = 7$ , Fig. S8F). Effects on raw but not normalized  
481 amplitudes indicated that adenosine tonically controlled GABAergic connections onto eGFP-  
482 neurons. This tonic control was only engaged when trains were repeated every 20 s and not every  
483 60 s indicating that the tone of adenosine controlling GABAergic connections onto eGFP- neurons  
484 was linked to the activity of these synapses.

485 In eGFP+ neurons (Fig. 7D-E), DPCPX increased raw eIPSCs amplitudes during both trains at 5 Hz  
486 repeated every 20 s ( $P_{\text{eGFP+, 5 Hz}} = 1.56 \cdot 10^{-2}$ ,  $n = 9$ , Fig. 7D) and trains at 50 Hz repeated every 60 s  
487 ( $P_{\text{eGFP+, 50 Hz}} = 3.70 \cdot 10^{-2}$ ,  $n = 7$ , Fig. 7E). At both frequencies, the effect of DPCPX on eGFP+ neurons  
488 was statistically significant ( $p\text{-value} < 0.05$ ), but the hypothesis of a difference did not  
489 unambiguously correspond to the most parsimonious explanation of the data (only AIC but not  
490 AICc was lower, see Table S8). In these eGFP+ neurons, no effect of DPCPX was detected on  
491 eIPSCs amplitudes normalized to the firsts eIPSCs of the control trains (Fig. S8) at 5 Hz every 20 s  
492 ( $P_{\text{eGFP+, 5 Hz}} = 0.191$ ,  $n = 9$ , Fig. S8L), but a weak effect was detected at 50 Hz every 60 s ( $P_{\text{eGFP+, 50 Hz}}$   
493  $= 3.70 \cdot 10^{-2}$ ,  $n = 7$ , Fig. S8O). Effects on raw but not normalized amplitudes indicated that  
494 adenosine tonically controlled GABAergic connections onto eGFP+ neurons. On these neurons,  
495 effect of adenosine was kept when trains were repeated every 60 s.

496 Interestingly, the tonic control by adenosine was significantly larger on connections onto eGFP-  
497 neurons than on connections onto eGFP+ neurons ( $P = 2.73 \cdot 10^{-8}$ ; Fig. 7F, Fig. 7F). The average  
498 increase in amplitude during 5 Hz trains was of  $47.3 \pm 2.9$  pA in eGFP- neurons whereas is was of  
499  $15.8 \pm 1.5$  pA in eGFP+ neurons.

500 These data indicated that A1 receptors could tonically inhibit GABAergic connections in LII.

501 This tonic inhibition was stronger on connections onto eGFP- neurons. In these neurons, this tonic  
502 control was only engaged when trains were repeated at short intervals.

503

## 504 **DISCUSSION**

505

506 Short-term plasticity is of major importance in information processing in sensory systems where  
507 the average number of spikes per unit of time encodes the intensity of adequate stimuli [1; 2; 7;  
508 51]. Using mice expressing eGFP under the control of the GAD65 promoter, we demonstrate that  
509 LII GABAergic connections onto putative inhibitory neurons (eGFP+) display different short-term  
510 plasticities than those onto putative excitatory neurons (eGFP-). Importantly, in the transgenic  
511 mice we used, ~80% of LII eGFP+ neurons were shown to be GABA-immunopositive and ~60% of  
512 GABA-immunopositive neurons express eGFP [10]. Therefore, eGFP+ neurons mainly represent  
513 GABAergic neurons, and eGFP- neurons excitatory neurons, although the matching is not perfect.  
514 This may however partly explain the larger data dispersion observed in eGFP- neurons (e.g. Fig.  
515 S4BD).

516 Nevertheless, the strong difference in short-term plasticity we observed when comparing eGFP-  
517 and eGFP+ neurons will probably have an impact on the excitation/inhibition balance within LII  
518 networks, and affect the processing of nociceptive information within the dorsal horn.



519

520 **Action-potential firing patterns**

521 Most of eGFP+ neurons we recorded from displayed a tonic action-potential firing pattern.

522 Other groups have reported this firing type in a majority or in a large proportion of LII neurons

523 identified as inhibitory by immunohistochemistry [52], expression of GAD67 [11], GAD65 [10; 24],

524 other genetic markers [18] or assumed from their morphology [16]. A very low proportion of

525 tonic-firing neurons has nevertheless been recorded in a single study in GAD67+ neurons [19].

526 We mostly recorded putative excitatory neurons displaying non-tonic firing, as did other groups

527 [16; 52]. Nevertheless, in our experiments, eGFP- neurons were mostly IB, whereas in the same

528 transgenic line these neurons mostly displayed a delayed firing [10]. Such difference may be

529 linked to distinct intracellular composition as well as distinct initial holding potential as it has

530 already been shown in lamina II [52]. Our data also indicate that both eGFP- and eGFP+ neurons

531 can fire at 50 Hz, and GABAergic neurons reliably trigger eIPSCs during stimulations at this

532 frequency.

533

534 **Inhibitory connections between LII interneurons**

535 Local inhibitory connections between LII neurons are mostly GABAergic, glycinergic transmission

536 occurring in only 1 / 15 inhibitory contacts recorded by Lu and Perl [33], and possibly in 1 / 3

537 inhibitory contacts recorded by Labrakakis et al. [27]. We therefore focused on GABAergic

538 connections since we aimed at examining the short-term plasticity of inhibitory connections

539 between LII neurons. Interestingly, spontaneously active inhibitory connections are dominated

540 by GABAergic transmission in most LII neurons, whether these are dominated by glycinergic

541 transmission in most neurons at laminae Iii/III border [48], glycinergic connections onto LII

542 neurons most likely originating from neurons the cell bodies of which are localized in other  
543 laminae.

544

#### 545 **Paired-pulse plasticity**

546 During paired-pulse stimulation experiments at short ISIs, GABAergic connections onto eGFP-  
547 neurons preferentially facilitated whereas, at long ISIs, GABAergic connections onto eGFP+  
548 neurons preferentially depressed. In primary cultures of rat laminae I-III, paired-pulse inhibition  
549 of GABAergic eIPSCs was preferentially observed (84%), but facilitation occasionally occurred  
550 (16%) [20]. Inhibitory eIPSPs in laminae II-V of Syrian hamsters, with no distinction between  
551 GABAergic and glycinergic transmissions, preferentially displayed facilitation [53]. These  
552 facilitating connections may correspond to glycinergic eIPSPs since LII glycinergic synapses  
553 display strong paired-pulse facilitation for 50 ms ISIs [24]. Thus, in LII, inhibitory connections  
554 showing paired-pulse facilitation may preferentially involve GABAergic synapses onto excitatory  
555 neurons, or glycinergic connections onto inhibitory neurons. In the dorsal-horn, paired-pulse  
556 depression of glycinergic eIPSCs has previously been shown in rat lamina I, with a maximal  
557 depression at ISIs of 150-200 ms and involving GABA<sub>B</sub> autoreceptors [8].

558

#### 559 **Frequency-dependent STP**

560 Our results indicate that GABAergic connections onto eGFP+ neurons strongly depress during  
561 trains of activity at both low (5 Hz) and high (50 Hz) frequency. In these neurons, trains at high  
562 frequency induce a larger depression reaching its maximum faster than during trains at low  
563 frequency. By contrast, GABAergic connections onto eGFP- neurons do not depress, allowing  
564 summated eIPSCs to reach larger amplitudes at high frequency.

565 We have reported previously that trains at 2.5 Hz induced a depression of eIPSCs in primary  
566 cultures of superficial dorsal-horn neurons from rats [20]. During 10 Hz stimulations, either a  
567 depression or a facilitation of eIPSPs were recorded in laminae II-V of Syrian hamsters [53].  
568 With 20 Hz stimulations, eIPSCs onto GABAergic interneurons in LII of adult mice showed a  
569 facilitation during the first train, and a depression during subsequent trains [27].  
570 Although in eGFP+ neurons we observed a tendency of such a facilitation-depression switch it  
571 was not statistically significant (Fig. S6), suggesting that the facilitation described by other studies  
572 may have involved a glycinergic component which has been shown to display a potentiation in  
573 LII neurons in response to 2 Hz stimulations [24]. Interestingly, we observed a facilitation-  
574 depression switch for the second eIPSCs during repeated trains at 50 Hz in eGFP- neurons (Fig.  
575 S6) suggesting that the facilitation observed in eGFP- neurons during paired-pulse stimulation at  
576 short intervals is replaced by other types of plasticities during repeated trains of stimulation at  
577 50 Hz. Such combination of short-term facilitation and depression at different repetitions,  
578 frequencies and time scales have been observed in other structures and may be relatively  
579 common [3].  
580 Interestingly, in recordings performed at room temperature, GABAergic eIPSCs recorded in  
581 eGFP+ neurons display slower decay kinetics than those recorded in eGFP- neurons [28]. Such  
582 slower kinetic would allow a better summation of overlapping eIPSCS. Nevertheless, in our  
583 conditions, eIPSCs kinetics were similar in eGFP+ and eGFP- neurons. Moreover, at high  
584 frequency, summated eIPSCs in eGFP- neurons reached larger amplitudes than summated eIPSCs  
585 in eGFP+ neurons, ruling out an involvement of different decay kinetics to explain a higher degree  
586 of summation of eIPSCs in eGFP- neurons.

587

## 588 **Modulation by GABA<sub>B</sub> and A1 receptors**

589 Our results indicate that GABAergic connections onto LII neurons can be under a tonic inhibitory  
590 control by A1 adenosine receptors. This inhibition is much stronger on connections onto eGFP-  
591 neurons than onto eGFP+ neurons. In eGFP- neurons, this inhibition only occurred when trains  
592 were delivered at short intervals. This suggests that the tone of adenosine inhibiting these  
593 connections is built-up by ongoing activity and decays when activity decreases. These data were  
594 consistent with our previous work showing that in cultured dorsal-horn neurons, presynaptic A1  
595 autoreceptors control a subset of GABAergic synapses in an activity-dependent manner [20].  
596 Tonic inhibition by adenosine receptors is not restricted to inhibitory transmission, glutamatergic  
597 synapses in LII are also under an inhibitory tone of adenosine, even under low electrical activity  
598 within the network [49].

599 Whereas inhibition of GABAergic connections by A1 receptors was tonic, we showed that GABA<sub>B</sub>  
600 receptors can be engaged in a phasic depression of GABAergic eIPSCs during trains of activity.  
601 This depression involving GABA<sub>B</sub> receptors is target-specific: occurring only in connections onto  
602 eGFP+ neurons. Interestingly, although GABA<sub>B</sub> receptors-mediated inhibition was phasic (it  
603 developed during trains), it was only seen when trains were applied at both low-frequency and  
604 short intervals (5 Hz, 20 s). This suggests that this inhibition is activity-dependent, but that  
605 underlying inhibitory mechanisms might have been surmounted by facilitation occurring during  
606 during trains at high-frequency (compare amplitudes Fig. 6D and 6E).

607 These results were consistent with previous work showing that presynaptic GABA<sub>B</sub> receptors  
608 decrease inhibitory synaptic transmission in the superficial dorsal-horn [8; 15; 20], and that a  
609 tone of GABA activating GABA<sub>B</sub> receptors can occur in the dorsal-horn under various conditions  
610 [14; 31; 35; 42].

611 Interestingly, intrathecal administration of GABA<sub>B</sub> and A1 agonists have antinociceptive effects  
612 [21; 45; 46] which may involve the types of modulation of short-term plasticity described in the  
613 present work.

614

615 **Impact of frequency and target-specific STPs of GABAergic connections onto LII neurons.**

616 GABAergic connections onto inhibitory or excitatory neurons display very distinct frequency-  
617 dependent STPs which can engage target-specific G-protein coupled receptors when bursts of  
618 activity occur at short intervals. Similar differential STPs involving synapses contacting excitatory  
619 and inhibitory neurons have been described in other structures of the central nervous system  
620 and have been shown to directly impact the dynamic properties of networks and their  
621 excitation/inhibition balance [4; 5; 25; 32; 36; 40; 41]. Therefore, these synapse-specific short-  
622 term plasticities and their modulation under various levels of activity are of crucial importance  
623 to understand information processing by a network receiving frequency-coded information from  
624 the periphery.

625 At low frequency, corresponding to the activity at low intensity of sensory stimulation under  
626 basal physiological conditions, the inhibitory control onto putative excitatory neurons remains  
627 constant whereas it depresses onto putative inhibitory neurons. At high frequency, the inhibitory  
628 control is increased by summation onto excitatory neurons whereas it remains constant onto  
629 inhibitory neurons. Since the latter fire action-potentials in a tonic manner, they are expected to  
630 be able to follow higher frequency of excitatory inputs. Therefore, in basal physiological  
631 conditions, both STP and firing properties of inhibitory interneurons of LII might concur to favor  
632 inhibitory controls of nociceptive information, particularly at high intensity of sensory

633 stimulation. Hence, the short-term plasticities within this network as well as their modulation by  
634 A1 and GABA<sub>B</sub> receptors might represent interesting targets in pain-alleviating strategies.

635

### 636 **Conflict of interest statement**

637 The authors declare no competing financial interests.

638

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645

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778 **Figure 1. Action-potential firing patterns in eGFP+ and eGFP- neurons.**

779 **A.** example of a tonic-firing eGFP+ neuron (left) and of an initial bursting eGFP- neuron (right). **B.**  
780 A majority of eGFP+ neurons were of tonic-firing type (Tonic) whereas a majority of eGFP-  
781 neurons were of initial bursting type (IB). Proportions of firing patterns were significantly  
782 different in eGFP+ and eGFP- neurons (Fisher's exact test,  $P = 6.3 \cdot 10^{-6}$ ;  $n = 67$  eGFP+ neurons and  
783 61 eGFP- neurons). **C.** Instantaneous firing frequency in 100 ms bins in response to 1 s-long 80  
784 pA current injections in eGFP- IB neurons (left) and eGFP+ Tonic neurons (right). **D.** Spiking in an  
785 eGFP- (left) and an eGFP+ (right) neuron during a 50 Hz train of simulated EPSCs. **E.** Probability of  
786 action-potential spiking per simulated EPSCs in eGFP- neurons ( $n = 7$ ) and eGFP+ neurons ( $n = 4$ ).

787

788 **Figure 2. GABAergic IPSCs recorded in eGFP+ and eGFP- neurons.**

789 **A.** Amplitude of eIPSCs is similar in eGFP+ (green) and eGFP- neurons (blue). **B.** Left: example of  
790 eIPSCs recorded in an eGFP+ and an eGFP- neuron (average of 10 eIPSCs; eIPSCs are scaled).  
791 Right: Weighted decay time-constant of GABAergic eIPSCs is similar in eGFP+ and eGFP- neurons.  
792 **C.** Amplitudes of mIPSCs are similar in eGFP+ and eGFP- neurons. **D.** Proportions of neurons  
793 displaying a paired-pulse facilitation ( $PPR > 1.1$ ), a paired-pulse depression ( $PPR < 0.9$ ), or no  
794 change in PPR ( $0.9-1.1$ ) at various inter-stimulation intervals (ISIs) in eGFP+ and eGFP- neurons.  
795 These proportions are significantly different in eGFP+ and eGFP- neurons (Log-linear analysis;  
796  $P=0.003$ ). **E.** PPRs as a function of ISIs were significantly different in eGFP+ and eGFP- neurons  
797 (Non-linear curve fitting,  $P = 7.20 \cdot 10^{-3}$ ). Curves correspond to fits with monoexponential  
798 functions. Numbers beside the points correspond to the number of neurons recorded.  
799

800 **Figure 3. Short-term plasticity of GABAergic connections during trains at 5 Hz.**

801 **A.** Representative average current trace recorded in an eGFP- neuron (top) and eGFP+ neuron  
802 (bottom). **B.** Relative amplitudes of GABAergic eIPSCs recorded in eGFP- and eGFP+ neurons  
803 (amplitudes normalized after eIPSCs of rank 1). **C. D.** Changes in eIPSCs amplitudes during the  
804 trains remained similar in the first five and the last five trains for connections on both eGFP- (C)  
805 and eGFP+ (D) neurons. Results of non-linear regressions used to compare conditions are given  
806 as  $P > 0.5$ : n.s. and  $P < 0.001$ : \*\*\*.  $n = 54$  eGFP- and  $n = 49$  eGFP+ neurons. Details of regressions  
807 and the corresponding analysis are given in Supplementary Table 1.

808

809 **Figure 4. Short-term plasticity of GABAergic connections during trains at 50 Hz.**

810 **A.** Representative average current trace recorded in an eGFP- neuron (left) and eGFP+ neuron  
811 (right). **B.** Relative amplitudes of GABAergic eIPSCs recorded in eGFP- and eGFP+ neurons

812 (amplitudes normalized after eIPSCs of rank 1). **C.** Same data as in B, but with eIPSCs measured  
813 from the basal current level before the first eIPSC (summated eIPSCs amplitudes). **D. E.** Changes  
814 in eIPSCs amplitudes during the trains remained similar in the first five and the last five trains for  
815 connections on both eGFP- (D) and eGFP+ (E) neurons. Results of non-linear regressions used to  
816 compare conditions are given as  $P > 0.5$ : n.s. and  $P < 0.001$ : \*\*\*.  $n = 27$  eGFP- and  $n = 19$  eGFP+  
817 neurons. Details of regressions and the corresponding analysis are given in Supplementary Table  
818 1.

819

820 **Figure 5. Distinct short-term plasticities in simultaneously recorded eGFP- and eGFP+ neurons.**

821 **A.** Short-term plasticity during stimulation trains at 5 Hz. Left: representative average current  
822 trace simultaneously recorded in an eGFP- neuron (blue) and eGFP+ neuron (green). Right: for  
823 each pair of eGFP- and eGFP+ neuron and for all ranks during the train, the difference in relative  
824 eIPSCs amplitude is calculated. This difference is significantly larger than zero indicating that the  
825 relative increase in amplitude during the train at 5 Hz is larger in eGFP- than in eGFP+ neurons ( $P$   
826  $< 0.001$ : \*\*\*;  $n = 6$  pairs).

827 **B.** Short-term plasticity during stimulation trains at 50 Hz. Left: representative average current  
828 trace simultaneously recorded in an eGFP- neuron (blue) and eGFP+ neuron (green). Right: for  
829 each pair of eGFP- and eGFP+ neuron and for all ranks during the train, the difference in relative  
830 eIPSCs amplitude is calculated. This difference is significantly larger than zero indicating that the  
831 relative increase in amplitude during the train at 50 Hz is larger in eGFP- than in eGFP+ neurons  
832 ( $P < 0.001$ : \*\*\*;  $n = 4$  pairs). Linear regressions of the data were compared with a linear regression  
833 with a slope set at 0 which would correspond to no differences in eIPSCs amplitudes in eGFP- and

834 eGFP+ neurons. Details of regressions and the corresponding analysis are given in Supplementary  
835 Table 1.

836

837 **Figure 6. Effect of a GABA<sub>B</sub> receptor antagonist on GABAergic eIPSCs during trains of**  
838 **stimulations.**

839 **A-E.** Average normalized amplitudes of GABAergic eIPSCs during trains of stimulations at 5 Hz  
840 and 50 Hz, before (blue and green) and during bath application of 10 μM CGP55845 (red), a  
841 GABA<sub>B</sub> receptors antagonist. For each neuron, trains of 11 stimulations were repeated 10 times  
842 in control condition and 10 times during bath application of 10 μM CGP55845. The type of neuron  
843 recorded (eGFP- or eGFP+), the frequency of stimulation (5 Hz or 50 Hz) and the interval between  
844 trains (20 s or 60 s) are given on the top of each panel. **A-B.** CGP55845 had no effect on GABAergic  
845 connections onto eGFP- neurons, neither during trains at 5 Hz (A, n = 6 neurons) nor during trains  
846 at 50 Hz (B, n = 12 neurons). **C-E.** In eGFP+ neurons CGP55845 significantly increased normalized  
847 eIPSCs amplitudes during 5 Hz trains repeated every 20 s (C, n = 8 neurons) but neither during 50  
848 Hz trains repeated every 60 s (D, n = 6 neurons) nor 50 Hz trains repeated every 20 s (E, n = 7  
849 neurons). Results of non-linear regressions used to compare conditions are given as P > 0.5: n.s.,  
850 0.05 < P < 0.01: \*, and P < 0.001: \*\*\*. Details of regressions and the corresponding analysis are  
851 given in Supplementary Table 1.

852

853 **Figure 7. Effect of an A1 adenosine receptor antagonist on GABAergic eIPSCs during trains of**  
854 **stimulations**

855 **A-E.** Average raw amplitudes of GABAergic eIPSCs during trains at 5 Hz and 50 Hz before (blue  
856 and green) and during bath application of 10 μM DPCPX (pink), an A1 receptor antagonist. For

857 each neuron, trains of 11 stimulations were repeated 10 times in control condition and 10 times  
858 during bath application of DPCPX. The type of neuron recorded (eGFP- or eGFP+), the frequency  
859 of stimulation (5 Hz or 50 Hz) and the interval between trains (20 s or 60 s) are given on the top  
860 of each panel. **A-C.** In eGFP- neurons, DPCPX significantly increased raw eIPSCs amplitudes during  
861 5 Hz trains repeated every 20 s (A, n = 9 neurons) and 50 Hz trains repeated every 20 s (C, n = 9  
862 neurons) but not during 50 Hz trains repeated every 60 s (B, n = 7 neurons). This indicated a tonic  
863 inhibition of GABAergic connections onto eGFP- neurons by adenosine depending on the delay  
864 between trains of stimulation. **D-E.** In eGFP+ neurons, DPCPX significantly increased raw eIPSCs  
865 amplitudes during 5 Hz trains repeated every 20 s (D, n = 9 neurons) and 50 Hz trains repeated  
866 every 60 s (E, n = 7 neurons). This indicated a tonic inhibition of GABAergic connections onto  
867 eGFP+ neurons. **F.** Average increase in raw eIPSCs amplitudes in presence of DPCPX with respect  
868 to control conditions. For trains at 5 Hz, the increase in amplitude is larger in eGFP- neurons than  
869 in eGFP+ neurons. Evolution of eIPSCs amplitudes during trains were compared with non-linear  
870 regression. Details of regressions and the corresponding analysis are given in Supplementary  
871 Table 1.

872

## 873 **Supplementary Figures and table legends**

### 874 **Supplementary figure S1. Data quantification, analysis and statistics.**

875 Stimulation protocols were applied ten times in each neuron and the within-cell average across  
876 trials was calculated by averaging eIPSCs amplitudes from these ten repetitions. These within-  
877 cell average across ten trials were used for statistical analysis. Non-linear regression analysis was  
878 used to analyze changes in eIPSCs amplitudes during stimulation train protocols. To compare two  
879 different conditions (e.g. eGFP- vs. eGFP+ or presence/absence of antagonists) data from each

880 conditions were fitted with Equation 3, either individually (sum of two functions) or pooled (one  
881 single function). When the sum of two individual fits provided statistically significant  
882 improvements with respect to the fit of pooled data, the two conditions were considered as  
883 having distinct effects. To define whether STP was expressed during trains of stimulations, i.e.  
884 whether significant changes in amplitude occurred during the train, fits with Equation 3 were  
885 compared with linear fits with slope values forced to 0. When fits with Equation 3 provided  
886 statistically significant improvements with respect to the linear fit with slope value forced to 0,  
887 the connections were considered as displaying a significant STP during the corresponding  
888 protocol. Details of all models (number of neurons, degree of freedom, Residual sum of squares,  
889 AIC, AICc, BIC) as well as details of model comparison (F, P, differences in AIC, AICc and BIC) are  
890 given in Supplementary table 1. Individual data of all neurons were always used for curve fitting  
891 and average values were used for illustration.

892

### 893 **Supplementary figure S2.**

#### 894 **Action-potential firing patterns in eGFP+ (left) and eGFP- (right) neurons.**

895 A majority of eGFP+ neurons were of tonic-firing type (Tonic) whereas a majority of eGFP-  
896 neurons were of initial bursting type (n = 67 eGFP+ neurons and 61 eGFP- neurons).

897

### 898 **Supplementary figure S3.**

#### 899 **Short-term plasticity of GABAergic connections during trains at 5 Hz and 50 Hz.**

900 **A.** Average raw eIPSCs amplitudes of GABAergic eIPSCs recorded in eGFP- neurons (blue) and  
901 eGFP+ neurons (green) during 5 Hz protocols. **B.** Relative amplitudes of GABAergic eIPSCs  
902 recorded in eGFP- and eGFP+ neurons (amplitudes normalized after eIPSCs of rank 1, same

903 neurons as in A). **C.** Average raw eIPSCs amplitudes of GABAergic eIPSCs recorded in eGFP-  
904 neurons (blue) and eGFP+ neurons (green) during 50 Hz protocols. **D.** Relative amplitudes of  
905 GABAergic eIPSCs recorded in eGFP- and eGFP+ neurons (amplitudes normalized after eIPSCs of  
906 rank 1, same neurons as in C). **E. F.** Same data as in C-D, but with eIPSCs measured from the basal  
907 current level before the first eIPSC (summated eIPSCs amplitudes). Results of non-linear  
908 regressions used to compare conditions are given as  $P > 0.5$ : n.s. and  $P < 0.001$ : \*\*\*. Details of  
909 regressions and the corresponding analysis are given in Supplementary Table 1. For 5 Hz  
910 protocols:  $n = 54$  eGFP- and  $n = 49$  eGFP+ neurons. For 50 Hz protocols:  $n = 27$  eGFP- and  $n = 19$   
911 eGFP+ neurons.

912

913 **Supplementary figure S4. Short-term plasticity of GABAergic connections during trains at 5 Hz**  
914 **and 50 Hz: individual neurons.**

915 **Left:** Average raw eIPSCs amplitudes of GABAergic eIPSCs. **Right:** Relative amplitudes of  
916 GABAergic eIPSCs (amplitudes normalized after eIPSCs of rank 1, same neurons as left).

917 **A-D:** train of stimulations at 5 Hz. Same data as in Fig. 3.  $n = 54$  eGFP- and  $n = 49$  eGFP+ neurons.

918 **E-H:** trains of stimulations at 50 Hz. Same data as in Fig. 4.  $n = 27$  eGFP- and  $n = 19$  eGFP+ neurons.

919 **A, B, E, F:** eIPSCs recorded in eGFP- neurons. **C, D, G, H:** eIPSCs recorded in eGFP+ neurons.

920 Average amplitudes from individual neurons are in grey.

921

922 **Supplementary figure S5. Short-term plasticity of GABAergic connections during trains of**  
923 **stimulations at 5 Hz with increased amplitudes of stimulation.**

924 For this experiment, stimulation amplitudes were set at 0.45 mA (versus 0.21 mA on average for  
925 all other experiments). **A.** Average raw eIPSCs amplitudes of GABAergic eIPSCs recorded in eGFP-



926 neurons (blue) and eGFP+ neurons (green). **B.** Relative amplitudes of GABAergic eIPSCs recorded  
927 in eGFP- and eGFP+ neurons (amplitudes normalized after eIPSCs of rank 1, same neurons as in  
928 A). n = 11 eGFP- and n = 11 eGFP+ neurons.

929 Evolution of eIPSCs amplitudes during the trains were significantly different in eGFP- and eGFP+  
930 neurons as it was observed with lower amplitudes of stimulation (Fig. 3). This indicated that  
931 changes in stimulation efficacy during trains of stimulations were unlikely to influence our results.

932

933 **Supplementary figure S6. Relative amplitude of rank 2 eIPSCs in the first five and the last five**  
934 **trains.**

935 **A.** During 5 Hz trains, eIPSC2/eIPSC1 remained similar in the first five and the last five trains for  
936 connections on both eGFP- and eGFP+ neurons ( $P_{\text{eGFP-}} = 0.678$ ,  $P_{\text{eGFP+}} = 0.223$ , Wilcoxon Signed  
937 Rank Test for Paired Data). **B.** During 50 Hz trains, eIPSC2/eIPSC1 remained similar in the first five  
938 and the last five trains for connections on eGFP+ neurons ( $P_{\text{eGFP+}} = 0.098$ , Wilcoxon Signed Rank  
939 Test for Paired Data), but significantly decreased by 18% for eGFP- neurons ( $P_{\text{eGFP-}} = 0.048$ ,  
940 Wilcoxon Signed Rank Test for Paired Data). This suggests that the facilitation observed in eGFP-  
941 neurons during paired-pulse stimulation at short intervals (Fig. 2E) is replaced by other types of  
942 plasticities during repeated trains of stimulation at 50 Hz.

943

944 **Supplementary figure S7. Effect of a GABA<sub>B</sub> receptor antagonist on GABAergic eIPSCs during 5**  
945 **Hz and 50 Hz trains.**

946 Average amplitudes of GABAergic eIPSCs during trains at 5 Hz and 50 Hz before (blue and green)  
947 and during bath application of 10  $\mu\text{M}$  CGP55845 (orange), a GABA<sub>B</sub> receptors antagonist. For  
948 each neuron, trains of 11 stimulations were repeated 10 times in control condition and 10 times

949 during bath application of CGP55845. **A, D, G, J, M.** Type of neuron (eGFP- or eGFP+), frequency  
950 of stimulation (5 Hz or 50 Hz) and interval between trains (20 s or 60 s) applied in B-C, E-F, H-I, K-  
951 L, N-O, respectively. **B, E, H, K, N.** Average raw eIPSCs amplitudes. **C, F, I, L, O.** Average relative  
952 eIPSCs amplitudes. **A-F:** CGP55845 had no effect on GABAergic connections onto eGFP- neurons,  
953 neither during trains at 5 Hz (A-C, n = 6 neurons) nor during trains at 50 Hz (D-F n = 12 neurons).  
954 **G-O:** in eGFP+ neurons CGP55845 did not changed raw eIPSCs amplitudes (H, K, N), but it  
955 significantly increased relative eIPSCs amplitude during 5 Hz trains repeated every 20 s (I, n = 8  
956 neurons), indicating a phasic inhibition involving GABA<sub>B</sub> receptors during these trains. Evolution  
957 of eIPSCs amplitudes during trains were compared with non-linear regression. Details of  
958 regressions and the corresponding analysis are given in Supplementary Table 1. P > 0.5: n.s. and  
959 P < 0.001: \*\*\*.

960

961 **Supplementary figure S8. Effect of an A1 adenosine receptor antagonist on GABAergic eIPSCs**  
962 **during 5 Hz and 50 Hz trains.**

963 Average amplitudes of GABAergic eIPSCs during trains at 5 Hz and 50 Hz before (blue and green)  
964 and during bath application of 10  $\mu$ M DPCPX (pink), an A1 receptor antagonist. For each neuron,  
965 trains of 11 stimulations were repeated 10 times in control condition and 10 times during bath  
966 application of DPCPX. **A, D, G, J, M.** Type of neuron (eGFP- or eGFP+), frequency of stimulation (5  
967 Hz or 50 Hz) and interval between trains (20 s or 60 s) applied in B-C, E-F, H-I, K-L, N-O,  
968 respectively. **B, E, H, K, N.** Average raw eIPSCs amplitudes. **C, F, I, L, O.** Average relative eIPSCs  
969 amplitudes. **B, H.** DPCPX significantly increased raw eIPSCs amplitudes recorded in eGFP- neurons  
970 during trains at 5 Hz repeated every 20 s (B, n = 9 neurons) and 50 Hz repeated every 20 s (H, n =  
971 9 neurons) but had no effect on raw eIPSCs amplitudes recorded during trains at 50 Hz repeated

972 every 60 s (E, n = 7 neurons). This indicated a tonic inhibition of GABAergic connections onto  
973 eGFP- neurons by adenosine depending on the delay between trains of stimulation. **K, N**, in  
974 eGFP+ neurons, DPCPX significantly increased raw eIPSCs amplitudes during 5 Hz trains repeated  
975 every 20 s (K) and 50 Hz trains repeated every 60 s (N). This indicated a tonic inhibition of  
976 GABAergic connections onto eGFP+ neurons. **C, F, I, L, O** Except during 50 Hz trains repeated  
977 every 60 s where a weak significant effect was recorded (O), no effect of DPCPX was detected in  
978 relative eIPSCs amplitudes, neither in eGFP- nor in eGFP+ neurons indicating that inhibition  
979 involving A1 receptors did not changed during the train. Evolution of eIPSCs amplitudes during  
980 trains were compared with non-linear regression. Details of regressions and the corresponding  
981 analysis are given in Supplementary Table 1.  $P > 0.5$ : n.s.,  $0.05 > P > 0.01$ : \*,  $P < 0.001$ : \*\*\*.

982

### 983 **Supplementary tables 1-7. Data analysis, model comparison and statistics.**

984 The tables correspond to the different figures illustrating the comparisons.

985 The column **Figure** indicates the panel of the figure illustrating the conditions compared.

986 In these figures, the averaged eIPSCs amplitudes in the two conditions are illustrated, but

987 statistical analysis were performed using averaged eIPSCs amplitudes of each neurons. These

988 values were calculated by averaging eIPSCs amplitudes of the same rank obtained from

989 reiterations of the protocols. The reiterations used for the average are indicated in the column

990 **Protocol repetition** (usually iterations 1-10). For protocols using trains of stimulations, the

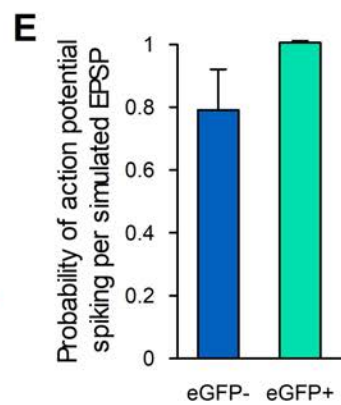
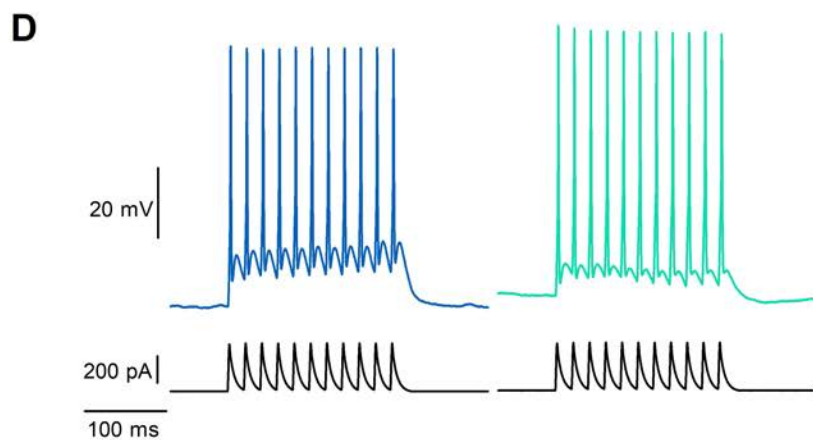
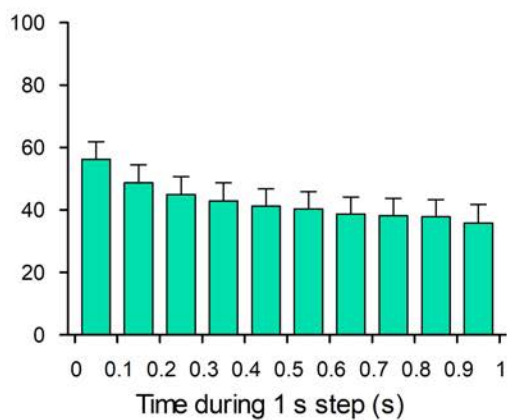
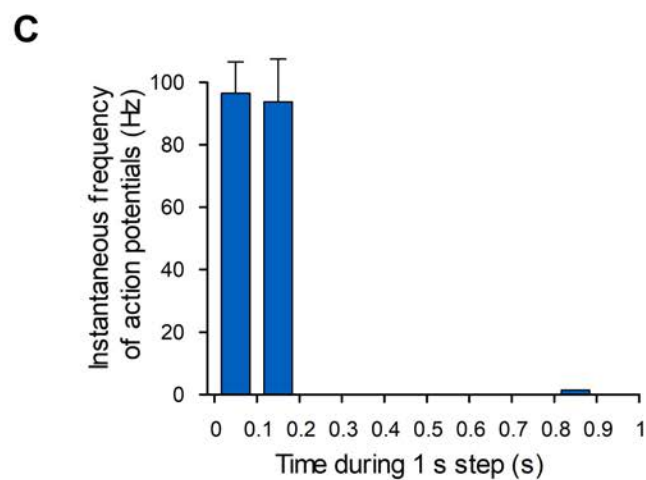
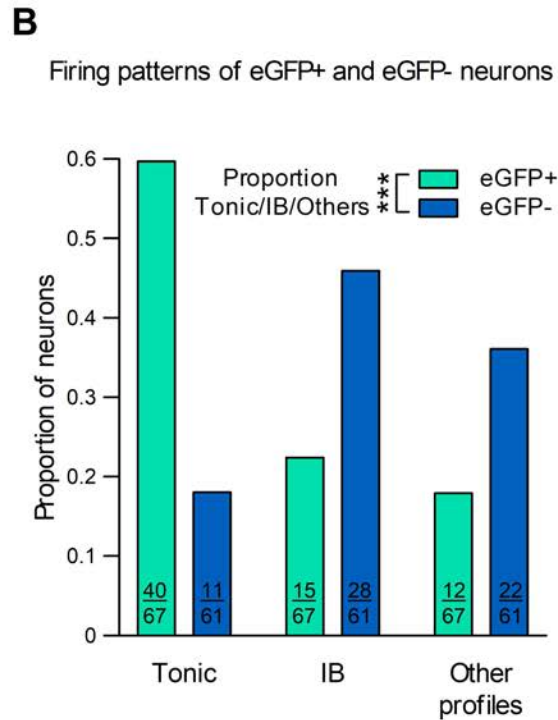
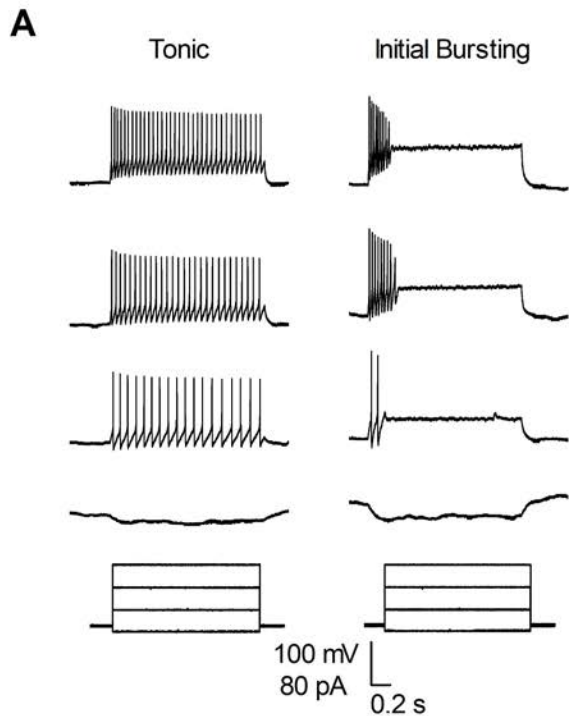
991 **frequency** of stimulations is given (5 Hz or 50 Hz) as well as the time between protocols (20 s or

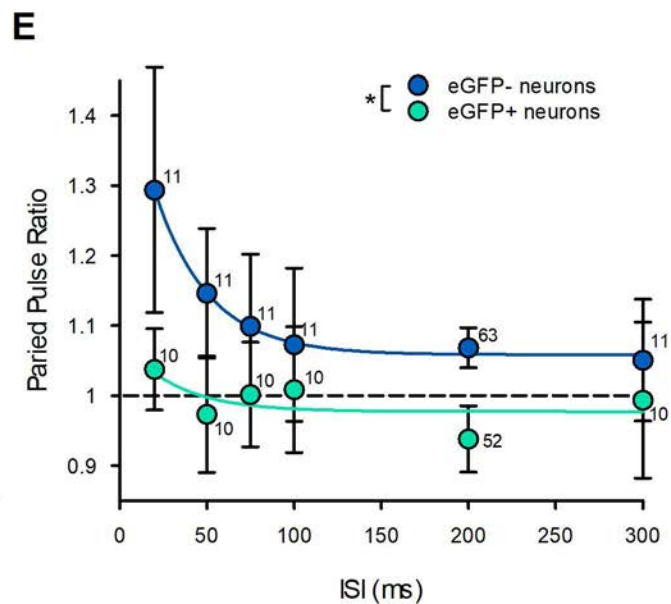
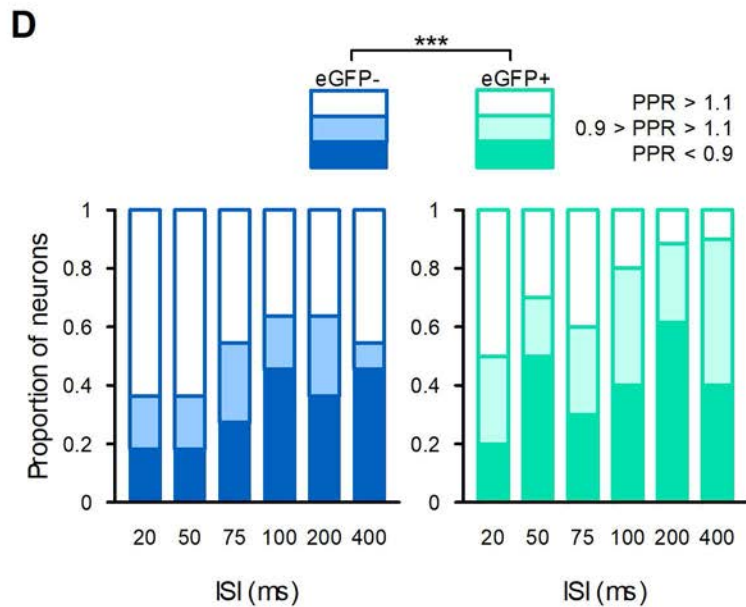
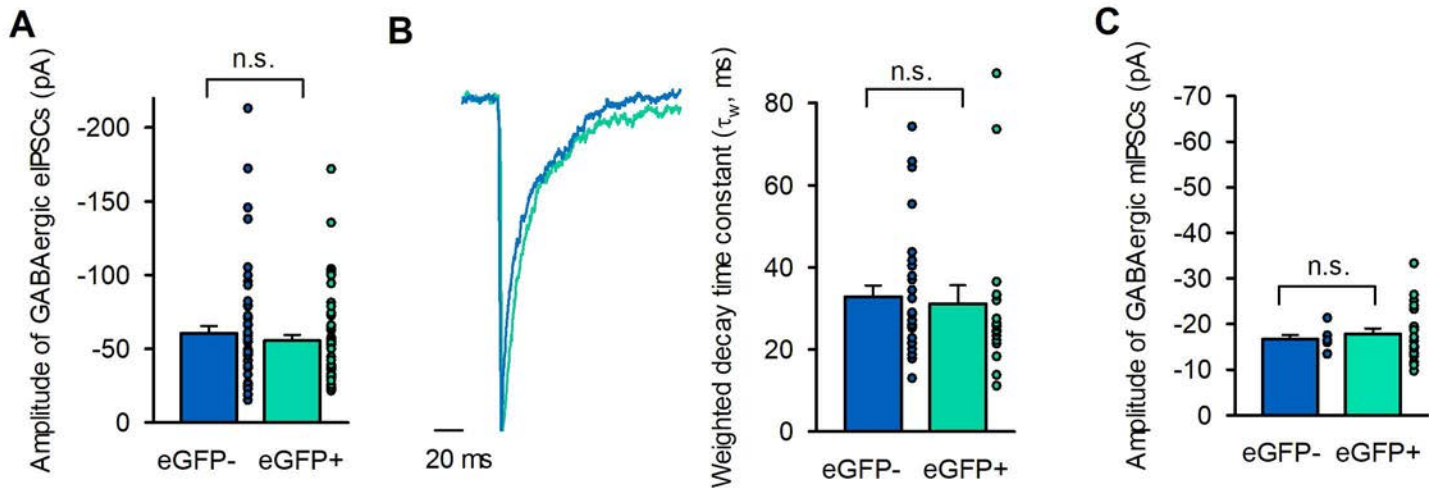
992 60 s) in the column **Protocol interval**. The **Type of data** column indicates whether data were

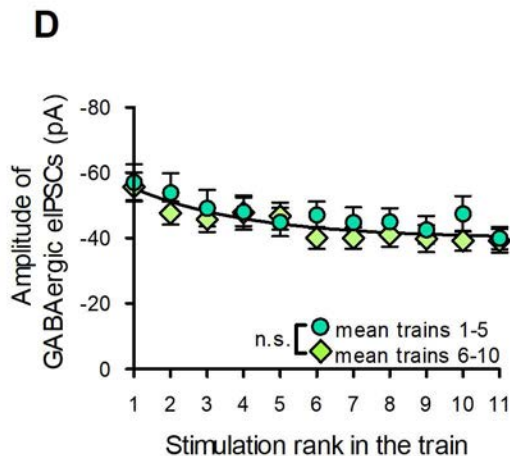
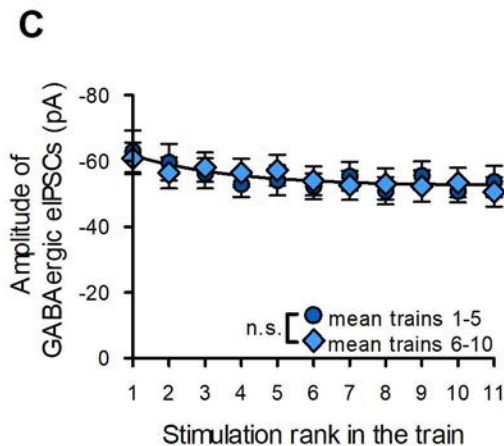
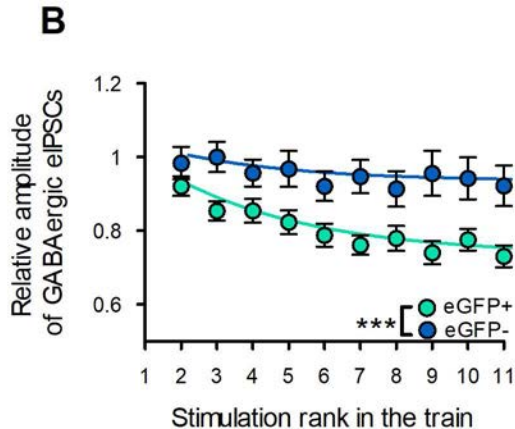
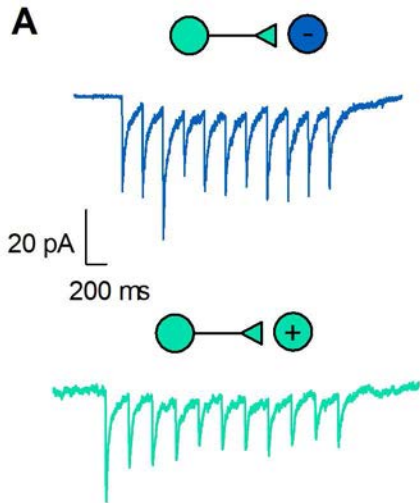
993 averaged raw eIPSCs amplitudes (Raw) or averaged normalized amplitudes (Norm). The **eGFP**

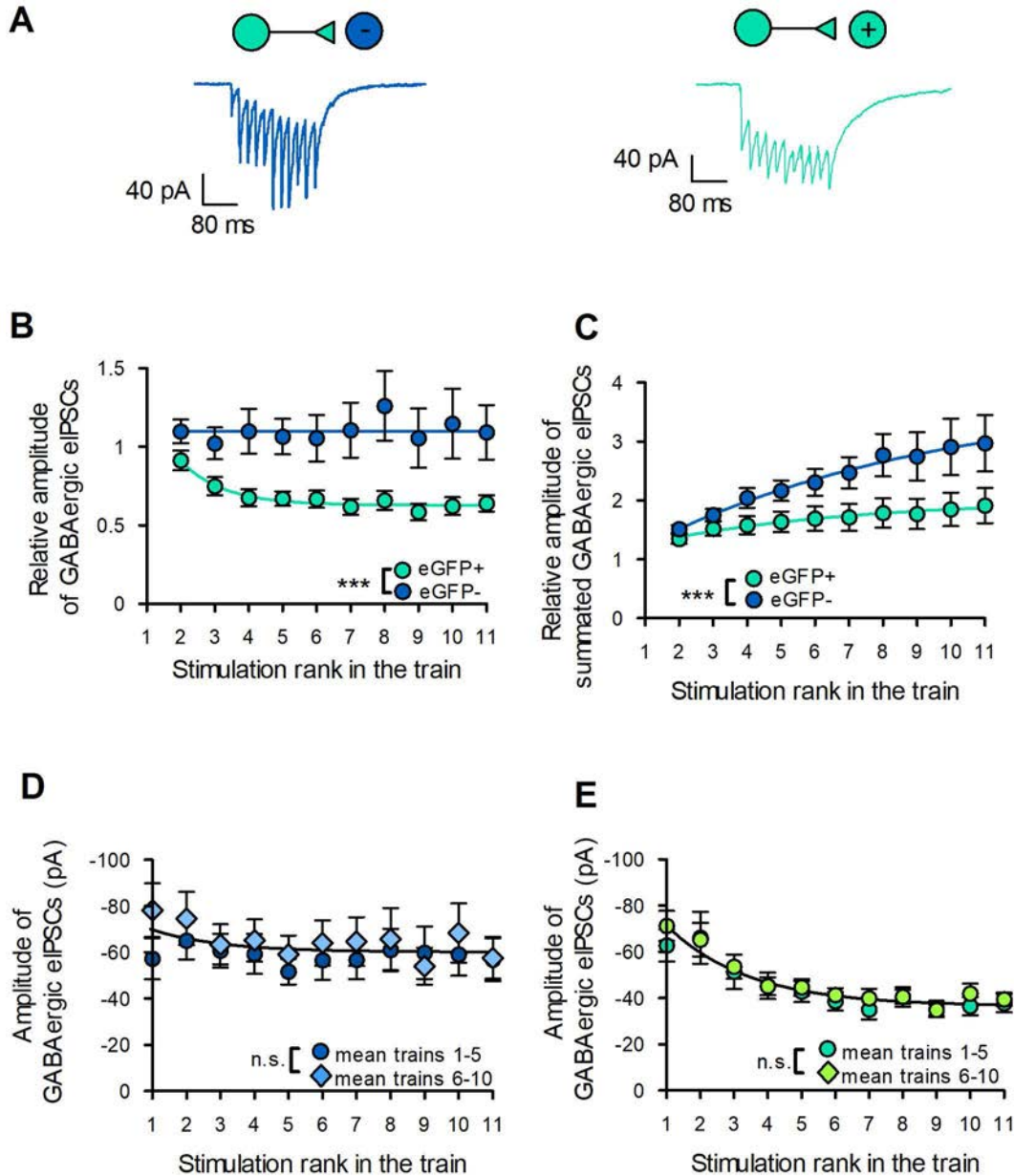
994 **condition** column indicates whether the data compared were from eGFP- (-) or eGFP+ (+)

995 neurons or both (+ and -). The **Drug condition** column indicates whether the data compared were  
996 recorded without (**Control**) or with CGP55845 or DPCPX, or both. The **Model** column indicates  
997 the two models compared. **N neurons** corresponds to the number of neurons (eGFP- (-) or eGFP+  
998 (+)). **N obs** corresponds to the number of observations. In experiments with trains of  
999 stimulations, the number of observations per neuron and per condition was of 11 for raw data  
1000 and 10 for normalized data. **k** corresponds to the number of parameters in the model. A1, A2 and  
1001 A3 are the parameters calculated by non-linear regression (two A1, A2 and A3 are given when a  
1002 sum of two regressions are used). **SS** is the residual sum of squares calculated with the model. **df**  
1003 is the degree of freedom of the model. **AIC** is Akaike information criterion; **AICc** is the AIC  
1004 corrected for small samples; **BIC** is the bayesian information criterion. **F** is the value of the F-  
1005 distribution of the comparison between the two models. **p** is the corresponding p-value. **dAICc** is  
1006 the decrease in AICc provided by the model indicated in that line with respect to the model in  
1007 the line above. A positive value indicate that the model correspond to the most parsimonious  
1008 explanation with respect to the model in the line above.  
1009





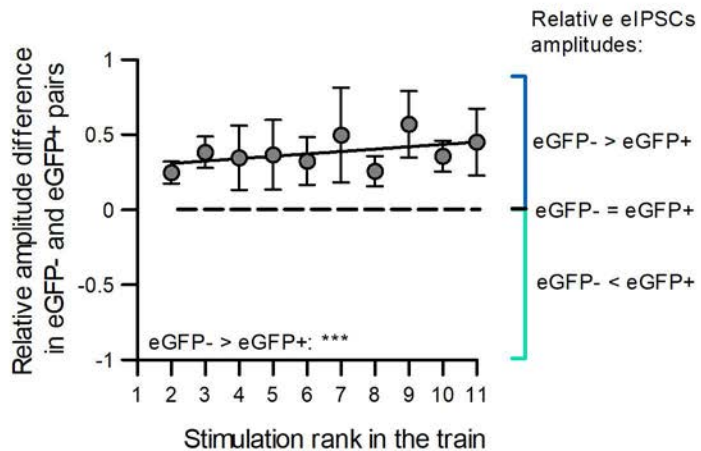
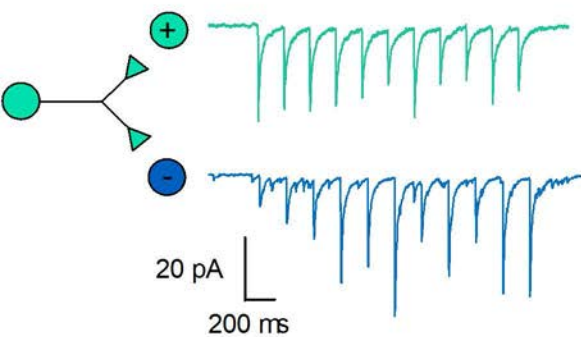




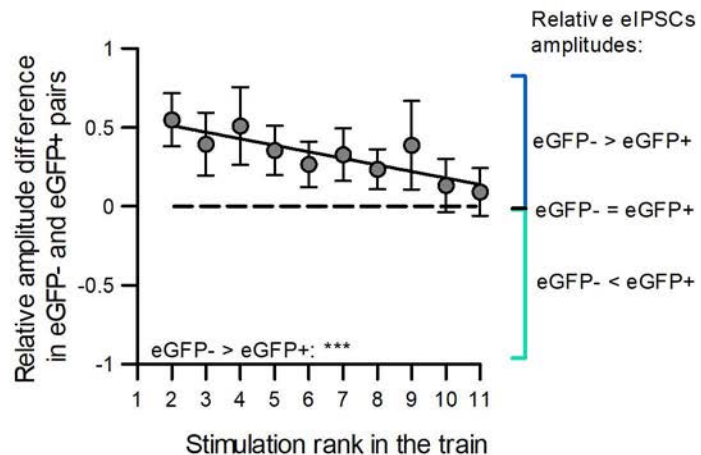
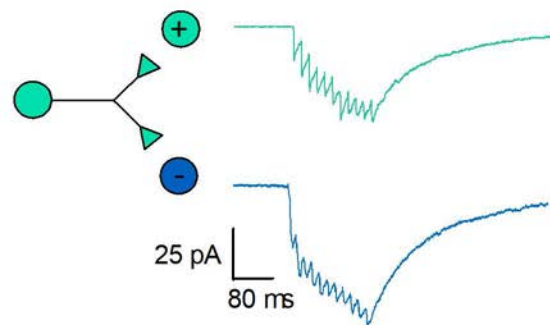


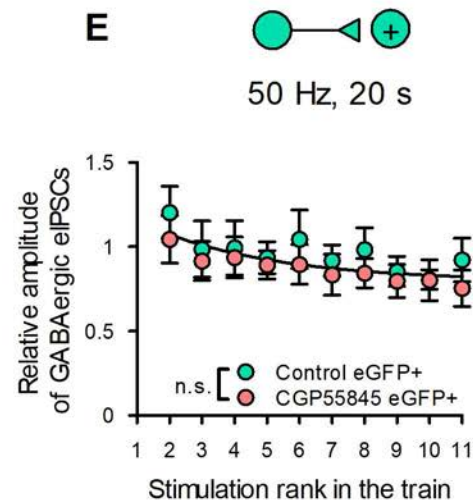
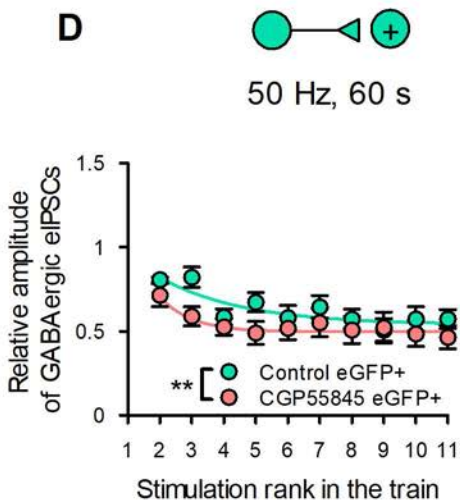
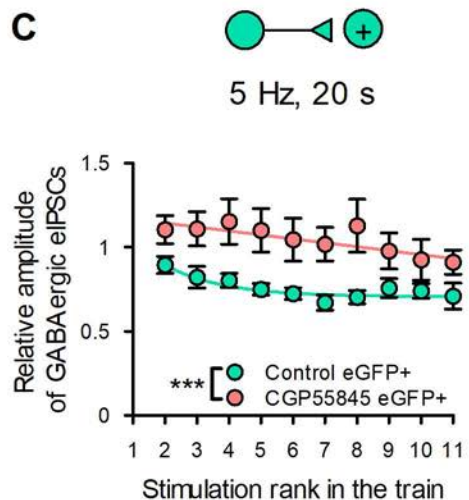
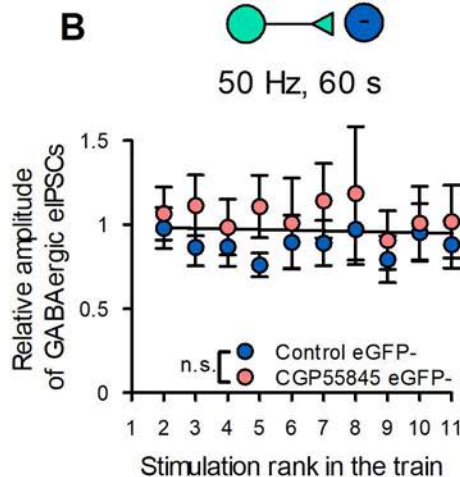
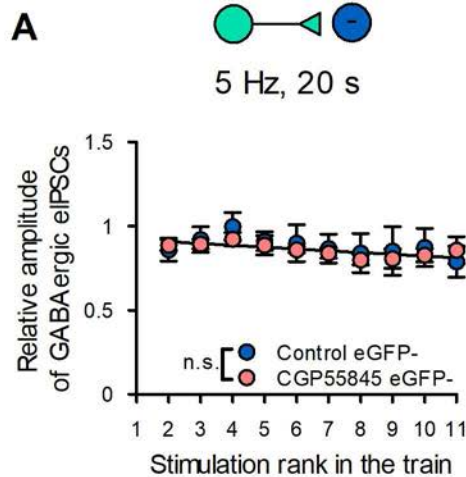
**A**

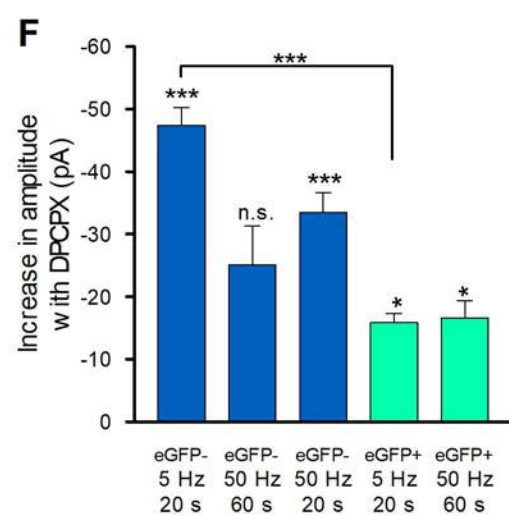
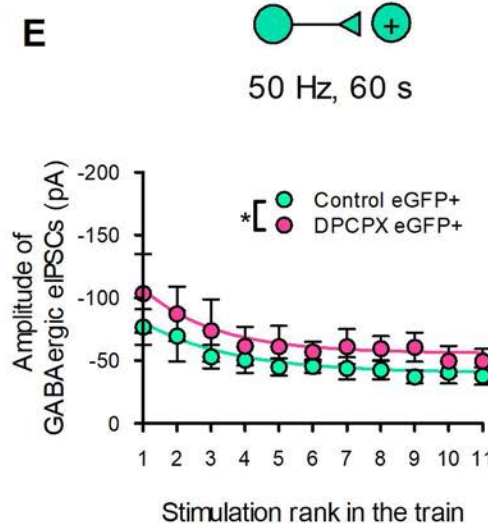
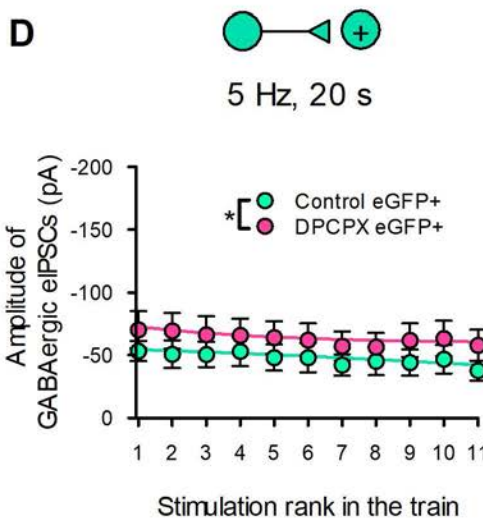
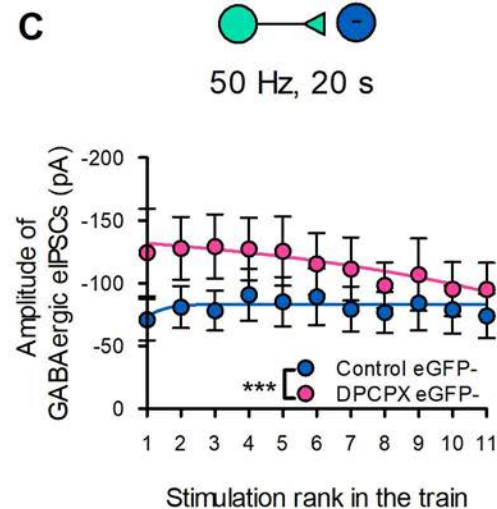
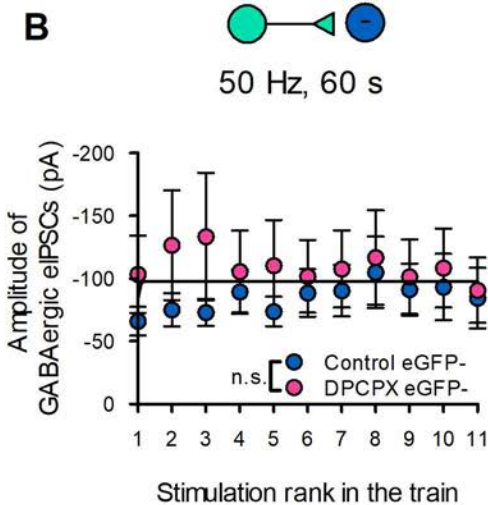
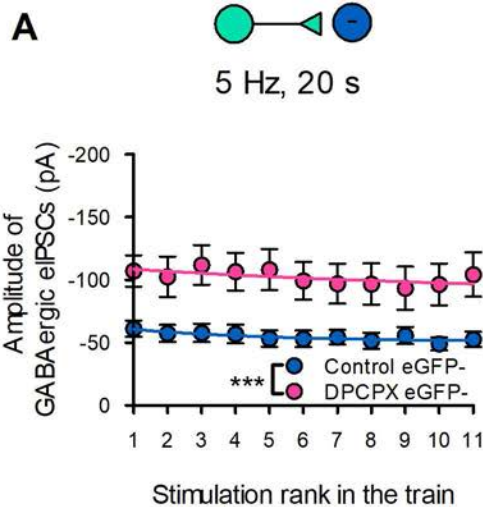
5 Hz

**B**

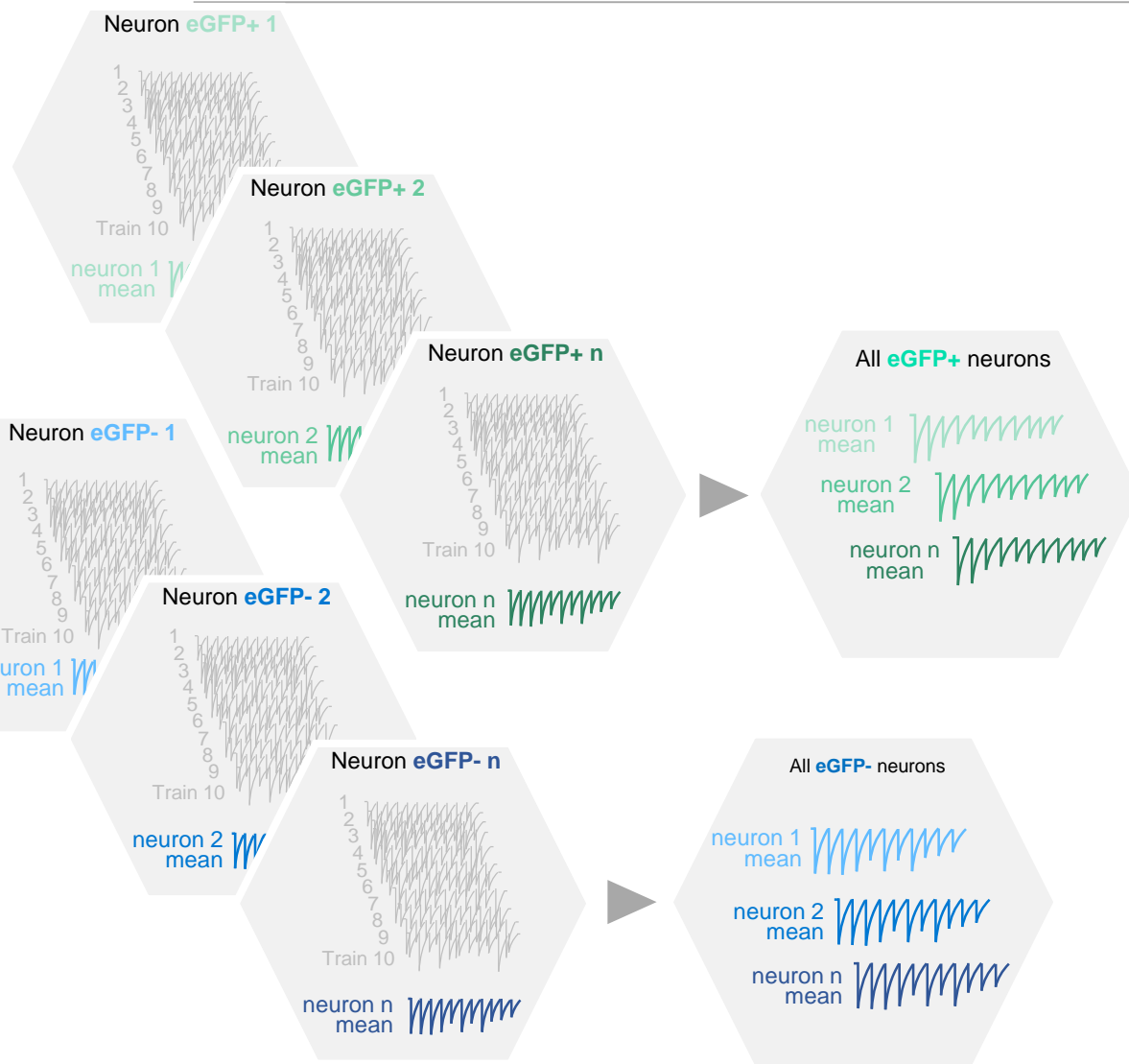
50 Hz





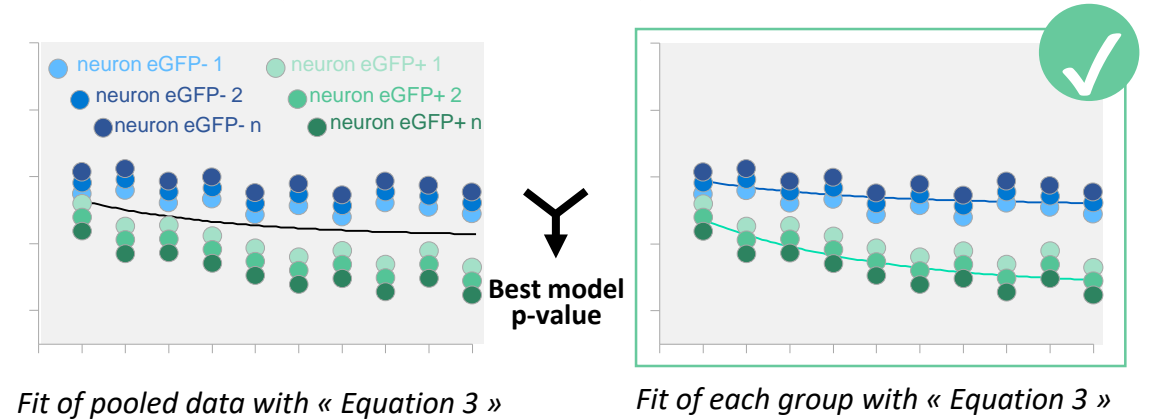


## Data quantification and analysis

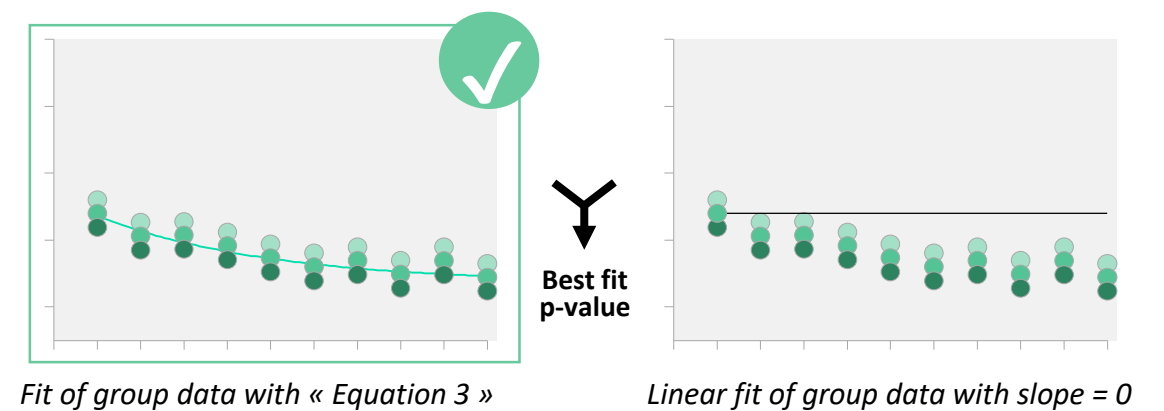


## Curve fitting and model comparison

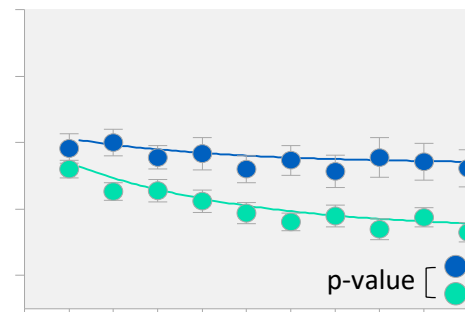
### Groups similar or statistically different?



### Plasticity or no plasticity during the train?



### Illustrations: Curve fits superimposed with average amplitudes

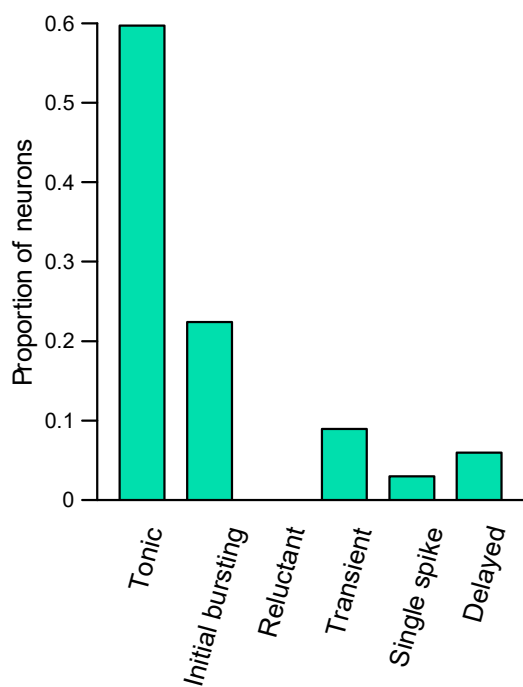


p-value of model comparisons are symbolized in the figures and given in the text. Other criteria of model/fit goodness (BIC, AIC, AICc) are given in the supplementary tables.

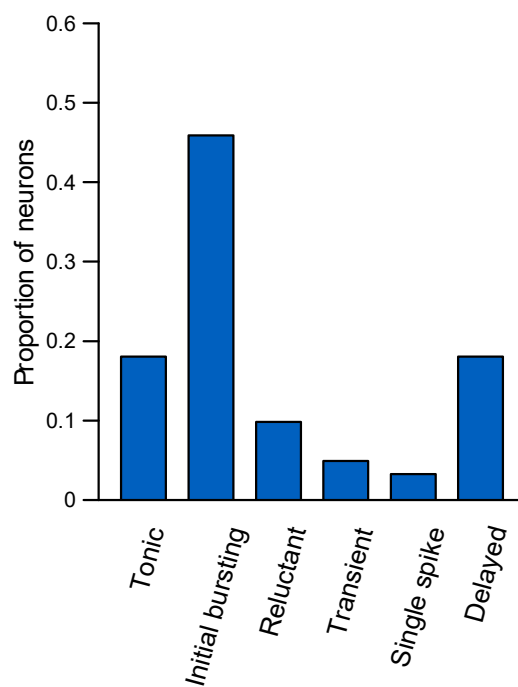
### Supplementary figure S1. Data quantification, analysis and statistics.

Stimulation protocols were applied ten times in each neuron and the within-cell average across trials was calculated by averaging eIPSCs amplitudes from these ten repetitions. These within-cell average across ten trials were used for statistical analysis. Non-linear regression analysis was used to analyze changes in eIPSCs amplitudes during stimulation train protocols. To compare two different conditions (e.g. eGFP- vs. eGFP+ or presence/absence of antagonists) data from each conditions were fitted with Equation 3, either individually (sum of two functions) or pooled (one single function). When the sum of two individual fits provided statistically significant improvements with respect to the fit of pooled data, the two conditions were considered as having distinct effects. To define whether STP was expressed during trains of stimulations, i.e. whether significant changes in amplitude occurred during the train, fits with Equation 3 were compared with linear fits with slope values forced to 0. When fits with Equation 3 provided statistically significant improvements with respect to the linear fit with slope value forced to 0, the connections were considered as displaying a significant STP during the corresponding protocol. Details of all models (number of neurons, degree of freedom, Residual sum of squares, AIC, AICc, BIC) as well as details of model comparison (F, P, differences in AIC, AICc and BIC) are given in Supplementary table 1. Individual data of all neurons were always used for curve fitting and average values were used for illustration.

Firing patterns of eGFP+ neurons



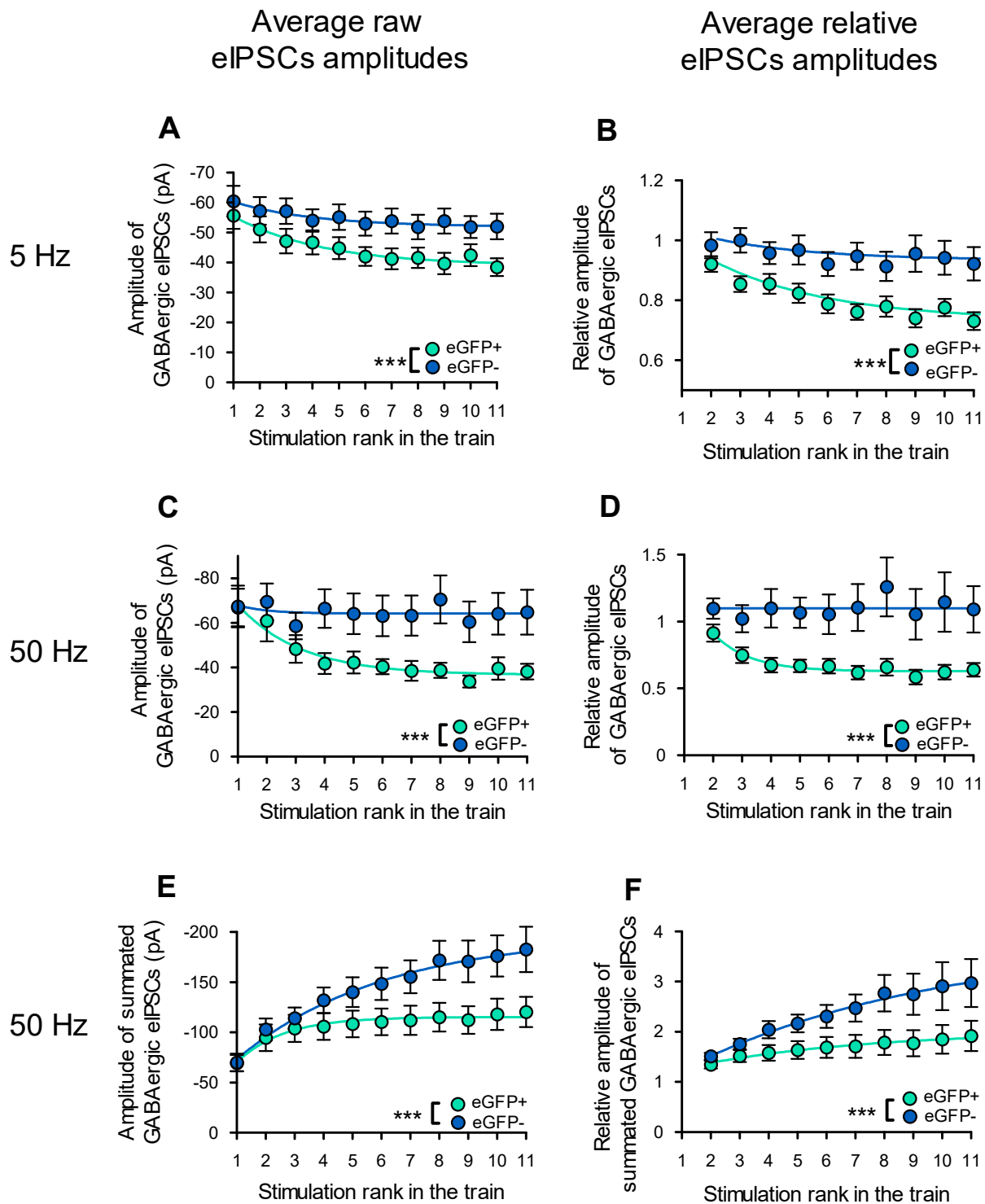
Firing patterns of eGFP- neurons



**Supplementary figure S2.**

Action-potential firing patterns in eGFP+ (left) and eGFP- (right) neurons.

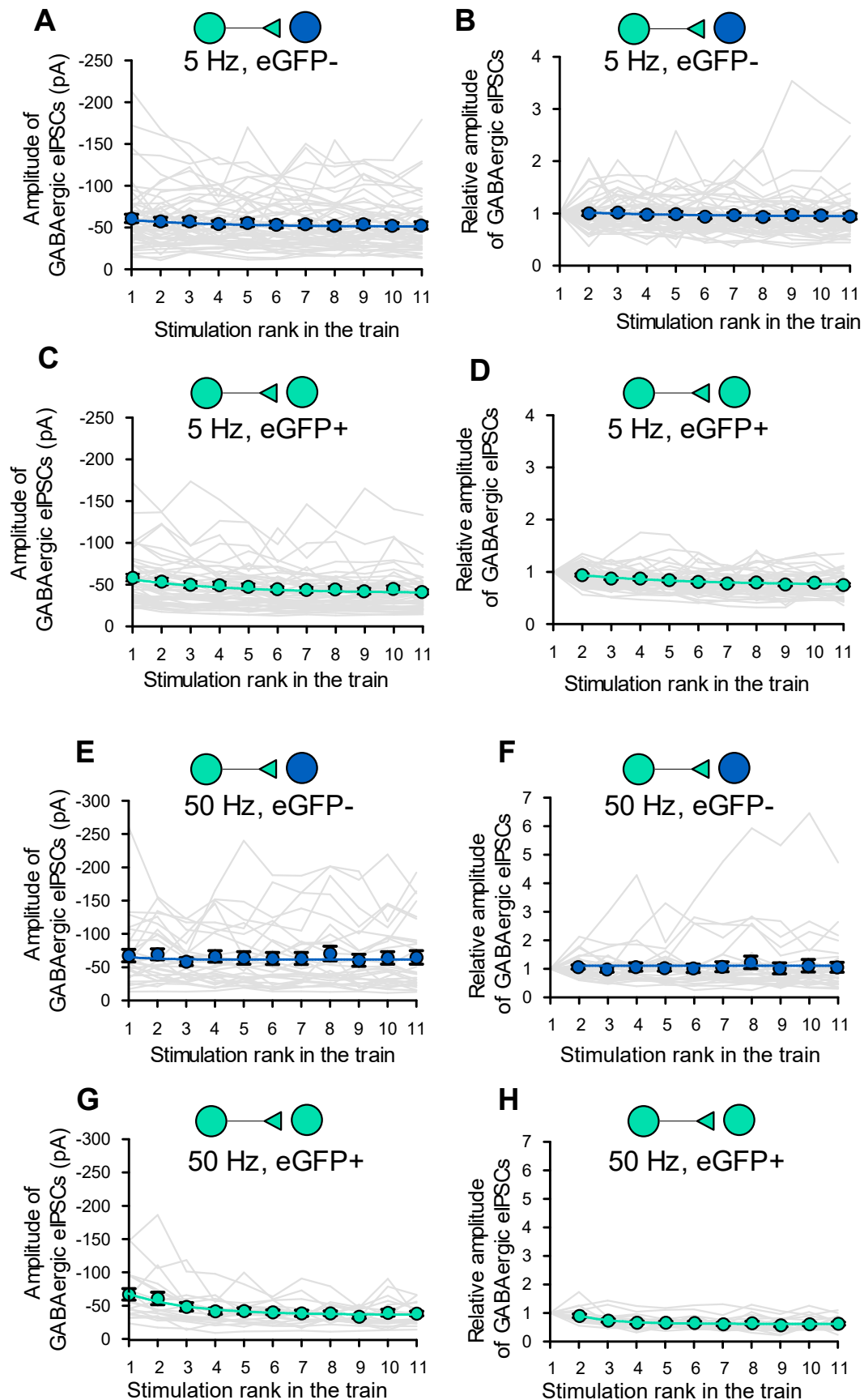
A majority of eGFP+ neurons were of tonic-firing type (Tonic) whereas a majority of eGFP- neurons were of initial bursting type (n = 67 eGFP+ neurons and 61 eGFP- neurons).



### Supplementary figure S3.

#### Short-term plasticity of GABAergic connections during trains at 5 Hz and 50 Hz.

A. Average raw eIPSCs amplitudes of GABAergic eIPSCs recorded in eGFP- neurons (blue) and eGFP+ neurons (green) during 5 Hz protocols. B. Relative amplitudes of GABAergic eIPSCs recorded in eGFP- and eGFP+ neurons (amplitudes normalized after eIPSCs of rank 1, same neurons as in A). C. Average raw eIPSCs amplitudes of GABAergic eIPSCs recorded in eGFP- neurons (blue) and eGFP+ neurons (green) during 50 Hz protocols. D. Relative amplitudes of GABAergic eIPSCs recorded in eGFP- and eGFP+ neurons (amplitudes normalized after eIPSCs of rank 1, same neurons as in C). E. F. Same data as in C-D, but with eIPSCs measured from the basal current level before the first eIPSC (summated eIPSCs amplitudes). Results of non-linear regressions used to compare conditions are given as  $P > 0.5$ : n.s. and  $P < 0.001$ : \*\*\*. Details of regressions and the corresponding analysis are given in Supplementary Table 1. For 5 Hz protocols:  $n = 54$  eGFP- and  $n = 49$  eGFP+ neurons. For 50 Hz protocols:  $n = 27$  eGFP- and  $n = 19$  eGFP+ neurons.



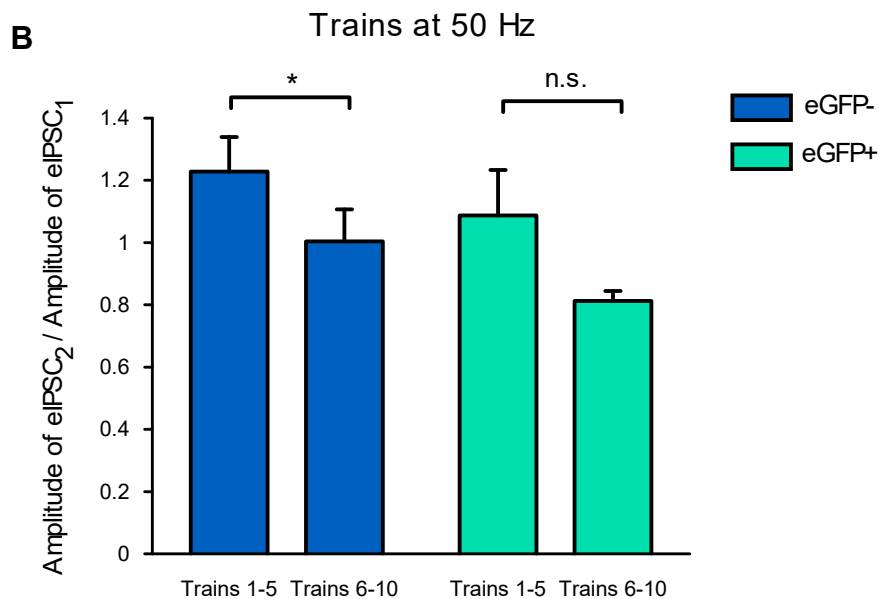
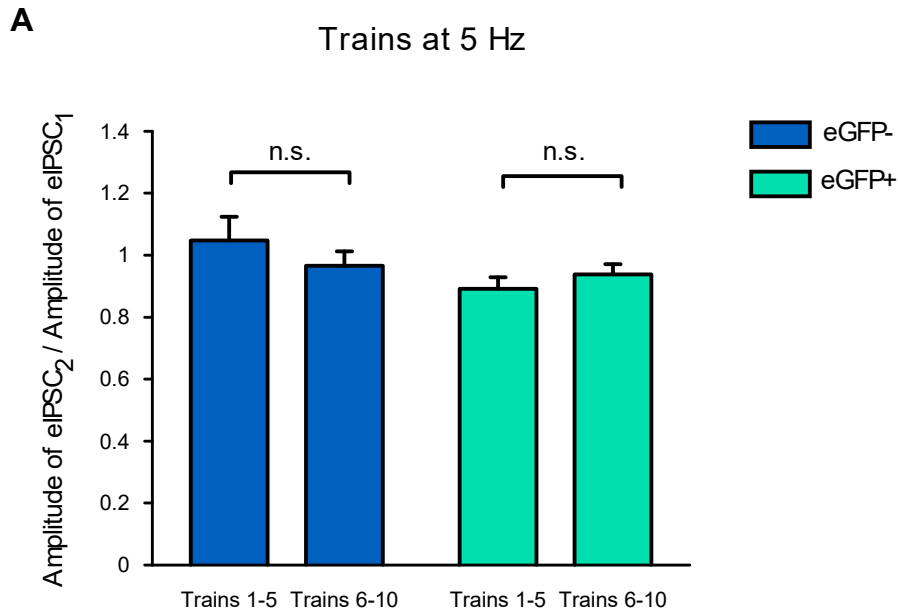
**Supplementary figure S4.**

**Short-term plasticity of GABAergic connections during trains at 5 Hz and 50 Hz: individual neurons.**

Left: Average raw eIPSCs amplitudes of GABAergic eIPSCs. Right: Relative amplitudes of GABAergic eIPSCs (amplitudes normalized after eIPSCs of rank 1, same neurons as left).

A-D: train of stimulations at 5 Hz. Same data as in Fig. 3.  $n = 54$  eGFP- and  $n = 49$  eGFP+ neurons. E-H: trains of stimulations at 50 Hz. Same data as in Fig. 4.  $n = 27$  eGFP- and  $n = 19$  eGFP+ neurons.

A, B, E, F: eIPSCs recorded in eGFP- neurons. C, D, G, H: eIPSCs recorded in eGFP+ neurons. Average amplitudes from individual neurons are in grey.

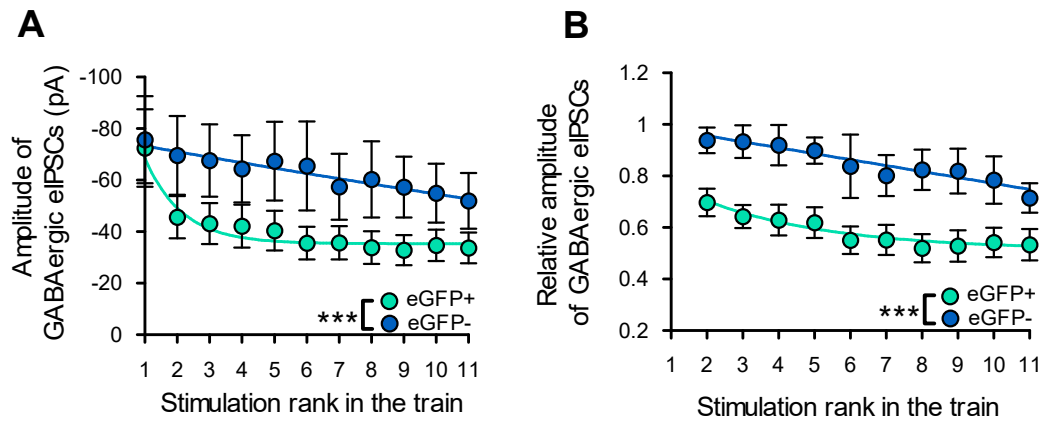


**Supplementary figure S5.**

**Relative amplitude of rank 2 eIPSCs in the first five and the last five trains.**

A. During 5 Hz trains, eIPSC<sub>2</sub>/eIPSC<sub>1</sub> remained similar in the first five and the last five trains for connections on both eGFP<sup>-</sup> and eGFP<sup>+</sup> neurons (PeGFP<sup>-</sup> = 0.678, PeGFP<sup>+</sup> = 0.223, Wilcoxon Signed Rank Test for Paired Data). B. During 50 Hz trains, eIPSC<sub>2</sub>/eIPSC<sub>1</sub> remained similar in the first five and the last five trains for connections on eGFP<sup>+</sup> neurons (PeGFP<sup>+</sup> = 0.098, Wilcoxon Signed Rank Test for Paired Data), but significantly decreased by 18% for eGFP<sup>-</sup> neurons (PeGFP<sup>-</sup> = 0.048, Wilcoxon Signed Rank Test for Paired Data). This suggests that the facilitation observed in eGFP<sup>-</sup> neurons during paired-pulse stimulation at short intervals (Fig. 2E) is replaced by other types of plasticities during repeated trains of stimulation at 50 Hz.



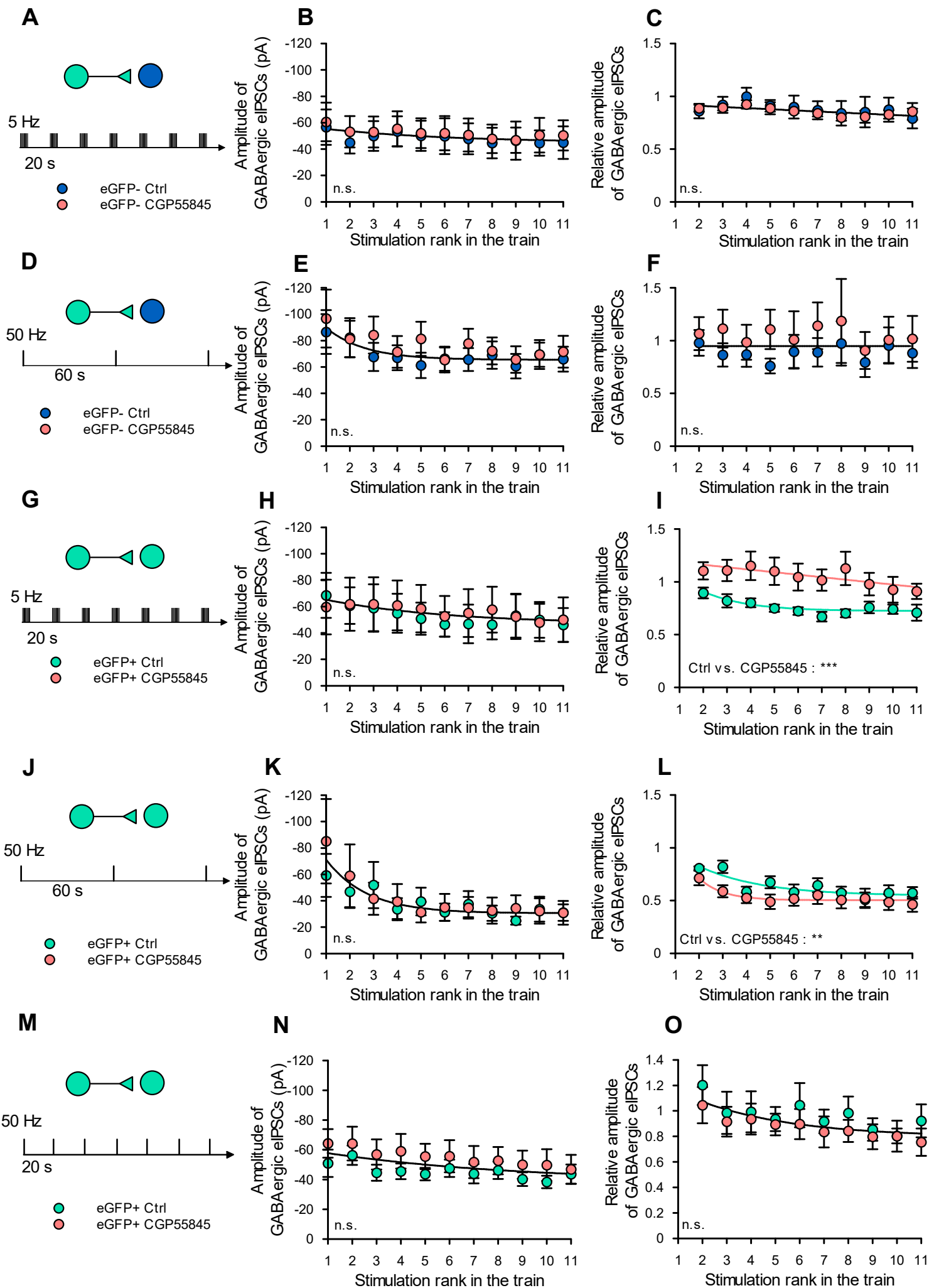


**Supplementary figure S6.**

**Short-term plasticity of GABAergic connections during trains of stimulations at 5 Hz with increased amplitudes of stimulation.**

For this experiment, stimulation amplitudes were set at 0.45 mA (versus 0.21 mA on average for all other experiments). A. Average raw eIPSCs amplitudes of GABAergic eIPSCs recorded in eGFP- neurons (blue) and eGFP+ neurons (green). B. Relative amplitudes of GABAergic eIPSCs recorded in eGFP- and eGFP+ neurons (amplitudes normalized after eIPSCs of rank 1, same neurons as in A). n = 11 eGFP- and n = 11 eGFP+ neurons.

Evolution of eIPSCs amplitudes during the trains were significantly different in eGFP- and eGFP+ neurons as it was observed with lower amplitudes of stimulation (Fig. 3). This indicated that changes in stimulation efficacy during trains of stimulations were unlikely to influence our results.



**Supplementary figure S7.**

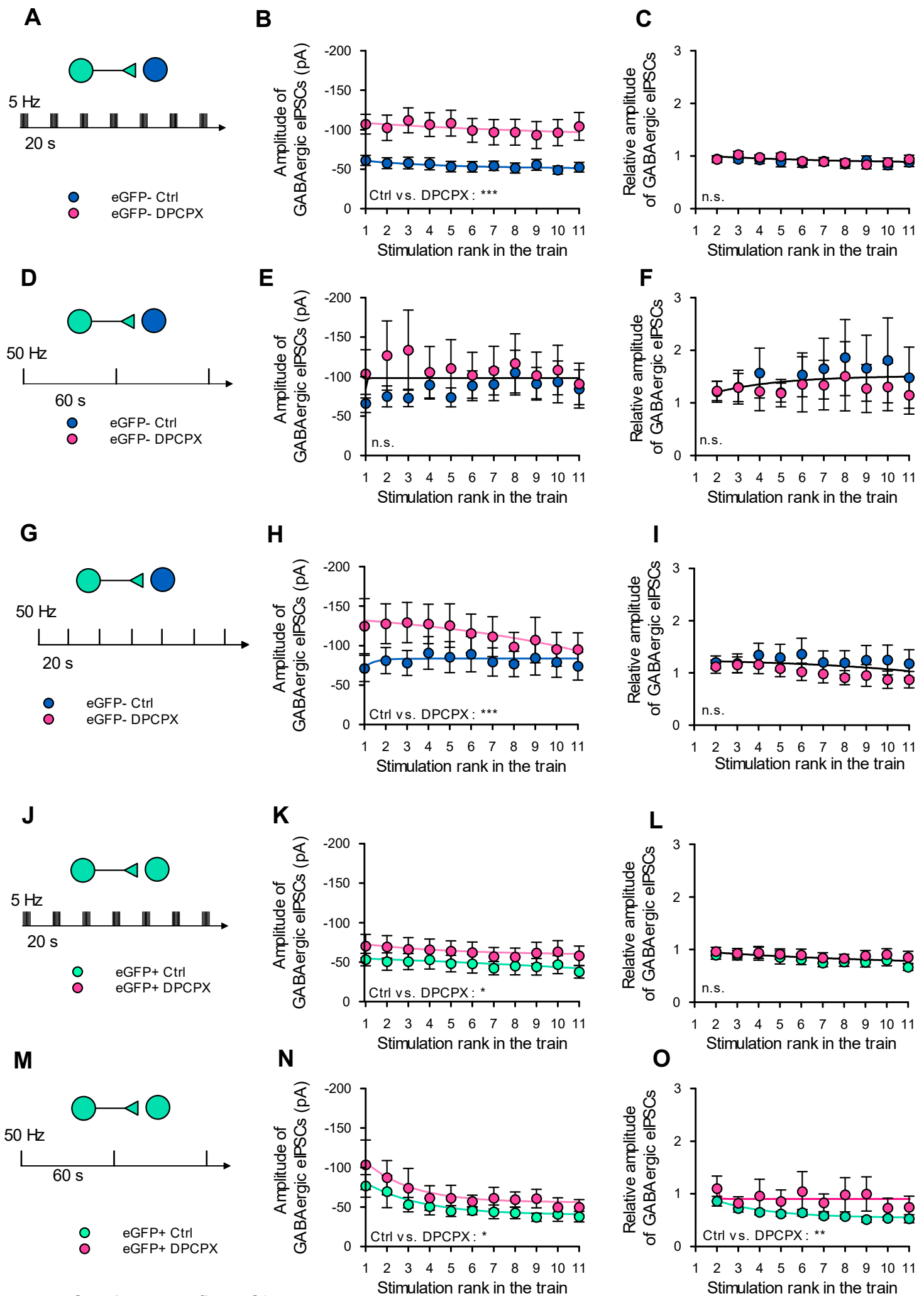
**Effect of a GABAB receptor antagonist on GABAergic eIPSCs during 5 Hz and 50 Hz trains.**

legend next page

### Supplementary figure S7.

#### Effect of a GABA<sub>B</sub> receptor antagonist on GABAergic eIPSCs during 5 Hz and 50 Hz trains.

Average amplitudes of GABAergic eIPSCs during trains at 5 Hz and 50 Hz before (blue and green) and during bath application of 10  $\mu$ M CGP55845 (orange), a GABA<sub>B</sub> receptors antagonist. For each neuron, trains of 11 stimulations were repeated 10 times in control condition and 10 times during bath application of CGP55845. A, D, G, J, M. Type of neuron (eGFP- or eGFP+), frequency of stimulation (5 Hz or 50 Hz) and interval between trains (20 s or 60 s) applied in B-C, E-F, H-I, K-L, N-O, respectively. B, E, H, K, N. Average raw eIPSCs amplitudes. C, F, I, L, O. Average relative eIPSCs amplitudes. A-F: CGP55845 had no effect on GABAergic connections onto eGFP- neurons, neither during trains at 5 Hz (A-C, n = 6 neurons) nor during trains at 50 Hz (D-F n = 12 neurons). G-O: in eGFP+ neurons CGP55845 did not changed raw eIPSCs amplitudes (H, K, N), but it significantly increased relative eIPSCs amplitude during 5 Hz trains repeated every 20 s (I, n = 8 neurons), indicating a phasic inhibition involving GABA<sub>B</sub> receptors during these trains. Evolution of eIPSCs amplitudes during trains were compared with non-linear regression. Details of regressions and the corresponding analysis are given in Supplementary Table 7. P > 0.5: n.s. and P < 0.001: \*\*\*.



**Supplementary figure S8.**

**Effect of an A1 adenosine receptor antagonist on GABAergic eIPSCs during 5 Hz and 50 Hz trains.**

legend next page

### Supplementary figure S8.

#### Effect of an A1 adenosine receptor antagonist on GABAergic eIPSCs during 5 Hz and 50 Hz trains.

legend next page

Average amplitudes of GABAergic eIPSCs during trains at 5 Hz and 50 Hz before (blue and green) and during bath application of 10  $\mu$ M DPCPX (pink), an A1 receptor antagonist. For each neuron, trains of 11 stimulations were repeated 10 times in control condition and 10 times during bath application of DPCPX. A, D, G, J, M. Type of neuron (eGFP- or eGFP+), frequency of stimulation (5 Hz or 50 Hz) and interval between trains (20 s or 60 s) applied in B-C, E-F, H-I, K-L, N-O, respectively. B, E, H, K, N. Average raw eIPSCs amplitudes. C, F, I, L, O. Average relative eIPSCs amplitudes. B, H. DPCPX significantly increased raw eIPSCs amplitudes recorded in eGFP- neurons during trains at 5 Hz repeated every 20 s (B, n = 9 neurons) and 50 Hz repeated every 20 s (H, n = 9 neurons) but had no effect on raw eIPSCs amplitudes recorded during trains at 50 Hz repeated every 60 s (E, n = 7 neurons). This indicated a tonic inhibition of GABAergic connections onto eGFP- neurons by adenosine depending on the delay between trains of stimulation. K, N, in eGFP+ neurons, DPCPX significantly increased raw eIPSCs amplitudes during 5 Hz trains repeated every 20 s (K) and 50 Hz trains repeated every 60 s (N). This indicated a tonic inhibition of GABAergic connections onto eGFP+ neurons. C, F, I, L, O Except during 50 Hz trains repeated every 60 s where a weak significant effect was recorded (O), no effect of DPCPX was detected in relative eIPSCs amplitudes, neither in eGFP- nor in eGFP+ neurons indicating that inhibition involving A1 receptors did not change during the train. Evolution of eIPSCs amplitudes during trains were compared with non-linear regression. Details of regressions and the corresponding analysis are given in Supplementary Table 8.  $P > 0.5$ : n.s.,  $0.05 > P > 0.01$ : \*,  $P < 0.001$ : \*\*\*.

**Supplementary Table 1. Non linear regressions FIGURE 2**

Figure	Stimulation protocol				Model parameters determined by non linear curve fitting											Model comparison stats							
	Paired-Pulse ISI: 20, 50, 75, 100, 200, 300 ms	Type of data	eGFP condition	Drug condition	Model	N neurons	N	obs	k	A1 eGFP-	A2 eGFP-	A3 eGFP-	A1 eGFP+	A2 eGFP+	A3 eGFP+	SS	df	AIC	AICc	BIC	F	p	dAICc
2E		PPR	- and +	Control	"equation 2" pooled data	63-	223	3	1.29	1.01	34.04	1.29	1.01	34.04	25.41	220	156.48	164.67	170.11				
					sum of two "equation 2"	52+	223	6	1.53	1.07	28.46	1.06	0.95	68.52	24.04	217	150.12	164.65	173.97	4.1222	<b>7.20E-03</b>	<b>0.02</b>	

**Supplementary Table 2. Non linear regressions FIGURE 3**

Figure	Stimulation protocol				Model parameters determined by non linear curve fitting											Model comparison stats								
	Frequency protocol interval	Protocol repetition	Type of data	eGFP condition	Drug condition	Model	N neurons	N	obs	k	A1 eGFP-	A2 eGFP-	A3 eGFP-	A1 eGFP+	A2 eGFP+	A3 eGFP+	SS	df	AIC	AICc	BIC	F	p	dAICc
S3A	5 Hz	20 s	1-10	Raw	- and +	Control	sum of two exponential fits, same intercept A1	54-	1133	5	-58.06	7.80	6.46	-58.06	18.23	2.56	928309.68	1128	10828.04	10840.12	10858.24			
							sum of two "Equation3"	49+	1133	6	-60.20	8.32	3.12	-55.42	16.42	3.38	927586.17	1127	10829.16	10843.26	10864.39	0.8791	3.49E-01	-3.14
S3A	5 Hz	20 s	1-10	Raw	- and +	Control	"Equation3" pooled data	54-	1133	3	-57.93	12.17	3.29	-57.93	12.17	3.29	957197.83	1130	10858.76	10866.80	10878.89			
							sum of two "Equation3"	49+	1133	6	-60.20	8.32	3.12	-55.42	16.42	3.38	927586.17	1127	10829.16	10843.26	10864.39	11.9925	<b>9.88E-08</b>	<b>23.54</b>
S3A	5 Hz	20 s	1-10	Raw	-	Control	Linear fit slope=0	54-	594	1	-54.56	0.00				573961.45	593	5772.52	5776.54	5781.30				
							Exponential (Equation3)	594	3	-60.20	8.32	3.12				570329.12	591	5772.75	5780.82	5790.30	1.8820	1.53E-01	-4.28	
S3A	5 Hz	20 s	1-10	Raw	+	Control	Linear fit slope=0	49+	539	1				-44.60	0.00		369871.83	538	5053.93	5057.95	5062.51			
							Exponential (Equation3)	539	3				-55.42	16.42	3.38	357257.05	536	5039.23	5047.30	5056.39	9.4631	<b>9.14E-05</b>	<b>10.65</b>	
3B, S3B	5 Hz	20 s	1-10	Norm	- and +	Control	"Equation3" pooled data	54-	1030	3	0.96	-0.13	3.79	0.96	-0.13	3.79	93.83	1027	463.28	471.32	483.03			
							sum of two "Equation3"	49+	1030	6	0.99	-0.07	3.48	0.92	-0.19	3.99	87.89	1024	401.99	416.10	436.56	23.0422	<b>1.94E-14</b>	<b>55.21</b>
3B, S3B	5 Hz	20 s	1-10	Norm	-	Control	Linear fit slope=0	54-	540	1	0.95	0.00				66.92	539	408.87	412.89	417.45				
							Exponential (Equation3)	540	3	0.99	-0.07	3.48				66.72	537	411.27	419.34	428.44	0.7974	4.51E-01	-6.451	
3B, S3B	5 Hz	20 s	1-10	Norm	+	Control	Linear fit slope=0	49+	490	1				0.80	0.00		22.66	489	-111.61	-107.59	-103.22			
							Exponential (Equation3)	490	3				0.92	-0.19	3.99	21.33	487	-137.19	-129.11	-120.42	15.1532	<b>4.13E-07</b>	<b>21.52</b>	
3C	5 Hz	20 s	1-10	Raw	-	Control	"Equation3" pooled data	50-	1100	3	-61.52	9.25	2.74	-61.52	9.25	2.74	1141933.80	1097	10769.35	10777.39	10789.36			
							sum of two "Equation3"		1100	6	-63.28	10.39	1.65	-59.81	12.94	9.61	1141274.39	1094	10774.71	10788.82	10809.74	0.2107	8.89E-01	-11.43
3D	5 Hz	20 s	1-10	Raw	+	Control	"Equation3" pooled data	43+	946	3	-55.61	15.11	3.31	-55.61	15.11	3.31	748490.65	943	9005.83	9013.87	9025.24			
							sum of two "Equation3"		946	6	-54.05	16.14	3.78	-57.19	14.23	2.89	745656.50	940	9008.24	9022.36	9042.21	1.1909	3.12E-01	-8.49

**Supplementary Table 3. Supplementary Figure 6 Non linear regressions stimulation at 5Hz at increased stimulation amplitude**

Figure	Stimulation protocol				Model parameters determined by non linear curve fitting											Model comparison stats								
	Frequency protocol interval	Protocol repetition	Type of data	eGFP condition	Drug condition	Model	N neurons	N	obs	k	A1 eGFP-	A2 eGFP-	A3 eGFP-	A1 eGFP+	A2 eGFP+	A3 eGFP+	SS	df	AIC	AICc	BIC	F	p	dAICc
S6A	5 Hz	20 s	1-10	Raw	- and +	Control	"Equation3" pooled data	11-	242	3	-71.30	26.74	2.53	-71.30	26.74	2.53	351728.28	239	2456.93	2465.10	2470.89			
							sum of two "Equation3"	11+	242	6	-71.22	35.90	1.14	-73.38	160.72	73.12	319721.69	236	2439.84	2454.32	2464.27	7.8751	<b>4.98E-05</b>	<b>10.78</b>
S6B	5 Hz	20 s	1-10	Norm	- and +	Control	"Equation3" pooled data	11-	220	3	0.82	-0.29	8.59	0.82	-0.29	8.59	14.31	217	31.21	39.40	44.78			
							sum of two "Equation3"	11+	220	6	0.95	-114.51	4954.04	0.70	-0.19	3.58	10.40	214	-33.11	-18.58	-9.35	26.8655	<b>8.70E-15</b>	<b>57.98</b>



Supplementary Table 7. Non linear regressions FIGURE 6 and supplementary figure S7

Figure	Stimulation protocol						Model parameters										Model comparison stats						
	Freq.	protocol interval	Protocol repetitio	Type of data	eGFP condition	Drug condition	Model	N neurons	N obs	k	A1 control	A2 control	A3 control	A1 CGP55845	A2 CGP55845	A3 CGP55845	SS	df	AIC	AICc	BIC	F	p
S7B	5 Hz	20 s	1-10	Raw	-	Control & "Equation3" pooled data	6	132	3	-55.81	10.62	5.56	-55.81	10.62	5.56	96495.70	129	1253.07	1261.38	1264.60			
						CGP55845 sum of two "Equation3"	6	132	6	-52.61	3459.37	4153.92	-59.07	10.23	2.98	96024.87	126	1258.42	1273.32	1278.60	0.2059	8.92E-01	-11.94
6A, S7C	5 Hz	20 s	1-10	Norm	-	Control & "Equation3" pooled data	6	120	3	0.92	-1.32	120.00	0.92	-1.32	120.00	3.63	117	-71.40	-63.05	-60.25			
						CGP55845 sum of two "Equation3"	6	120	6	0.93	-1.75	145.23	0.91	-0.13	8.31	3.61	114	-66.02	-51.02	-46.50	0.1962	8.99E-01	-12.03
S7E	50 Hz	60 s	1-10	Raw	-	Control & "Equation3" pooled data	12	264	3	-91.97	24.14	1.64	-91.97	24.14	1.64	427700.08	261	2708.22	2716.37	2722.52			
						CGP55845 sum of two "Equation3"	12	264	6	-88.18	23.18	1.42	-95.27	25.24	2.12	424394.75	258	2712.17	2726.61	2737.20	0.6698	5.71E-01	-10.23
6B, S7F	50 Hz	60 s	1-10	Norm	-	Control & "Equation3" pooled data	12	240	3	1.02	-0.06	0.04				98.52	237	475.41	483.58	489.33			
						CGP55845 sum of two "Equation3"	12	240	6	0.97	-0.10	0.06	1.09	-0.12	12.47	96.74	234	477.02	491.51	501.39	1.4381	2.32E-01	-7.928
S7H	5 Hz	20 s	1-10	Raw	+	Control & "Equation3" pooled data	8	176	3	-64.68	18.77	5.30	-64.68	18.77	5.30	326569.82	173	1832.03	1840.26	1844.71			
						CGP55845 sum of two "Equation3"	8	176	6	-68.78	21.47	2.39	-61.77	-7.10	-9.55	325296.18	170	1837.34	1852.01	1859.53	0.2219	8.81E-01	-11.74
6C, S7I	5 Hz	20 s	1-10	Norm	+	Control & "Equation3" pooled data	8	160	3	1.00	-0.37	13.53				12.67	157	56.28	64.54	68.58			
						CGP55845 sum of two "Equation3"	8	160	6	0.90	-0.19	1.83	1.15	-85.77	3662.80	9.20	154	11.19	25.92	32.71	19.3142	<b>1.11E-10</b>	<b>38.62</b>
S7K	50 Hz	60 s	1-10	Raw	+	Control & "Equation3" pooled data	6	132	3	-71.90	40.46	1.73	-71.90	40.46	1.73	115572.90	129	1276.88	1285.20	1288.41			
						CGP55845 sum of two "Equation3"	6	132	6	-58.81	30.01	3.00	-85.57	53.08	1.30	113173.57	126	1280.11	1295.01	1300.29	0.8904	4.48E-01	-9.82
6D, S7L	50 Hz	60 s	1-10	Norm	+	Control & "Equation3" pooled data	6	120	3	0.77	-0.24	1.86				3.12	117	-89.37	-81.02	-78.22			
						CGP55845 sum of two "Equation3"	6	120	6	0.83	-0.28	2.67	0.72	-0.21	1.00	2.80	114	-96.39	-81.39	-76.88	4.3568	<b>6.05E-03</b>	<b>0.373</b>
S7N	50 Hz	20 s	1-10	Raw	+	Control & "Equation3" pooled data	7	154	3	-58.68	19.42	7.85	-58.68	19.42	7.85	69256.72	151	1385.76	1394.03	1397.91			
						CGP55845 sum of two "Equation3"	7	154	6	-52.86	13.12	5.00	-64.52	30.88	12.81	65781.93	148	1383.83	1398.60	1405.09	2.6059	5.40E-02	-4.57
6E, S7O	50 Hz	20 s	1-10	Norm	+	Control & "Equation3" pooled data	7	140	3	1.08	-0.28	3.90	1.08	-0.28	3.90	13.10	137	73.64	81.93	85.41			
						CGP55845 sum of two "Equation3"	7	140	6	1.17	-0.27	1.91	1.01	-0.34	7.46	12.77	134	76.07	90.92	96.66	1.1538	3.30E-01	-8.982
6CE, S7IO	50Hz	20s Ctrl	1-10	Norm	+	Control & "Equation3" pooled data	7	150	3	1.12	-2.12	80.00				15.44	147	92.67	100.95	104.72			
						CGP55845 sum of two "Equation3"	8	150	6	1.17	-0.27	1.91	1.15	-4.78	200.25	15.12	144	95.48	110.27	116.55	1.0335	3.80E-01	-9.32
6DE, S7LO	50Hz	20s Ctrl	1-10	Norm	+	Control & "Equation3" pooled data	7	130	3	1.01	-0.28	2.45				12.12	127	68.48	76.80	79.95			
						CGP55845 sum of two "Equation3"	6	130	6	1.17	-0.27	1.91	0.83	-0.28	2.67	8.60	124	29.81	44.73	49.88	16.9489	<b>2.75E-09</b>	<b>32.07</b>



Supplementary Table 8. Non linear regressions FIGURE 7 and supplementary figure S8

Figure	Stimulation protocol					Drug condition	Model parameters									Model comparison stats								
	Frequency	protocol interval	Protocol repetition	Type of data	eGFP condition		Model	N neurons	N obs	k	A1 control	A2 control	A3 control	A1 DPCPX	A2 DPCPX	A3 DPCPX	SS	df	AIC	AICc	BIC	F	p	dAICc
<b>7A, S8B</b>	5 Hz	20 s	1-10	Raw	-	Control &	9	198	3	-84.54	14.12	7.73	-84.54	14.12	7.73	347778.43	195	2049.17	2057.38	2062.32				
						DPCPX		198	6	-60.78	9.91	4.15	-108.73	19.22	10.73	236918.36	192	1979.17	1993.76	2002.18	29.9472	<b>6.28E-16</b>	<b>63.62</b>	
<b>S8C</b>	5 Hz	20 s	1-10	Norm	-	Control &	9	180	3	0.97	-0.13	6.15	0.97	-0.13	6.15	7.90	177	-43.96	-35.73	-31.19				
						DPCPX		180	6	0.95	-0.09	3.82	0.99	-0.17	7.79	7.85	174	-39.10	-24.45	-16.75	0.3670	7.77E-01	-11.29	
<b>7B, S8E</b>	50 Hz	60 s	1-10	Raw	-	Control &	7	154	3	-84.67	-13.61	0.04	-84.67	-13.61	0.04	786345.89	151	1759.92	1768.18	1772.06				
						DPCPX		154	6	-65.61	-28.94	3.47	-119.29	7673.78	3955.37	755965.17	148	1759.85	1774.62	1781.11	1.9826	1.19E-01	-6.43	
<b>S8F</b>	50 Hz	60 s	1-10	Norm	-	Control &	7	140	3	1.20	0.31	3.11	1.20	0.31	3.11	197.12	137	453.20	461.50	464.97				
						DPCPX		140	6	1.19	0.56	3.90	1.23	0.06	0.06	194.39	134	457.26	472.10	477.85	0.6257	6.00E-01	-10.6	
<b>7C, S8H</b>	50 Hz	20 s	1-10	Raw	-	Control &	9	198	3	-104.53	-1.83	-3.93	-104.53	-1.83	-3.93	860620.94	195	2228.57	2236.78	2241.73				
						DPCPX		198	6	-71.04	-10.90	0.48	-130.34	-18.57	-8.89	798562.47	192	2219.76	2234.34	2242.77	4.9736	<b>2.40E-03</b>	<b>2.44</b>	
<b>S8I</b>	50 Hz	20 s	1-10	Norm	-	Control &	9	180	3	1.22	-4.07	199.98				64.41	177	333.82	342.05	346.60				
						DPCPX		180	6	1.19	0.06	0.69	1.17	-3.72	100.57	61.59	174	331.77	346.42	354.12	2.6551	5.01E-02	-4.366	
<b>7D, S8K</b>	5 Hz	20 s	1-10	Raw	+	Control &	9	198	3	-62.16	17.69	8.91	-62.16	17.69	8.91	238830.83	195	1974.76	1982.97	1987.91				
						DPCPX		198	6	-52.92	-17.40	-18.80	-71.29	13.45	4.14	226298.52	192	1970.09	1984.68	1993.10	3.5443	<b>1.56E-02</b>	-1.71	
<b>S8L</b>	5 Hz	20 s	1-10	Norm	+	Control &	9	180	3	0.94	-0.27	10.26				14.42	177	64.45	72.68	77.22				
						DPCPX		180	6	0.9199	-2.6885	108.479	0.96761	-0.1152	3.43286	14.03	174	65.55	80.20	87.90	1.60	1.91E-01	-7.52	
<b>7E, S8N</b>	50 Hz	60 s	1-10	Raw	+	Control &	7	154	3	-90.97	45.05	2.33	-90.97	45.05	2.33	199289.92	151	1548.53	1556.80	1560.68				
						DPCPX		154	6	-77.94	39.98	2.62	-104.10	50.32	2.12	188222.67	148	1545.73	1560.50	1566.99	2.9007	<b>3.70E-02</b>	-3.70	
<b>S8O</b>	50 Hz	60 s	1-10	Norm	+	Control &	7	140	3	0.91	-0.30	5.65	0.91	-0.30	5.65	33.82	137	206.44	214.73	218.20				
						DPCPX		140	6	0.84	-0.32	2.63	1.10	-0.21	0.06	30.85	134	199.55	214.40	220.14	4.3077	<b>6.18E-03</b>	<b>0.338</b>	
<b>7F</b>	5 Hz	20 s	1-10	Raw	- and +	DPCPX	9-	198	3	-89.81	15.26	6.69	-89.81	15.26	6.69	419133.26	195	2086.12	2094.33	2099.27				
						9+		198	6	-108.73	19.22	10.73	-71.29	13.45	4.14	343921.71	192	2052.96	2067.55	2075.98	13.9960	<b>2.73E-08</b>	<b>26.78</b>	

## Supplementary tables 1-8.

### Data analysis, model comparison and statistics.

The tables correspond to the different figures illustrating the comparisons. The column **Figure** indicates the panel of the figure illustrating the conditions compared. In these figures, the averaged eIPSCs amplitudes in the two conditions are illustrated, but statistical analysis were performed using averaged eIPSCs amplitudes of each neurons. These values were calculated by averaging eIPSCs amplitudes of the same rank obtained from reiterations of the protocols. The reiterations used for the average are indicated in the column **Protocol repetition** (usually iterations 1-10). For protocols using trains of stimulations, the **frequency** of stimulations is given (5 Hz or 50 Hz) as well as the time between protocols (20 s or 60 s) in the column **Protocol interval**. The **Type of data** column indicates whether data were averaged raw eIPSCs amplitudes (Raw) or averaged normalized amplitudes (Norm). The **eGFP condition** column indicates whether the data compared were from eGFP- (-) or eGFP+ (+) neurons or both (+ and -). The **Drug condition** column indicates whether the data compared were recorded without (**Control**) or with CGP55845 or DPCPX, or both. The **Model** column indicates the two models compared. **N neurons** corresponds to the number of neurons (eGFP- (-) or eGFP+ (+)). **N obs** corresponds to the number of observations. In experiments with trains of stimulations, the number of observations per neuron and per condition was of 11 for raw data and 10 for normalized data. **k** corresponds to the number of parameters in the model. A1, A2 and A3 are the parameters calculated by non-linear regression (two A1, A2 and A3 are given when a sum of two regressions are used). **SS** is the residual sum of squares calculated with the model. **df** is the degree of freedom of the model. **AIC** is Akaike information criterion; **AICc** is the AIC corrected for small samples; **BIC** is the bayesian information criterion. **F** is the value of the F-distribution of the comparison between the two models. **p** is the corresponding p-value. **dAICc** is the decrease in AICc provided by the model indicated in that line with respect to the model in the line above. A positive value indicate that the model correspond to the most parsimonious explanation with respect to the model in the line above.