

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

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- 3 tune excitatory/inhibitory balance in the spinal nociceptive system
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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 $GABA_B$ 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_B 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 Aδ-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, alteration of inhibition and 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 display short-term plasticity in order to process frequency-coded sensory information. 63 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].

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

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

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

82

83 Methods

84 Animals

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

animals were housed at room temperature (22-25°C) with a 12hr light/dark cycle with free access
to food and water. All procedures used were in accordance with laws for laboratory animal
welfare and approved by the local ethical committee of the University of Strasbourg (CREMEAS;
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⁻¹). Under deep 97 anesthesia, intracardiac perfusion was performed with oxygenated ice-cold (~4°C) sucrose 98 artificial cerebrospinal fluid (sACSF) bubbled with carbogen ($95\% O_2$, $5\% CO_2$) containing (in mM): 99 248 sucrose, 26 NaHCO₃, 11 glucose, 2 KCl, 2 CaCl₂, 1.3 MgSO₄, 1.25 KH₂PO₄, 2.5 kynurenic acid. 100 The lumbar part (L3-L5) of the spinal cord was removed by laminectomy and 400 μ M 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 CaCl₂, 2 MgCl₂, 26 NaHCO₃, 1.25 NaH₂PO₄, 10 glucose.

104

105 Patch-clamp recordings

After recovery (~1 h), slices were transferred to the recording chamber, maintained at $32 \pm 1^{\circ}$ C and continuously perfused by oxygenated ACSF at 3-4 ml/min. In order to isolate GABAergics IPSCs, glycinergic and glutamatergic ionotropic transmissions were blocked by adding in ACSF 1 μ M strychnine and 10 μ M 6-cyano-7nitroquinoxaline-2,3-dione (CNQX) respectively. Whole-cell patch recordings were made from inhibitory (eGFP+) and putative excitatory (eGFP-) LII neurons. Recording and extracellular stimulation electrodes (4-7 M Ω) were pulled from borosilicate glass 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

Our recordings started in the current-clamp mode. Holding current was adjusted in order to keep 124 125 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 μ M bicuculline and 1 μ 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-fibrers 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 ± 0.01 mA) via a patch-pipette filled with ACSF. This stimulation electrode was placed at a distance of 139 140 20-150 µm 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 elPSCs.

The same criteria were applied for simultaneous recordings of pairs of eGFP- and eGFP+ neuronsconnected by the same presynaptic neuron.

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

156

157 Data quantification and analysis

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

Averaging amplitudes of raw eIPSCs gives greater weight to connections in which eIPSCs display larger amplitudes. Therefore, in addition to analyzing raw eIPSCs amplitudes, we calculated normalized eIPSCs amplitudes. These were calculated by normalizing for each neuron the averaged eIPSCs amplitudes to the averaged amplitude of the first eIPSCs in the train. This allowed to analyze relative changes in amplitude during trains regardless of the initial raw 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 (τ_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 τ_f and τ_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_B 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 ±

192 SEM.

For some experiments, we recorded miniature IPSCs. These synaptic events were detected using WinEDR (V3.8.6, Strathclyde Electrophysiology Software, John Dempster, University of Strathclyde, Glasgow, UK) with an amplitude threshold detection algorithm and were visually inspected for validity. Peak amplitude of miniature IPSCs was determined using WinWCP (version 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

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

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

For paired-pulse protocols, the proportions of neurons displaying a facilitation (PPR>1.1), a depression (PPR<0.9) or no change in PPR (0.9-1.1) were analyzed with a log-linear analysis performed on the 3-way contingency table of the proportion of facilitation, ISI and eGFP+/eGFPcondition using Statistica 13 (StatSoft, USA). The χ^2 values and p-values given in the text correspond to those of marginal associations between the two parameters examined. To compare amplitudes of the two first eIPSCs during stimulation trains protocols, Wilcoxon Signed 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)*EXP(-X/A3))

where A1 corresponded to Y value for ISI=0; A2 corresponded to the asymptotic value of the fit;
and A3 corresponded to the exponential decay constant of PPR value as a function of ISI.
Parameters A1, A2 and A3 were determined by nonlinear curve fitting using KyPlot 6.0 (KyensLab,
Tokyo, Japan). A global fit on the same data was also performed using the same equation. This
global fit was compared to the sum of fits from separated data obtained from eGFP+ and from
eGFP- neurons.

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

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

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

where A1 corresponded to Y value of the 1st eIPSCs of the train, A2 corresponded to the change in amplitude for the curve asymptote, A3 corresponded to the constant of the exponential function.

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

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 [38].

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 [38]. Fits of pooled data are illustrated (in black) when the two conditions were not different whereas individual fits are illustrated (in color) when the two

conditions were significantly different. For illustration purpose, these fits are illustrated together
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 < p254 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 µM strychnine and 10 µM CNQX were present 261 in all our electrophysiologial experiments to isolate GABAergic elPSCs. CGP55845 ((2S)-3-[[(1S)-262 1-(3,4-Dichlorophenyl)ethy]amino-2hydroxypropyl](phenylmethyl)phosphinic acid 263 hydrochloride; 10 μ M, Tocris) and DPCPX (8-Cyclopentyl-1,3-dipropylxanthine solid; 10 μ M, 264 Sigma) were used to block GABA_B receptors and Adenosine A1 receptors, respectively. In a subset 265 of experiments, eEPSCs were recorded in presence of strychnine (1 μ M) and bicuculline (10 μ M, 266 sigma). Miniature IPSCs were recorded in the presence of tetrodotoxin (TTX) (0.5μM, 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

antagonists, analysis of changes in normalized eIPSCs amplitudes allowed to detect modulations
 engaged during the train (phasic modulation). All substances were diluted to their final
 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 eFGP- and eGFP+ neurons were significantly different (Fisher's exact test, $P = 6.3 \cdot 10^{-1}$ 284 ⁶, 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 ± 13.0 % in eGFP- neurons (n 288 = 7) and of 100.0 ± 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.

292 GABAergic synaptic transmission in LII

Average amplitude of pharmacologically-isolated eIPSCs (Fig. 2A) was not significantly different when recorded in eGFP- neurons (-60.5 ± 5.1 pA; n = 54) or in eGFP+ neurons (-55.6 ± 4.4 pA; n = 49); unpaired Student's *t*-test, *t* = -0.709, df = 101, P = 0.48. eIPSCs were evoked at similar stimulation amplitude intensity in eGFP- neurons (0.21 ± 0.01 mA; n = 53) or in eGFP+ (0.21 ± 0.01 mA; n = 45), unpaired Student's *t*-test, *t* = 0.176, df = 96, P = 0.86. The weighted decay time constant (τ_w) measured for isolated eIPSCs (Fig. 2B) was not significantly different when recorded in eGFP- neurons (32.8 ± 2.8 ms; n = 29) or in eGFP+ neurons

- 300 (31.1 ± 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
- 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

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

307 Short-term synaptic plasticity was first examined using paired-pulse stimulation protocols with308 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; χ^2 = 11.97; P = 0.003), with a larger proportion of paired-pulse facilitation in eGFP-313 neurons.

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

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

324

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

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

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

Rank Test for Paired Data, Fig. S6). Therefore, for each neuron, amplitudes of eIPSCs of the samerank 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 eGFPneurons and 11 eGFP+ neurons, $P_{normalized} = 8.70 \cdot 10^{-15}$, Table S3, Fig. S5) indicating that changes 343 344 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 (Pnormalized= 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 ($P_{normalized} = 2.04 \cdot 10^{-6}$, 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.

We examined the short-term synaptic plasticity expressed by GABAergic connections onto LII neurons during their repetitive activation at high frequency (n = 27 eGFP- neurons and n = 19 eGFP+ neurons). We applied trains of 11 stimulations at 50 Hz (Fig. 4A). These trains were 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 (PeGFP-362 = 0.278; P_{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 ($P_{eGFP-} = 0.048$, $P_{eGFP+} = 0.098$, 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 = 3.73·10⁻⁸, Table S4, Fig. S3C). In eGFP- neurons, amplitudes of normalized eIPSCs did not 372 373 significantly change indicating that no plasticity was expressed during the train (P_{normalized} = 0.647, 374 Table S1.4). By contrast, the amplitude of normalized eIPSCs recorded in eGFP+ neurons 375 significantly decreased during the train ($P_{normalized} = 9.03 \cdot 10^{-6}$, 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 = 2.76·10⁻¹⁰, Table S5, Fig. 3B, 4B) indicating that the characteristics of the effects depended on the 384 385 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 values were reached at the 11th stimulation with 5 Hz protocols and before the 5th stimulation 389 390 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 elPSCs during trains at 50 Hz

During 50 Hz trains, the eIPSCs had not fully returned to baseline at the onset of the following stimulation, a situation that resulted in their summation. We therefore reanalyzed our data measuring summed eIPSCs amplitudes from the basal holding current of each train (Fig. 4C, S3E; statistics Table S4). Evolution of summed eIPSCs amplitudes during 50 Hz trains was significantly different in eGFP- or eGFP+ neurons for both normalized amplitudes (P = $4.90 \cdot 10^{-7}$, Fig. 4C) and 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 ($P_{normalized} = 1.15 \cdot 10^{-5}$, Fig. 3E; $P_{raw} = 3.98 \cdot 10^{-10}$, 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 amplitudes. Importantly, only 86.5 % of the asymptotic value was reached at the end of the train (i.e. 11^{th} eIPSC) suggesting that further summation was still possible with longer lasting trains. In eGFP+ neurons, no significant increase was detected for normalized summed eIPSCs (P_{normalized} = 0.076, Fig. 4C) but raw summated eIPSCs significantly increased during 50 Hz trains (P_{raw} = 0.67·10⁻³, Fig. S3E). In these neurons, the increase in raw summed amplitude was of 44.47 pA and 95 % of the asymptotic value was reached before the 6th eIPSC indicating that maximal summation is reached with few eIPSCs.

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

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

419

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

We next defined whether connections from a given presynaptic neuron displayed distinct shortterm plasticities when the postsynaptic neuron was eGFP- or eGFP+. To this end, we performed simultaneous recordings of eGFP- and eGFP+ neurons and searched for a presynaptic neuron connecting the recorded ones. For each pair of neuron, we substracted the normalized eIPSCs amplitudes recorded on eGFP+ neurons from those recorded on eGFP- neurons (Fig. 5). For both 5 Hz and 50 Hz trains, the relative eIPSCs amplitude difference was significantly larger

427 than zero ($P_{5Hz} = 1.26 \cdot 10^{-5}$, n = 6 pairs; $P_{50Hz} = 6.21 \cdot 10^{-6}$, 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_B receptors

434 We next assessed whether the short-term plasticity of GABAergic connections onto LII neurons 435 may involve GABA_B 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_B 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 (PeGFP-, 5 Hz = 0.892, 441 n = 6; $P_{eGFP-, 50 Hz} = 0.571$, n = 12; $P_{eGFP+, 5 Hz} = 0.881$, n = 8; $P_{eGFP+, 50 Hz} = 0.448$; Fig. S7B, E, H, K, N), 442 indicating that GABA_B receptors were not tonically controlling these connections under these 443 conditions.

Since GABA_B receptors may be activated in a phasic manner by the GABA released during the train of activity, we also analyzed effects of CGP55845 on eIPSCs amplitudes normalized to the firsts eIPSCs of the control trains (Fig. 6, S7, Table S8). Normalized eIPSCs recorded in eGFPneurons were not modified in presence of CGP55845 during both trains at 5 Hz and at 50 Hz ($P_{eGFP-, 5 Hz} = 0.900$, n = 6; $P_{eGFP-, 50 Hz} = 0.232$, n = 12, Fig. 6A, B). Interestingly, in presence of CGP55845, amplitudes of normalized eIPSCs recorded in eGFP+ neurons during trains at 5 Hz were significantly increased by 38 % with respect to control trains ($P = eGFP+, 5 Hz = 1.11 \cdot 10^{-10}$, n = 451 8, Fig. 6C). A weak effect of CGP55845 was observed during trains at 50 Hz ($P_{eGFP+, 50 \text{ Hz}} = 6.05 \cdot 10^{-10}$ 452 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.

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

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

467

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

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

474 In eGFP- neurons (Fig. 7A-C), DPCPX significantly increased raw eIPSCs amplitudes during trains at 5 Hz repeated every 20 s ($P_{eGFP-, 5 Hz} = 6.28 \cdot 10^{-16}$, n = 9, Fig. 7A) and trains at 50 Hz repeated 475 every 20 s ($P_{eGFP-, 50 \text{ Hz}} = 2.40 \cdot 10^{-3}$, n = 9, Fig. 7C) but not during trains at 50 Hz repeated every 60 476 477 s (P 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_{eGFP-, 5 Hz} = 0.777, n = 9, Fig. S8C), nor at 50 Hz every 20 s (P_{eGFP-, 50 Hz} = 0.050, n = 9, Fig. S8I), 480 nor at 50 Hz every 60 s (P 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 ($P_{eGFP+, 5 Hz} = 1.56 \cdot 10^{-2}$, n = 9, Fig. 7D) and trains at 50 Hz repeated every 60 s $(P_{eGFP+, 50 Hz} = 3.70 \cdot 10^{-2})$, n = 7, Fig. 7E). At both frequencies, the effect of DPCPX on eGFP+ neurons 487 488 was statistically significant (p-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 (P_{eGFP+, 5 Hz} = 0.191, n = 9, Fig. S8L), but a weak effect was detected at 50 Hz every 60 s (P_{eGFP+, 50 Hz} 492 $= 3.70 \cdot 10^{-2}$, n = 7, Fig. S8O). Effects on raw but not normalized amplitudes indicated that 493 494 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 ±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.

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

Local inhibitory connections between LII neurons are mostly GABAergic, glycinergic transmission occurring in only 1 / 15 inhibitory contacts recorded by Lu and Perl [33], and possibly in 1 / 3 inhibitory contacts recorded by Labrakakis et al. [27]. We therefore focused on GABAergic connections since we aimed at examining the short-term plasticity of inhibitory connections between LII neurons. Interestingly, spontaneously active inhibitory connections are dominated by GABAergic transmission in most LIIo neurons, whether these are dominated by glycinergic transmission in most neurons at laminae IIi/III border [48], glycinergic connections onto LII neurons most likely originating from neurons the cell bodies of which are localized in otherlaminae.

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_B 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].

Interestingly, in recordings performed at room temperature, GABAergic eIPSCs recorded in eGFP+ neurons display slower decay kinetics than those recorded in eGFP- neurons [28]. Such slower kinetic would allow a better summation of overlapping eIPSCS. Nevertheless, in our conditions, eIPSCs kinetics were similar in eGFP+ and eGFP- neurons. Moreover, at high frequency, summated eIPSCs in eGFP- neurons reached larger amplitudes than summated eIPSCs in eGFP+ neurons, ruling out an involvement of different decay kinetics to explain a higher degree of summation of eIPSCs in eGFP- neurons.

587

588 Modulation by GABA_B 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 GABAB 600 receptors can be engaged in a phasic depression of GABAergic eIPSCs during trains of activity. 601 This depression involving GABA_B receptors is target-specific: occurring only in connections onto 602 eGFP+ neurons. Interestingly, although $GABA_B$ 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).

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

Interestingly, intrathecal administration of GABA_B and A1 agonists have antinociceptive effects
[21; 45; 46] which may involve the types of modulation of short-term plasticity described in the
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_B receptors might represent interesting targets in pain-alleviating strategies.

636 **Conflict of interest statement**

637 The authors declare no competing financial interests.

638

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646 **References**

- 647 [1] Adrian ED. The recovery process of excitable tissues: Part I. J Physiol 1920;54(1-2):1-31.
- 648 [2] Adrian ED, Zotterman Y. The impulses produced by sensory nerve endings: Part 3. Impulses
 649 set up by Touch and Pressure. J Physiol 1926;61(4):465-483.
- [3] Anwar H, Li X, Bucher D, Nadim F. Functional roles of short-term synaptic plasticity with an
 emphasis on inhibition. Curr Opin Neurobiol 2017;43:71-78.
- [4] Bartley AF, Dobrunz LE. Short-term plasticity regulates the excitation/inhibition ratio and the
 temporal window for spike integration in CA1 pyramidal cells. Eur J Neurosci
 2015;41(11):1402-1415.
- [5] Buonomano DV. Decoding temporal information: A model based on short-term synaptic
 plasticity. J Neurosci 2000;20(3):1129-1141.
- [6] Buonomano DV. The biology of time across different scales. Nat Chem Biol 2007;3(10):594-597.

- [7] Campero M, Serra J, Ochoa JL. C-polymodal nociceptors activated by noxious low
 temperature in human skin. J Physiol 1996;497 (Pt 2):565-572.
- [8] Chery N, De Koninck Y. GABA(B) receptors are the first target of released GABA at lamina I
 inhibitory synapses in the adult rat spinal cord. J Neurophysiol 2000;84(2):1006-1011.
- [9] Cordero-Erausquin M, Coull JA, Boudreau D, Rolland M, De Koninck Y. Differential maturation
 of GABA action and anion reversal potential in spinal lamina I neurons: impact of chloride
 extrusion capacity. J Neurosci 2005;25(42):9613-9623.
- [10] Cui L, Kim YR, Kim HY, Lee SC, Shin HS, Szabo G, Erdelyi F, Kim J, Kim SJ. Modulation of
 synaptic transmission from primary afferents to spinal substantia gelatinosa neurons by
 group III mGluRs in GAD65-EGFP transgenic mice. J Neurophysiol 2011;105(3):1102 1111.
- [11] Daniele CA, MacDermott AB. Low-threshold primary afferent drive onto GABAergic
 interneurons in the superficial dorsal horn of the mouse. J Neurosci 2009;29(3):686-695.
- [12] Dunwiddie TV, Diao L, Proctor WR. Adenine nucleotides undergo rapid, quantitative
 conversion to adenosine in the extracellular space in rat hippocampus. J Neurosci
 1997;17(20):7673-7682.
- [13] Froemke RC, Merzenich MM, Schreiner CE. A synaptic memory trace for cortical receptive
 field plasticity. Nature 2007;450(7168):425-429.
- [14] Fukuhara K, Katafuchi T, Yoshimura M. Effects of baclofen on mechanical noxious and
 innocuous transmission in the spinal dorsal horn of the adult rat: in vivo patch-clamp
 analysis. Eur J Neurosci 2013;38(10):3398-3407.
- [15] Grudt TJ, Henderson G. Glycine and GABAA receptor-mediated synaptic transmission in rat
 substantia gelatinosa: inhibition by mu-opioid and GABAB agonists. J Physiol 1998;507 (
 Pt 2):473-483.
- 683 [16] Grudt TJ, Perl ER. Correlations between neuronal morphology and electrophysiological
 684 features in the rodent superficial dorsal horn. J Physiol 2002;540(Pt 1):189-207.
- 685 [17] Guan Y. Spinal cord stimulation: neurophysiological and neurochemical mechanisms of
 686 action. Curr Pain Headache Rep 2012;16(3):217-225.
- [18] Hantman AW, van den Pol AN, Perl ER. Morphological and physiological features of a set of
 spinal substantia gelatinosa neurons defined by green fluorescent protein expression. J
 Neurosci 2004;24(4):836-842.
- [19] Heinke B, Ruscheweyh R, Forsthuber L, Wunderbaldinger G, Sandkuhler J. Physiological,
 neurochemical and morphological properties of a subgroup of GABAergic spinal lamina II
 neurones identified by expression of green fluorescent protein in mice. J Physiol
 2004;560(Pt 1):249-266.

- 694 [20] Hugel S, Schlichter R. Convergent control of synaptic GABA release from rat dorsal horn
 695 neurones by adenosine and GABA autoreceptors. J Physiol 2003;551(Pt 2):479-489.
- 696 [21] Jacobson KA, Gao ZG. Adenosine receptors as therapeutic targets. Nat Rev Drug Discov
 697 2006;5(3):247-264.
- [22] Jensen MP, Brownstone RM. Mechanisms of spinal cord stimulation for the treatment of pain:
 Still in the dark after 50 years. Eur J Pain 2019;23(4):652-659.
- [23] Kato G, Kawasaki Y, Ji RR, Strassman AM. Differential wiring of local excitatory and inhibitory
 synaptic inputs to islet cells in rat spinal lamina II demonstrated by laser scanning
 photostimulation. J Physiol 2007;580(Pt.3):815-833.
- [24] Kloc ML, Pradier B, Chirila AM, Kauer JA. NMDA receptor activation induces long-term
 potentiation of glycine synapses. PLoS One 2019;14(9):e0222066.
- [25] Klyachko VA, Stevens CF. Excitatory and feed-forward inhibitory hippocampal synapses work
 synergistically as an adaptive filter of natural spike trains. PLoS Biol 2006;4(7):e207.
- [26] Kuhlman SJ, Olivas ND, Tring E, Ikrar T, Xu X, Trachtenberg JT. A disinhibitory microcircuit
 initiates critical-period plasticity in the visual cortex. Nature 2013;501(7468):543-546.
- [27] Labrakakis C, Lorenzo LE, Bories C, Ribeiro-da-Silva A, De Koninck Y. Inhibitory coupling
 between inhibitory interneurons in the spinal cord dorsal horn. Mol Pain 2009;5:24.
- [28] Labrakakis C, Rudolph U, De Koninck Y. The heterogeneity in GABAA receptor-mediated
 IPSC kinetics reflects heterogeneity of subunit composition among inhibitory and
 excitatory interneurons in spinal lamina II. Front Cell Neurosci 2014;8:424.
- [29] Letzkus JJ, Wolff SB, Meyer EM, Tovote P, Courtin J, Herry C, Luthi A. A disinhibitory
 microcircuit for associative fear learning in the auditory cortex. Nature
 2011;480(7377):331-335.
- [30] Light AR, Perl ER. Reexamination of the dorsal root projection to the spinal dorsal horn
 including observations on the differential termination of coarse and fine fibers. J Comp
 Neurol 1979;186(2):117-131.
- [31] Lin Q, Peng YB, Willis WD. Role of GABA receptor subtypes in inhibition of primate
 spinothalamic tract neurons: difference between spinal and periaqueductal gray inhibition.
 J Neurophysiol 1996;75(1):109-123.
- [32] Lovett-Barron M, Turi GF, Kaifosh P, Lee PH, Bolze F, Sun XH, Nicoud JF, Zemelman BV,
 Sternson SM, Losonczy A. Regulation of neuronal input transformations by tunable
 dendritic inhibition. Nat Neurosci 2012;15(3):423-430, S421-423.
- [33] Lu Y, Perl ER. A specific inhibitory pathway between substantia gelatinosa neurons receiving
 direct C-fiber input. J Neurosci 2003;23(25):8752-8758.

- [34] Lu Y, Perl ER. Modular organization of excitatory circuits between neurons of the spinal
 superficial dorsal horn (laminae I and II). J Neurosci 2005;25(15):3900-3907.
- [35] Malan TP, Mata HP, Porreca F. Spinal GABA(A) and GABA(B) receptor pharmacology in a
 rat model of neuropathic pain. Anesthesiology 2002;96(5):1161-1167.
- [36] Moreno A, Morris RGM, Canals S. Frequency-Dependent Gating of Hippocampal-Neocortical
 Interactions. Cereb Cortex 2016;26(5):2105-2114.
- [37] Motanis H, Seay MJ, Buonomano DV. Short-Term Synaptic Plasticity as a Mechanism for
 Sensory Timing. Trends Neurosci 2018;41(10):701-711.
- [38] Motulsky HJ, Christopoulos A. Fitting Models to Biological Data Using Linear and Nonlinear
 Regression. San Diego: GraphPad Software Inc., 2003.
- [39] Polgar E, Durrieux C, Hughes DI, Todd AJ. A quantitative study of inhibitory interneurons in
 laminae I-III of the mouse spinal dorsal horn. PLoS One 2013;8(10):e78309.
- [40] Pouille F, Scanziani M. Routing of spike series by dynamic circuits in the hippocampus.
 Nature 2004;429(6993):717-723.
- [41] Royer S, Zemelman BV, Losonczy A, Kim J, Chance F, Magee JC, Buzsaki G. Control of
 timing, rate and bursts of hippocampal place cells by dendritic and somatic inhibition. Nat
 Neurosci 2012;15(5):769-775.
- [42] Salio C, Merighi A, Bardoni R. GABAB receptors-mediated tonic inhibition of glutamate
 release from Abeta fibers in rat laminae III/IV of the spinal cord dorsal horn. Mol Pain
 2017;13:1744806917710041.
- [43] Santos SF, Luz LL, Szucs P, Lima D, Derkach VA, Safronov BV. Transmission efficacy and
 plasticity in glutamatergic synapses formed by excitatory interneurons of the substantia
 gelatinosa in the rat spinal cord. PLoS One 2009;4(11):e8047.
- [44] Santos SF, Rebelo S, Derkach VA, Safronov BV. Excitatory interneurons dominate sensory
 processing in the spinal substantia gelatinosa of rat. J Physiol 2007;581(Pt 1):241-254.
- [45] Sawynok J, LaBella FS. On the involvement of GABA in the analgesia produced by baclofen,
 muscimol and morphine. Neuropharmacology 1982;21(5):397-403.
- [46] Schenone S, Brullo C, Musumeci F, Bruno O, Botta M. A1 receptors ligands: past, present
 and future trends. Curr Top Med Chem 2010;10(9):878-901.
- [47] Spiess AN, Neumeyer N. An evaluation of R2 as an inadequate measure for nonlinear models
 in pharmacological and biochemical research: a Monte Carlo approach. BMC Pharmacol
 2010;10:6.
- [48] Takazawa T, MacDermott AB. Synaptic pathways and inhibitory gates in the spinal corddorsal horn. Ann N Y Acad Sci 2010;1198:153-158.

- [49] Tian L, Ji G, Wang C, Bai X, Lu Y, Xiong L. Excitatory synaptic transmission in the spinal
 substantia gelatinosa is under an inhibitory tone of endogenous adenosine. Neurosci Lett
 2010;477(1):28-32.
- [50] Todd AJ. Plasticity of inhibition in the spinal cord. Handb Exp Pharmacol 2015;227:171-190.
- [51] Torebjork HE, Hallin RG. Responses in human A and C fibres to repeated electrical
 intradermal stimulation. J Neurol Neurosurg Psychiatry 1974;37(6):653-664.
- [52] Yasaka T, Tiong SY, Hughes DI, Riddell JS, Todd AJ. Populations of inhibitory and excitatory
 interneurons in lamina II of the adult rat spinal dorsal horn revealed by a combined
 electrophysiological and anatomical approach. Pain 2010;151(2):475-488.
- [53] Zhang W, Schneider SP. Short-term modulation at synapses between neurons in laminae II V of the rodent spinal dorsal horn. J Neurophysiol 2011;105(6):2920-2930.
- [54] Zheng J, Lu Y, Perl ER. Inhibitory neurones of the spinal substantia gelatinosa mediate
 interaction of signals from primary afferents. J Physiol 2010;588(Pt 12):2065-2075.
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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.

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

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

819

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

B. Short-term plasticity during stimulation trains at 50 Hz. Left: representative average current
trace simultaneously recorded in an eGFP- neuron (blue) and eGFP+ neuron (green). Right: for
each pair of eGFP- and eGFP+ neuron and for all ranks during the train, the difference in relative
eIPSCs amplitude is calculated. This difference is significantly larger than zero indicating that the
relative increase in amplitude during the train at 50 Hz is larger in eGFP- than in eGFP+ neurons
(P < 0.001: ***; n = 4 pairs). Linear regressions of the data were compared with a linear regression
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_B receptor antagonist on GABAergic elPSCs 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_B 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 μ M 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

Figure 7. Effect of an A1 adenosine receptor antagonist on GABAergic elPSCs during trains of
 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 μ M 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.

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 withincell 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 880 881 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 statistically significant improvements with respect to the linear fit with slope value forced to 0, 886 887 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.

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 = 19911 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

Supplementary figure S5. Short-term plasticity of GABAergic connections during trains of
 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.

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.

932

933 Supplementary figure S6. Relative amplitude of rank 2 elPSCs 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 ($P_{eGFP-} = 0.678$, $P_{eGFP+} = 0.223$, 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 ($P_{eGFP+} = 0.098$, Wilcoxon Signed Rank 939 Test for Paired Data), but significantly decreased by 18% for eGFP- neurons ($P_{eGFP-} = 0.048$, 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_B receptor antagonist on GABAergic elPSCs during 5
945 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 μ M CGP55845 (orange), a GABA_B receptors antagonist. For 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 $GABA_{B}$ 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

Supplementary figure S8. Effect of an A1 adenosine receptor antagonist on GABAergic elPSCs 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 µM 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 = 1971 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 972 973 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.















D



Control eGFP+

6

Stimulation rank in the train

9

8

0.5

0

1 2 3





(+)



50 Hz, 20 s





Data quantification and analysis

Curve fitting and model comparison







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 withincell 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.



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: elPSCs recorded in eGFP- neurons. C, D, G, H: elPSCs recorded in eGFP+ neurons. Average amplitudes from individual neurons are in grey.

Trains at 5 Hz





Supplementary figure S5.

Relative amplitude of rank 2 eIPSCs in the first five and the last five trains. A. During 5 Hz trains, eIPSC2/eIPSC1 remained similar in the first five and the last five trains for connections on both eGFP- and eGFP+ neurons (PeGFP- = 0.678, PeGFP+ = 0.223, Wilcoxon Signed Rank Test for Paired Data). B. During 50 Hz trains, eIPSC2/eIPSC1 remained similar in the first five and the last five trains for connections on eGFP+ neurons (PeGFP+ = 0.098, Wilcoxon Signed Rank Test for Paired Data), but significantly decreased by 18% for eGFP- neurons (PeGFP- = 0.048, Wilcoxon Signed Rank Test for Paired Data). This suggests that the facilitation observed in eGFP- 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 elPSCs during 5 Hz and 50 Hz trains. legend next page

Supplementary figure S7. Effect of a GABAB receptor antagonist on GABAergic elPSCs 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 μ M CGP55845 (orange), a GABA_B 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 GABAB 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: ***.



Effect of an A1 adenosine receptor antagonist on GABAergic elPSCs during 5 Hz and 50 Hz trains. legend next page

Supplementary figure S8.

Effect of an A1 adenosine receptor antagonist on GABAergic elPSCs 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 µM 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 changed 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

	Stimulation protoco	bl						N	/lodel pai	rameters	determin	ed by non	linear cur	ve fitting						Model co	nparison stats	
Figure	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	р	dAICc
2E		PPR	- and +	Control	"equation 2" pooled data sum of two "equation 2"	63- 52+	223 223	3 6	1.29 1.53	1.01 1.07	34.04 28.46	1.29 1.06	1.01 0.95	34.04 68.52	25.41 24.04	220 217	156.48 150.12	164.67 164.65	170.11 173.97	4.1222	7.20E-03	0.02

Supplementary Table 2. Non linear regressions FIGURE 3

Stimu	ation p	rotocol								Ν	Model pa	rameters	s determi	ned by nor	n linear cu	rve fittin	g					Model cor	nparison stats	
Figure	Fregency	protocol interval	Protocol repetition	Type of data	e GFP 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	р	dAICc
S3A	5 Hz	20 s	1-10	Raw	- and +	Control	sum of two exponential fits, same intercept A1 sum of two "Equation3"	54- 49+	1133 1133	5	-58.06 -60.20	7.80 8.32	6.46 3.12	-58.06 -55.42	18.23 16.42	2.56 3.38	928309.68 927586.17	1128 1127	10828.04 10829.16	10840.12 10843.26	10858.24 10864.39	0.8791	3.49E-01	-3.14
S3A	5 Hz	20 s	1-10	Raw	- and +	Control	"Equation3" pooled data sum of two "Equation3"	54- 49+	1133 1133	3 6	-57.93 -60.20	12.17 8.32	3.29 3.12	-57.93 -55.42	12.17 16.42	3.29 3.38	957197.83 927586.17	1130 1127	10858.76 10829.16	10866.80 10843.26	10878.89 10864.39	11.9925	9.88E-08	23.54
S3A	5 Hz	20 s	1-10	Raw	-	Control	Linear fit slope=0 Exponential (Equation3)	54-	594 594	1	-54.56 -60.20	0.00 8.32	3.12				573961.45 570329.12	593 591	5772.52 5772.75	5776.54 5780.82	5781.30 5790.30	1.8820	1.53E-01	-4.28
S3A	5 Hz	20 s	1-10	Raw	+	Control	Linear fit slope=0 Exponential (Equation3)	49+	539 539	1 3				-44.60 -55.42	0.00 16.42	3.38	369871.83 357257.05	538 536	5053.93 5039.23	5057.95 5047.30	5062.51 5056.39	9.4631	9.14E-05	10.65
3B, S3E	5 Hz	20 s	1-10	Norm	- and +	Control	"Equation3" pooled data sum of two "Equation3"	54- 49+	1030 1030	3 6	0.96 0.99	-0.13 -0.07	3.79 3.48	0.96 0.92	-0.13 -0.19	3.79 3.99	93.83 87.89	1027 1024	463.28 401.99	471.32 416.10	483.03 436.56	23.0422	1.94E-14	55.21
3B, S3E	5 Hz	20 s	1-10	Norm	-	Control	Linear fit slope=0 Exponential (Equation3)	54-	540 540	1 3	0.95 0.99	0.00 -0.07	3.48				66.92 66.72	539 537	408.87 411.27	412.89 419.34	417.45 428.44	0.7974	4.51E-01	-6.451
3B, S3E	5 Hz	20 s	1-10	Norm	+	Control	Linear fit slope=0 Exponential (Equation3)	49+	490 490	1 3				0.80 0.92	0.00 -0.19	3.99	22.66 21.33	489 487	-111.61 -137.19	-107.59 -129.11	-103.22 -120.42	15.1532	4.13E-07	21.52
зc	5 Hz	20 s	1-10 1-5	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			
3D	5 Hz	20 s	6-10 1-10 1-5	Raw	+	Control	"Equation3" pooled data	43+	1100 946	3	-63.28 -55.61	10.39	1.65 3.31	-59.81 -55.61	12.94 15.11	9.61 3.31	1141274.39 748490.65	1094 943	9005.83	9013.87	9025.24	0.2107	8.89E-01	-11.43
			6-10				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

	Stir	mulation protocol						М	lodel pa	aramete	rs determ	ined by no	on linear c	urve fittin	g					Model co	mparison stats	
	igure	qency otocol erval otocol oetition	be of a	FP Idition	Jg Idition	N			eGFP-	eGFP-	eGFP-	eGFP+	eGFP+	eGFP+								
	ш	Fre pro Pro rep	dat	6G Cor	Dr.	Model neurons	N obs	k	A1	A2	A3	A1	A2	A3	SS	df	AIC	AICc	BIC	F	р	dAICc
	C A	E U = 20 < 1 10	David	and	Control	"Equation3" pooled data 11-	242	3 -7	1.30	26.74	2.53	-71.30	26.74	2.53	351728.28	239	2456.93	2465.10	2470.89			
3	бА	5 HZ 20 § 1-10	KdW	- anu +	Control	sum of two "Equation3" 11+	242	6 -7	1.22	35.90	1.14	-73.38	160.72	73.12	319721.69	236	2439.84	2454.32	2464.27	7.8751	4.98E-05	10.78
			Norm	andi	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			
S	6B	5 Hz 20 s 1-10	NOT	- and +	Control	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	8.70E-15	57.98

Supplementary Table 4. Non linear regressions FIGURE 4

	Stimu	lation p	rotocol							М	lodel p	aramete	ers detern	nined by no	on linear o	urve fittin	g					Model cor	nparison stats	
Figure	Fregency	protocol interval	Protocol repetitio	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
\$30	50 Hz	60 s	1-10	Raw	- and +	Control	"Equation3" pooled data sum of two "Equation3"	27- 19+	517 517	3-6 6-6	8.28 8.03	14.98 3.88	1.82 1.01	-68.28 -68.49	14.98 31.86	1.82 2.18	855892.23 795499.75	514 511	5307.11 5275.28	5315.19 5289.50	5324.10 5305.02	12.9313	3.73E-08	25.69
\$30	50 Hz	60 s	1-10	Raw	-	Control	Linear fit slope=0 Exponential (Equation3)	27-	308 308	1 -6 3 -6	54.71 58.03	0.00 3.88	1.01				682823.28 682430.74	307 305	3250.86 3254.69	3254.90 3262.82	3258.32 3269.61	0.0877	9.16E-01	-7.92
\$30	50 Hz	60 s	1-10	Raw	+	Control	Linear fit slope=0 Exponential (Equation3)	19+	209 209	1 3				-44.45 -68.49	0.00 31.86	2.18	132402.80 113069.01	208 206	1945.43 1916.44	1949.49 1924.64	1952.12 1929.81	17.6121	8.69E-08	24.85
4B, S	3D 50 Hz	60 s	1-10	Norm	- and +	Control	"Equation3" pooled data sum of two "Equation3"	27- 19+	470 470	3 1. 6 1.	.02 .10	-0.10 0.10	0.07 8.17	1.02 0.91	-0.10 -0.28	0.07 1.26	233.23 212.36	467 464	1012.46 974.40	1020.55 988.64	1029.08 1003.47	15.2018	1.88E-09	31.91
4B, S	3D 50 Hz	60 s	1-10	Norm	-	Control	Linear fit slope=0 Exponential (Equation3)	27-	280 280	1 1. 3 1.	.10 .10	0.00 0.10	8.17				201.79 201.65	279 277	706.89 710.69	710.93 718.84	714.16 725.23	0.0956	9.09E-01	-7.909
4B, S	3D 50 Hz	60 s	1-10	Norm	+	Control	Linear fit slope=0 Exponential (Equation3)	19+	190 190	1 3				0.68 0.91	-0.28	1.26	11.96 10.57	189 187	17.83 -1.77	21.89 6.45	24.32 11.22	12.3666	9.03E-06	15.45
S3E	50 Hz	60 s	1-10	Raw Summ.	- and +	Control	"Equation3" pooled data sum of two "Equation3"	27- 19+	506 506	3 -7 6 -7	'4.18 '4.27	-86.24 -123.33	3.95 5.07	-74.18 -71.05	-86.24 -44.47	3.95 1.60	2907009.78 2698637.74	503 500	5823.95 5792.32	5832.03 5806.54	5840.86 5821.90	12.8690	4.12E-08	25.49
S3E	50 Hz	60 s	1-10	Raw Summ.	-	Control	Linear fit slope=0 Exponential (Equation3)	27-	297 297	1 -1 3 -7	.42.14 '4.27	0.00 -123.33	5.07				2405477.14 2076127.99	296 294	3519.71 3479.98	3523.75 3488.11	3527.10 3494.75	23.3195	3.98E-10	35.64
S3E	50 Hz	60 s	1-10	Raw Summ.	+	Control	Linear fit slope=0 Exponential (Equation3)	19+	209 209	1 3				-106.84 -71.05	0.00 -44.47	1.60	659375.99 622509.74	208 206	2280.97 2272.94	2285.03 2281.14	2287.65 2286.31	6.0999	2.67E-03	3.89
4C, S	3F 50 Hz	60 s	1-10 ır	rm Sum	- and +	Control	"Equation3" pooled data sum of two "Equation3"	27- 19+	450 450	3 1. 6 1.	.46 .51	1.62 2.29	8.17 8.70	1.46 1.38	1.62 0.64	8.17 6.06	876.68 815.54	457 454	1585.15 1558.62	1593.24 1572.87	1601.59 1587.38	11.0962	4.90E-07	20.37
4C, S	3F 50 Hz	60 s	1-10 ır	rm Sum	-	Control	Linear fit slope=0 Exponential (Equation3)	27-	270 270	1 2. 3 1.	.36 .51	0.00 2.29	8.70				731.22 671.52	269 267	1039.23 1020.23	1043.27 1028.38	1046.42 1034.62	11.8691	1.15E-05	14.89
4C, S	3F 50 Hz	60 s	1-10 ır	rm Sum	+	Control	Linear fit slope=0 Exponential (Equation3)	19+	180 180	1	-			1.68 1.38	0.00 0.64	6.06	148.47 144.02	189 187	480.16 478.68	484.23 486.91	486.54 491.45	2.7350	6.76E-02	-2.682
4D	50 Hz	60 s	1-10 1-5	Raw	-	Control	"Equation3" pooled data	22-	484	3 -6	9.41	9.97	2.04	-69.41	9.97	2.04	943143.46	481	5047.78	5055.86	5064.51	1 2002	2 705 04	0.05
	50.11	60 -	6-10 1-10	Davis		Contral	"Equation3" pooled data	17+	484 374	6 -6 3 -7	0.37 1.91	2.61 34.62	2.88	-79.15 -71.91	34.62	1.65 2.42	935579.40 237187.61	478 371	5049.88 3482.55	5064.12 3490.65	5079.16 3498.24	1.2882	2./8E-01	-8.25
4E	50 Hz	6U S	1-5 6-10	кам	+	Control	sum of two "Equation3"		374	6 -6	57.00	33.18	3.23	-77.60	37.33	1.80	235260.35	368	3485.49	3499.80	3512.96	1.0049	3.91E-01	-9.15

Supplementary Table 5. Non linear regression comparion 5 Hz with 50 Hz FIGURE 3 and 4

	Stimulation	orotocol								Mod	lel paramet	ers determ	ined by no	n linear c	urve fitting	1					Model co	mparison stats	
	Freq	num	Data		Drug		N																
Figu	e ency interva	l ber	type	eGFP	condition	Model	neurons	Ν	obs	k A1	5Hz A2 5H	z A3 5Hz	A1 50Hz	A2 50Hz	A3 50Hz	SS	df	AIC	AICc	BIC	F	р	dAICc
20.4	5 Hz 200 60	- 1 10	Norm		Control	"Equation3" pooled	data 54@5Hz	8	20	3 1.02	-0.02	0.98	1.02	-0.02	0.98	272.71	817	1432.32	1440.37	1451.16			
30,4	50Hz 205 60	5 1-10	NOTTI	-	Control	sum of two "Equatio	n3" 27@50H	8	20	6 0.99	-0.07	3.48	1.10	0.00	0.06	268.48	814	1425.50	1439.63	1458.46	4.2777	5.23E-03	0.738
20.4	5Hz 202 CO	- 1 10	N		Constant	"Equation3" pooled	data 49@5Hz	6	80	3 0.91	-0.20	2.52	0.91	-0.20	2.52	34.09	877	-97.54	-89.48	-79.45			
3B, 4	50Hz 205 60	5 1-10	Norm	+	Control	sum of two "Equatio	n3" 19@50H	6	80	6 0.92	-0.19	3.99	0.91	-0.28	1.26	31.77	874	-139.40	-125.23	-107.75	16.3811	2.76E-10	35.75

Supplementary Table 6. Linear regressions FIGURE 5

	Stimu	lation p	orotocol			N								Model com	parison stats	
Figure	Fregency	protocol interval	Protocol repetition Type of data	eGFP condition	Model		N obs	k	SS	df	AIC	AICc	BIC	F	р	dAICc
5A	5 Hz	20 s	1-10 Relativ		y=0x+0	6pairs	10	0	1.99	10	14.25	16.75	14.56			
					y=A1+A2*x		10	2	0.07	8	-15.02	-5.02	-14.11	107.4780	1.66E-06	21.78
5B	50 Hz	60 s	1-10 Relativ		y=0x+0	4pairs	10	0	1.25	10	9.59	12.09	9.89			
					y=A1+A2*x		10	2	0.05	8	-17.74	-7.74	-16.83	87.7696	3.61E-06	19.83

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

	Stimu	lation p	rotocol		_	-				Model	paramete	ers	ń	ń	Ń						Model cor	nparison stats	
Figure	Freq.	protocol interval	Protocol repetitio	Type of data	eGFP conditio	Drug condition	Model	N neurons	N obs	ہ A1 control	A2 control	A3 control	A1 CGP5584	A2 CGP5584	A3 CGP5584	SS	df	AIC	AICc	BIC	F	р	dAICc
\$7B	5 Hz	20 s	1-10	Raw	-	Control & CGP55845	"Equation3" pooled data sum of two "Equation3"	6	132 132	3 -55.81 6 -52.61	10.62 3459.37	5.56 4153.92	-55.81 -59.07	10.62 10.23	5.56 2.98	96495.70 96024.87	129 126	1253.07 1258.42	1261.38 1273.32	1264.60 1278.60	0.2059	8.92E-01	-11.94
6A, S70	5 Hz	20 s	1-10	Norm	-	Control & CGP55845	"Equation3" pooled data sum of two "Equation3"	6	120 120	3 0.92 6 0.93	-1.32 -1.75	120.00 145.23	0.92 0.91	-1.32 -0.13	120.00 8.31	3.63 3.61	117 114	-71.40 -66.02	-63.05 -51.02	-60.25 -46.50	0.1962	8.99E-01	-12.03
S7E	50 Hz	60 s	1-10	Raw	-	Control & CGP55845	"Equation3" pooled data sum of two "Equation3"	12	264 264	3 -91.97 6 -88.18	24.14 23.18	1.64 1.42	-91.97 -95.27	24.14 25.24	1.64 2.12	427700.08 424394.75	261 258	2708.22 2712.17	2716.37 2726.61	2722.52 2737.20	0.6698	5.71E-01	-10.23
6B, S7I	50 Hz	60 s	1-10	Norm	-	Control & CGP55845	"Equation3" pooled data sum of two "Equation3"	12	240 240	3 1.02 6 0.97	-0.06 -0.10	0.04 0.06	1.09	-0.12	12.47	98.52 96.74	237 234	475.41 477.02	483.58 491.51	489.33 501.39	1.4381	2.32E-01	-7.928
\$7H	5 Hz	20 s	1-10	Raw	+	Control & CGP55845	"Equation3" pooled data sum of two "Equation3"	8	176 176	3 -64.68 6 -68.78	18.77 21.47	5.30 2.39	-64.68 -61.77	18.77 -7.10	5.30 -9.55	326569.82 325296.18	173 170	1832.03 1837.34	1840.26 1852.01	1844.71 1859.53	0.2219	8.81E-01	-11.74
6C, S7	5 Hz	20 s	1-10	Norm	+	trol & CGP5	"Equation3" pooled data sum of two "Equation3"	8	160 160	3 1.00 6 0.90	-0.37 -0.19	13.53 1.83	1.15	-85.77	3662.80	12.67 9.20	157 154	56.28 11.19	64.54 25.92	68.58 32.71	19.3142	1.11E-10	38.62
\$7К	50 Hz	60 s	1-10	Raw	+	Control & CGP55845	"Equation3" pooled data sum of two "Equation3"	6	132 132	3 -71.90 6 -58.81	40.46 30.01	1.73 3.00	-71.90 -85.57	40.46 53.08	1.73 1.30	115572.90 113173.57	129 126	1276.88 1280.11	1285.20 1295.01	1288.41 1300.29	0.8904	4.48E-01	-9.82
6D, S71	. 50 Hz	60 s	1-10	Norm	+	Control & CGP55845	"Equation3" pooled data sum of two "Equation3"	6	120 120	3 0.77 6 0.83	-0.24 -0.28	1.86 2.67	0.72	-0.21	1.00	3.12 2.80	117 114	-89.37 -96.39	-81.02 -81.39	-78.22 -76.88	4.3568	6.05E-03	0.373
\$7N	50 Hz	20 s	1-10	Raw	+	Control & CGP55845	"Equation3" pooled data sum of two "Equation3"	7	154 154	3 -58.68 6 -52.86	19.42 13.12	7.85 5.00	-58.68 -64.52	19.42 30.88	7.85 12.81	69256.72 65781.93	151 148	1385.76 1383.83	1394.03 1398.60	1397.91 1405.09	2.6059	5.40E-02	-4.57
6E, S70	50 Hz	20 s	1-10	Norm	+	Control & CGP55845	"Equation3" pooled data sum of two "Equation3"	7	140 140	3 1.08 6 1.17	-0.28 -0.27	3.90 1.91	1.08 1.01	-0.28 -0.34	3.90 7.46	13.10 12.77	137 134	73.64 76.07	81.93 90.92	85.41 96.66	1.1538	3.30E-01	-8.982
6CE, \$710	50Hz 5Hz 2	20s Ctr 20s CGI	1-10	Norm	+	Control CGP55845	"Equation3" pooled data sum of two "Equation3"	7 8	150 150	3 1.12 6 1.17	-2.12 -0.27	80.00 1.91	1.15	-4.78	200.25	15.44 15.12	147 144	92.67 95.48	100.95 110.27	104.72 116.55	1.0335	3.80E-01	-9.32
6DE, S7LO	50Hz 50Hz	20s Ctr 60s Ctr	1-10	Norm	+	Control CGP55845	"Equation3" pooled data sum of two "Equation3"	7 6	130 130	3 1.01 6 1.17	-0.28 -0.27	2.45 1.91	0.83	-0.28	2.67	12.12 8.60	127 124	68.48 29.81	76.80 44.73	79.95 49.88	16.9489	2.75E-09	32.07

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

	Stimul	ation p	rotocol							Model	paramete	ers									Model com	nparison stats	
Figure	Fregency	protocol interval	Protocol repetition	Type of data	eGFP condition	Drug condition	Model	N neurons	N obs	포 A1 control	A7 control	A3 control	A1 DPCPX			SS	df	AIC	AICc	BIC	F	р	dAICc
7A, S8E	5 Hz	20 s	1-10	Raw	-	Control & DPCPX	"Equation3" pooled data sum of two "Equation3"	9	198 198	3 -84.54 6 -60.78	14.12 9.91	7.73 4.15	-84.54 -108.73	14.12 19.22	7.73 10.73	347778.43 236918.36	195 192	2049.17 1979.17	2057.38 1993.76	2062.32 2002.18	29.9472	6.28E-16	63.62
S8C	5 Hz	20 s	1-10	Norm	-	Control & DPCPX	"Equation3" pooled data sum of two "Equation3"	9	180 180	3 0.97 6 0.95	-0.13 -0.09	6.15 3.82	0.97 0.99	-0.13 -0.17	6.15 7.79	7.90 7.85	177 174	-43.96 -39.10	-35.73 -24.45	-31.19 -16.75	0.3670	7.77E-01	-11.29
7B, S8E	50 Hz	60 s	1-10	Raw	-	Control & DPCPX	"Equation3" pooled data sum of two "Equation3"	7	154 154	3 -84.67 6 -65.61	-13.61 -28.94	0.04 3.47	-84.67 -119.29	-13.61 7673.78	0.04 3955.37	786345.89 755965.17	151 148	1759.92 1759.85	1768.18 1774.62	1772.06 1781.11	1.9826	1.19E-01	-6.43
S8F	50 Hz	60 s	1-10	Norm	-	Control & DPCPX	"Equation3" pooled data sum of two "Equation3"	7	140 140	3 1.20 6 1.19	0.31 0.56	3.11 3.90	1.20 1.23	0.31 0.06	3.11 0.06	197.12 194.39	137 134	453.20 457.26	461.50 472.10	464.97 477.85	0.6257	6.00E-01	-10.6
7C, S8H	50 Hz	20 s	1-10	Raw	-	Control & DPCPX	"Equation3" pooled data sum of two "Equation3"	9	198 198	3 -104.53 6 -71.04	-1.83 -10.90	-3.93 0.48	-104.53 -130.34	-1.83 -18.57	-3.93 -8.89	860620.94 798562.47	195 192	2228.57 2219.76	2236.78 2234.34	2241.73 2242.77	4.9736	2.40E-03	2.44
S8I	50 Hz	20 s	1-10	Norm	-	Control & DPCPX	"Equation3" pooled data sum of two "Equation3"	9	180 180	3 1.22 6 1.19	-4.07 0.06	199.98 0.69	1.17	-3.72	100.57	64.41 61.59	177 174	333.82 331.77	342.05 346.42	346.60 354.12	2.6551	5.01E-02	-4.366
7D, S8	5 Hz	20 s	1-10	Raw	+	Control & DPCPX	"Equation3" pooled data sum of two "Equation3"	9	198 198	3 -62.16 6 -52.92	17.69 -17.40	8.91 -18.80	-62.16 -71.29	17.69 13.45	8.91 4.14	238830.83 226298.52	195 192	1974.76 1970.09	1982.97 1984.68	1987.91 1993.10	3.5443	1.56E-02	-1.71
S8L	5 Hz	20 s	1-10	Norm	+	Control & DPCPX	"Equation3" pooled data sum of two "Equation3"	9	180 180	3 0.94 6 0.9199	-0.27 -2.6885	10.26 108.479	0.96761	-0.1152	3.43286	14.42 14.03	177 174	64.45 65.55	72.68 80.20	77.22 87.90	1.60	1.91E-01	-7.52
7E, S8N	1 50 Hz	60 s	1-10	Raw	+	Control & DPCPX	"Equation3" pooled data sum of two "Equation3"	7	154 154	3 -90.97 6 -77.94	45.05 39.98	2.33 2.62	-90.97 -104.10	45.05 50.32	2.33 2.12	199289.92 188222.67	151 148	1548.53 1545.73	1556.80 1560.50	1560.68 1566.99	2.9007	3.70E-02	-3.70
S8O	50 Hz	60 s	1-10	Norm	+	Control & DPCPX	"Equation3" pooled data sum of two "Equation3"	7	140 140	3 0.91 6 0.84	-0.30 -0.32	5.65 2.63	0.91 1.10	-0.30 -0.21	5.65 0.06	33.82 30.85	137 134	206.44 199.55	214.73 214.40	218.20 220.14	4.3077	6.18E-03	0.338
7F	5 Hz	20 s	1-10	Raw	- and +	DPCPX	"Equation3" pooled data sum of two "Equation3"	9- 9+	198 198	3 -89.81 6 -108.73	15.26 19.22	6.69 10.73	-89.81 -71.29	15.26 13.45	6.69 4.14	419133.26 343921.71	195 192	2086.12 2052.96	2094.33 2067.55	2099.27 2075.98	13.9960	2.73E-08	26.78

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 elPSCs 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.