Title: Self-tuning of inhibition by endocannabinoids shapes spike-time precision in CA1 pyramidal neurons

Abbreviated title: Endocannabinoids tune spike-time precision

Authors: Franck Dubruc¹,², David Dupret³ and Olivier Caillard¹,²

1. Inserm, UMR_S 1072, 13015, Marseille, France
2. Aix-Marseille Université, UNIS, 13015, Marseille, France
3. MRC Anatomical Neuropharmacology Unit, Department of Pharmacology, University of Oxford, Mansfield Road, Oxford OX1 3TH, United Kingdom

Corresponding author: Olivier Caillard
Inserm UMR_S 1072, Unité de Neurobiologie des Canaux Ioniques et de la Synapse
Faculté de Médecine - Secteur Nord, Université Aix-Marseille CS80011, Bd Pierre Dramard
13344 Marseille Cedex 15 France
Tel: 00 33 4 91 69 88 62
Fax: 00 33 4 91 09 05 06
e-mail: olivier.caillard@univ-amu.fr

Number of Words: Abstract: 207 previously 224; Introduction: 624 previously 558; Results: 2487, previously 3496 Discussion: 1664 previously 2165

Number of Figures: 7; Number of Tables: 0; Number of Pages: 32

Author Contributions: F.D. and O.C. designed research; F.D. and O.C. performed research; F.D., D.D., and O.C. analyzed data; F.D., D.D. and O.C. wrote the paper.

Note to Editor: The place cell firing patterns depicted in figure 1 and replayed in vitro for the purpose of the present study were part of the data set collected, analyzed and published by Dupret et al. 2010. However, the in vivo firing patterns of the selected place cells used in the present study were not depicted, used in isolation for analysis purposes or published elsewhere.
Abstract

In the hippocampus, activity-dependent changes of synaptic transmission and spike-timing coordination are thought to mediate information processing for the purpose of memory formation. Here we investigated the self-tuning of intrinsic excitability and spiking reliability by CA1 hippocampal pyramidal cells via changes of their GABAergic inhibitory inputs and endocannabinoid signaling. Firing patterns of CA1 place cells, when replayed in vitro, induced an endocannabinoid-dependent transient reduction of spontaneous GABAergic activity, sharing the main features of depolarization-induced suppression of inhibition (DSI), and conditioned a transient improvement of spike-time precision during consecutive burst discharges. When evaluating the consequences of DSI on Excitatory Post-Synaptic Potential (EPSP)-spike coupling, we found that transient reductions of uncorrelated (spontaneous) or correlated (feed-forward) inhibition improved EPSP-Spike coupling probability. The relationship between EPSP-Spike timing reliability and inhibition was however more complex: transient reduction of correlated (feed-forward) inhibition disrupted or improved spike-timing reliability according to the initial spike coupling probability. Thus eCB-mediated tuning of pyramidal cell spike-time precision is governed not only by the initial level of global inhibition but also by the ratio between spontaneous and feed-forward GABAergic activities. These results reveal that eCB-mediated self-tuning of spike-timing by the pyramidal cells' own discharge can constitute an important contribution to place cell assemblies and memory formation in the hippocampus.

Keywords
Short-term plasticity; DSI; Feed-Forward inhibition; Spontaneous inhibition; Endocannabinoids; Spike Timing; EPSP-Spike coupling; CA1 place cell
Abbreviations:

2-AG: 2-arachidonoylglycerol; AM251: N-(Piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide; CB1R: Cannabinoid type 1 receptor; CCH: Carbachol; CCK: cholecystokinin; CVisi: Coefficient of Variation of the Interspike Interval; DC: Direct Current; DSI: Depolarization-induced Suppression of Inhibition; dDSI: dynamic DSI; eCB: endocannabinoids; EPSC: excitatory postsynaptic conductance; EPSP: excitatory postsynaptic potential; FFI: Feed-Forward Inhibition; FF-IPSC: Feed-forward inhibitory postsynaptic conductance; IPSC: inhibitory postsynaptic conductance; IPSP: inhibitory postsynaptic potential; ISI: Inter Spike Interval; PTX: picrotoxin; SP-IPSC: spontaneous inhibitory postsynaptic conductance; THL: Tetrahydrolipstatin; Vhold: Holding Membrane Potential; Vm: Membrane Potential;
Introduction

Learning-related brain computations rely on complex interactions between excitatory and inhibitory circuits, and possibly involve activity-dependent synaptic plasticity to refine the spike timing underlying firing synchronization and temporal coordination of cell assemblies. In the hippocampus, a key circuit for memory, pyramidal cells called 'place cells' fire in specific regions of the environment (i.e. place fields) and their joint activity is thought to provide representations of space used to guide behavior (O'keefe and Dostrovsky, 1971; O'keefe and Conway, 1978; Wilson and McNaughton, 1993). Cell assemblies forming distinct representations are thought to arise as a result of changes in synaptic weights between active place cells and precise spike-timing is expected to be instrumental for their temporal coordination (Wilson and McNaughton, 1994; Kentros et al., 1998; Robbe et al., 2006; O'Neill et al., 2008). However the mechanisms by which place cells could refine the spike-time precision of their firing patterns remain unknown.

In the hippocampus GABAergic interneurons orchestrate the activity of principal cells, notably through feed-forward and feed-back inhibition (for review, see Kullmann, 2011). The timing of inhibition, related to specific GABAergic innervation of distinct subcellular domains of pyramidal cells, may be tightly linked to excitation and may sharpen EPSP-spike coupling (Pouille and Scanziani, 2001; Glickfeld and Scanziani, 2006). However, variable firing of interneurons (Klausberger et al., 2005), action potential-independent (for review, see Ramirez and Kavalali, 2011) and asynchronous release (for review, see Jonas and Hefft, 2010) provide fluctuating inhibitory bombardment of principal cells that participate in action potential jitter in response to excitatory responses (Caillard, 2011).

Depolarization-induced suppression of inhibition (DSI) has been suggested to represent an important mechanism for learning and memory (for review, see Freund et al., 2003; Kano et al., 2009). This form of plasticity is mediated by endocannabinoid (eCB) signaling involving the cannabinoid type 1 receptor (CB1R), the activation of which is required for hippocampus-dependent long-term memory (Varvel et al., 2005; Puighermanal et al., 2009; Han et al., 2012). CB1Rs are predominantly expressed on axon terminals of cholecystokinin-expressing (CCK) interneurons (Katona et al., 1999; Tsou et al., 1999) known to provide local inhibition to pyramidal cells by means of asynchronous release of GABA (Losonczy et al., 2004; Foldy et al., 2006; Daw et al., 2009; Karson et al., 2009). During DSI, hippocampal CA1 pyramidal neurons release eCBs that transiently inhibit afferent GABAergic activity and the CB1R agonist 2-arachidonoylglycerol (2-AG) is known to mediate such a retrograde control (Gao et al., 2010; Tanimura et al., 2010). In knock-out mice
deficient for the 2-AG-degrading enzyme monoacylglycerol lipase, sustained levels of 2-AG correlate with enhanced spatial learning and hippocampal long-term potentiation (Pan et al., 2011). As these knock-out mice exhibit prolonged DSI of hippocampal CA1 pyramidal neurons (Pan et al., 2011), it was suggested that DSI may be an important mechanism for learning and memory. Yet whether in vivo hippocampal place cell firing patterns induce eCB-mediated DSI is controversial (Hampson et al., 2003). Moreover, the effects of DSI on spike-time precision remain to be determined.

Here we established the firing requirement for DSI using in vivo place cell patterns monitored during a spatial learning task (Dupret et al., 2010) and evaluated the effect of DSI on CA1 pyramidal cell firing and spike timing properties. We found that the in vitro replay of place cell discharge elicited an eCB-mediated transient reduction in GABA release depending on the number of action potentials fired, and improved their spike-train precision as measured by a decrease in the coefficient of variation during burst discharge. Moreover we employed dynamic current injections to mimic synaptic input events, with computational modeling, to identify the parameters and decipher the mechanisms underlying self-tuning of spike-time precision by coexisting correlated and uncorrelated GABAergic activities. We revealed that excitatory postsynaptic potential (EPSP)-spike coupling was governed by feed-forward inhibition imposing a biphasic tuning of spike-time precision, ultimately prevented by high-level spontaneous inhibition.
**Materials and Methods**

**Ethic Statement**

All rats were maintained on a 12 h light/dark cycle with food and water provided ad libitum. Animal research was approved by the local direction of veterinary services (Direction Départementale des Services Vétérinaires – Préfecture des Bouches du Rhône, France (O. Caillard, licence number A13-493). All experiments were carried out according to the European and institutional guidelines for the care and use of laboratory animals (Council Directive 86/609/EEC and French National Research Council).

**Hippocampal slice preparation and electrophysiological recordings**

Transverse hippocampal slices (350 µm thick) were obtained from 14- to 20-day old Wistar rats as previously described (Debanne et al., 2008). Experiments were performed at 32°C in ACSF containing the following (in mM): 125 NaCl, 26 NaHCO3, 3 CaCl2, 2.5 KCl, 2 MgCl2, 0.8 NaH2PO4, 10 D-glucose; and saturated with 95% O2 and 5% CO2. CA1 pyramidal cells were visualized using an Olympus BX-51 WI microscope equipped with differential interference contrast optics under infrared illumination and a water immersion lens (X60, 0.9 NA, Olympus). Electrophysiological recordings were performed in whole-cell configuration with a Multiclamp 700B amplifier (Axon Instruments), filtered at 5 kHz and digitized at 20 kHz via a PCI-6220 National Instrument interface controlled by IgorPro (Wavemetrics) and/or Digidata 1322A interface controlled by PClamp software (Axon Instruments). Patch pipettes had a resistance of 4-10 MΩ when filled with a KCl solution containing (in mM): 140 KCl, 5 HEPES, 2 EGTA, 2 Na2ATP, 0.3 NaGTP, and 2 MgCl2, pH 7.4 or with a low chloride solution containing (in mM): 140 Kgluconate, 10 HEPES, 2 EGTA, 2 Na2ATP, 0.3 NaGTP, and 2 MgCl2, pH 7.4. With the KCl-based solution, the reversal potential for GABA<sub>A</sub> receptors, determined by local pressure ejection of isoguvacine (0.5 mM) at different holding membrane potentials, was 3.7 ± 2.9 mV (n=4). With the low chloride-based solution, the reversal potential for GABA<sub>A</sub> receptors, determined by the same method was -73.7±1.7 mV (n=9).

Cells were recorded if the series resistance, measured throughout experiments, remained stable and < 40 MΩ. Stimulating pipettes were filled with extracellular saline. Picrotoxin (PTX, 100 mM) was prepared in ethanol and stored at -80°C. Kynurenate (200 mM) and carbachol (CCH, 5 mM) were prepared in distilled water and stored at -20°C. AM251 (10 mM) and tetrahydrolipstatin (THL, 1mM) were prepared in DMSO and stored at -20°C. Stocks solutions were thawed and diluted into the extracellular solution before use. Final DMSO concentrations were 0.05% and 0.1% for
extracellular and intracellular solutions, respectively. At these concentrations DSI was not affected by DMSO. DSI induced by depolarization of the cell in voltage-clamp at 0 mV for 1s was 27±4 % of control GABAergic charge (n=16) in the absence of DMSO. DSI was of comparable magnitude when cells were recorded with the intracellular or extracellular DMSO concentrations used in this study, respectively (28±5%, U(22)=41, Z=0.52 p=0.303; 32±3 %, U(21)=22, Z=1.49 p=0.069).

**Stimuli**

Voltage or direct current (DC) steps and GABAκ synaptic conductances were generated using the Igor Pro software (Wavemetrics), and converted to analog signals via a PCI 6723 National Instrument interface.

DSI was induced in 2 different modes. When DSI was induced in voltage-clamp mode, the cell was depolarized from a holding membrane potential of -80mV to 0mV for 1s. Otherwise DSI was induced by a series of action potential elicited in current-clamp mode. The transient switch to current-clamp mode was under the control of an analog signal, constructed with Igor Pro Software and injected to the external mode command of the Multiclamp 700B amplifier contemporaneously with the voltage-clamp or current-clamp external input commands. The holding current was preliminary set on the amplifier such that the resting membrane potential was kept at around -80mV for 125ms when switching to current clamp mode prior to the depolarizing DC steps.

The firing patterns presented in figures 1, 2 and 4 were extracted from the dataset used in Dupret et al. (2010). These patterns corresponded to 3 successive learning trials (trials number 21, 22 and 23) during each of which the rat had to retrieve three food rewards hidden in a cheeseboard maze, before returning to the start box and collecting an additional reward. All procedures were carried out in accordance with the Animals (Scientific Procedures) Act, 1986 (UK) and associated procedures under an approved project license. In vitro replay of these place cell firing patterns was obtained by delivering sequences of brief depolarization (1.25 ms / 2nA) followed by short hyperpolarization (1.25 ms / 2nA) at specific times (Fig. 1, Fig. 2C-F) in order to drive cell discharges at very short Inter Spike Intervals (ISI, see Fig. 2C for instance). The whole-cell amplifier switched to current-clamp mode 125 ms prior to each action potential and back to voltage-clamp mode, unless the interval to the next spike to be triggered was less than 250 ms. Following the elicited action potential discharge (34±7mV; mean peak±sem; n=5), the membrane potential (Vm) was transiently further hyperpolarized (-78.0±3.3mV) when compared to the preceding holding potential (-76.2±1.4mV). In behaving rats, CA1 pyramidal cells classically exhibit bursts of spikes
(Ranck, Jr., 1973) with ISI ranging from 2 to 6 ms (Harris et al., 2001). In line with this, the CA1 pyramidal cell activity from the study of Dupret et al. (2010) also revealed short ISI, as low as 2.5 ms. Hence the protocols we used were the most reliable for driving action potentials at precise times. The DC injected to hyperpolarize the cell was also set to avoid uncontrolled action potential discharge, which may be facilitated by the depolarizing envelope that follows the fast phase of action potential repolarization (Schwartzkroin, 1975; Storm, 1987) and depolarizing GABAergic postsynaptic potentials, that occurred frequently in our recording conditions (carbachol 5µM and GABA_A reversal potential = 3.7 ± 2.9 mV (n=4)).

**Neuronal and synaptic modeling**

GABAergic and glutamatergic synaptic events were modeled as a conductance with a dual exponential time-course of the form (1-exp(-t/τ_rise).exp(-t/τ_decay) where τ_ rise/decay is the rise/decay time constant. Spontaneous activity was created by convolution of the synaptic event template with the Poisson train occurrence of spontaneous events. τ_rise and τ_decay were 1 ms and 5.5 ms for excitatory postsynaptic conductances (EPGs) and 1.5 ms and 14.7 ms for inhibitory postsynaptic conductances (IPGs), respectively (Caillard, 2011). IPSG amplitude varied according to a binomial distribution with n=5 and p=0.2. Feed-forward IPSGs (FF-IPGs) were sent with a delay of 2 ms after EPGs (Pouille and Scanziani, 2001). The conductance instruction was then either converted to an analog signal to instruct the dynamic clamp amplifier or injected into the computational model. The latter was based on a leaky integrate and fire model (LIF) with random threshold (Holden, 1976; Manwani et al., 2002; Caillard, 2011): Cm.dV_i/dt = Σ g_i.(V_o-V_i) + Σ m_i I with C_m = 400 pF; V_o = -65 mV. A spike is generated when V_i reaches the action potential threshold (-45±1 mV; mean±SD), and the membrane potential resets to V_o. Resting membrane resistance was set at 200 MΩ. GABAergic and glutamatergic synaptic events, affecting both g_i and I when present, were included according to the equations I_{GABA} = g_{GABA}.(V_m - E_{GABA}) and I_{GLU} = g_{GLU}.(V_m - E_{GLU}). E_{GABA} and E_{GLU} were respectively set at -70 mV and 0 mV. All modeling was performed with Igor Pro Software.

**Dynamic clamp**

To add artificial IPSPs and EPSPs, we used a fast dynamic-clamp system using National Instruments data acquisition cards (PCI 6052E or PCI 6251) and NI-DAQmx 9.21, controlled by the QuB software (www.qub.buffalo.edu; Milescu et al., 2008). The dynamic-clamp system was running on an independent PC mounted with a dual 8 core Xeon processor @ 2.67 GHz. The Feedback loop
(F>90 kHz) continuously read the membrane potential V_m from the Multiclamp 700B, EPSG and/or
IPSG and the DC command from the PCI-6723 NIDAQ card controlled by Igor software on a
separate computer and generated an output according to the equation I_{sum} = EPSG(V_m-E_{Glu}) +
IPSG(V_m-E_{GABA}) + DC. E_{Glu} and E_{GABA} were set respectively at 0 and -75 mV.

Dynamic clamp experiments were performed in the presence of kynurenate (2 mM) and PTX
(100 µM). Depolarization induced suppression of spontaneous GABAergic inhibition (dDSI) was
modeled by an initial reduction of presynaptic activity to 10% of control, followed by a 30s
exponential recovery of GABAergic charge. A minimum of 6 dynamic DSI (dDSI) trials, with place
cell in vivo firing patterns associated with every other trial, were performed per recorded cell to
evaluate the contribution of both dDSI and in vivo place cell firing to EPSP-Spike coupling. EPSP-
Spike coupling was evaluated by measuring both EPSP-Spike latency and temporal jitter in control
conditions with dynamic GABAergic activity reduced approximately to 20% and 80% for DSI and
recovery, respectively.

For dynamic feed-forward inhibition experiments, the following protocol was performed: EPSG
peak amplitude was set so that spikes were successfully triggered with an average latency of
approximately 5ms after each EPSG; the largest FF-IPSG amplitude was then defined in order to
prevent action potential firing when recording 20 successive EPSG/IPSG sequences. A preliminary
EPSP-Spike probability curve was drawn online after testing a few IPSG amplitudes several times. It
served to define, for a given FF-IPSG amplitude, the number of EPSG/IPSG sequences that should
theoretically be injected into the cell in order to get one successful coupling, with the limit probability
of 0.05. Thereafter, EPSG/IPSG sequences with variable FF-IPSG amplitudes were injected in
random order, such that EPSP-Spike latencies at low EPSP-Spike coupling probabilities could
successfully be collected.

Analysis

Spontaneous synaptic activity and spike analysis were performed with Igor Pro (Wavemetrics,
Lake Oswego, OR, USA). A collection of Igor Pro functions (Neuromatic, Jason Rothman,
http://www.neuromatic.thinkrandom.com) were used in addition to homemade functions.

To quantify spontaneous GABAergic activity we integrated the current area under baseline for
each 500 ms bin of recordings in order to determine the total synaptic charge received by the cell
over time. This parameter captured changes in both the frequency and the amplitude of synaptic
events. DSI was measured as the change in the average charge in the 4 seconds that followed
action potential discharge or depolarization to 0 mV. DSI Magnitude depicted in figure 1 and 2 are defined as the % of reduction of GABAergic charge during this period of time.

Spike threshold was detected when dV / dt exceeded 10 mV / ms. Changes in excitability were evaluated for equivalent DC steps. Measurements of CV_{isi} were performed by averaging CV for each sequential interval along the discharge starting from the 2^{nd} spike. With this method, the reproducibility of the full discharge from one trial to another could be evaluated, independently of spike-frequency adaptation that was observed experimentally (Caillard, 2011). During the recordings dedicated to the measurement of CV_{isi} small and large DC steps alternated to allow DSI recovery and prevent its build-up over time.

Measurement of spike-time jitter at 20 spikes / s was performed by interpolating the CV_{isi} vs. average spike discharge with either the best linear or exponential fit. For EPSP-Spike coupling experiments, action potential coupling was evaluated, and latencies between the beginning of EPSG and spike threshold were collected. Jitter corresponded to the standard deviation of the mean latency.

Statistical comparisons were made using the Mann-Whitney U test. The degree of freedom (df), the value of the U test (u) as well as Z and p are provided in the result section using the following format: “U(df)=u , Z=Z value, p=p value”. Differences were considered significant when p<0.05. Latencies are reported as mean ± jitter where jitter corresponds to SD of the mean. Other data are reported as mean ± SEM.
Results

Place cell firing induces endocannabinoid-mediated decrease of GABAergic transmission

To determine whether waking firing patterns of place cells can induce DSI, spiking activity recorded *in vivo* was replayed *in vitro* while monitoring GABAergic activity in CA1 pyramidal cells. Neurons were recorded in the presence of kynurenic acid (2 mM) to block ionotropic glutamatergic receptors and monitor the temporal dynamics of GABAergic activity, together with carbachol (5 µM) to promote spontaneous inhibitory post-synaptic currents (IPSCs) (Pitler and Alger, 1992) and an elevated intracellular chloride concentration to reliably detect GABA<sub>A</sub> events. In these conditions, GABA<sub>A</sub> synaptic activity was detected as large inward currents when neurons were recorded at V<sub>m</sub>=-80 mV. The CA1 place cell firing patterns were obtained from rats performing a spatial learning task on a cheeseboard maze (Dupret et al., 2010), and converted into short current steps to elicit action potentials *in vitro* (see Methods). Infield place cell discharge (i.e., occurring inside the place field) observed across successive learning trials was followed *in vitro* by a transient reduction of the GABAergic input activity (Fig. 1A and 1B). The time integral of the spontaneous occurring currents – referred to as GABAergic charge (see methods) – was reduced within the 4 seconds following each place field traversal (Fig. 1B; 31±4%, 45±5% and 36±3% of baseline GABAergic charge after each replayed trial; p<0.05 for each place field traversal; U(8)=0, Z=2.31, p=0.014). This induction of DSI by infield place cell firing patterns was prevented by adding to the intracellular solution the tetrahydrodipstatin (THL, 1µM), an inhibitor of sn-1-diacylglycerol lipase responsible for 2-AG synthesis (Fig. 1C and 1D; p>0.05 for each place field traversal U(8)=5, Z=0.87, p=0.24; U(8)=2, Z=1.73, p=0.06; U(8)=6, Z=0.58, p=0.34). Thus the DSI induced by the replay of CA1 place cell discharge involved the endogenous CB1R agonist 2-AG. Hippocampal place cells can exhibit substantial differences in their discharge (McNaughton et al., 1983). We then replayed the patterns recorded from another less active cell (Fig. 1E-H). In this case however the discharge of fewer spikes failed to induce DSI (6 spikes discharged; DSI: 8±6%; U(18)=36, Z=0.40, p=0.35), suggesting that only the discharge of the most active place cells can induce DSI.

We next investigated how spiking activity governs the induction of the DSI and its magnitude. Pyramidal cells were driven with AP bursts defined by the number and frequency of APs (Fig 2). The DSI induced by the replay of an *in vivo* discharge of 29 APs (Fig 2A, top left panel; 53±7%, U(18)=0, Z=3.58, p<0.001) and by a depolarizing current eliciting a comparable number of spikes (Fig 2A, top right panel; 55±8%, U(12)=0, Z=2.88, p=0.001) were similar (U(15)=27, Z=0.0, p=0.500). In both
cases a lower number of elicited spikes failed to induce a DSI (Fig. 2A bottom panels; in vivo replay: $U(13)=11, Z=1.08, p=0.14$; constant depolarizing current: $U(8)=8, Z=0.0, p=0.56$). The DSI was indeed governed by the number of spikes discharged (Fig. 2B-D) and its induction was prevented both by the CB1 receptor antagonist AM251 (Fig. 2B, AM251 5µM; 0±3%; $U(10)=10, Z=0.52, p=0.34$) and the intracellular dialysis of THL (Fig. 2B, THL 1 µM; 1±3%; $U(6)=3, Z=0.65, p=0.35$). Within the 10–250 Hz frequency range however, a given number of APs induced a DSI of similar magnitude (Fig. 2D).

Taken together these results showed that place cell firing patterns replayed in vitro can induce an endocannabinoid-mediated DSI provided that the number of action potentials emitted is sufficiently high.

**Endocannabinoids mediate self-tuning of spike-timing in CA1 pyramidal neurons**

Next we determined the consequence of DSI on action potential firing and timing of CA1 pyramidal cells. We evoked sequences of bursts of action potentials at various intra-burst rates using DC injections every 30 s, and monitored spontaneous GABAergic activity in between (Fig. 2E1). We measured spike rate as a function of DC injection (Fig. 2E2) and calculated the mean coefficient of variation of inter-spike intervals along the discharge (CVISI, see Methods, Fig 2E3) to evaluate spike-train precision as a function of discharge rate before DSI. In order to evaluate excitability and CVISI during DSI, the last burst of the sequence was elicited 5s after the next to last burst, when DSI was maximal. When cells were recorded with a physiological, hyperpolarizing GABA<sub>A</sub> reversal potential, the DSI induced either by a constant depolarizing current or place cell patterns did not significantly change cell excitability (Fig. 2F; 98±3%, $U(20)=40, Z=0.76, p=0.22$) but improved intra-burst spike-time precision as shown by a smaller CVISI (Fig. 2G; 81±5%, $U(20)=10, Z=3.02, p=0.001$). In order to confirm that CB1-mediated reduction in GABAergic activity was required for the improvement of spike-train precision during DSI, we performed similar protocols while recording cells with an elevated intracellular chloride concentration to reliably detect GABA<sub>A</sub> events. In control conditions, the mean CVISI decreased following sustained burst discharge and related maximal DSI (Fig. 3A; 78±5% of control, $U(20)=10, Z=3.02, p=0.001$). In the presence of AM251 (Fig. 3B; 5 µM), sustained discharge did not induce DSI nor improved spike-train precision (97±5% of the CVISI measured in AM251,$U(16)=24, Z=0.84, P=0.200$). If the reduced GABAergic activity observed during DSI was responsible for the improvement in spike-train precision, DSI should not improve spike-train precision in the presence of the GABA<sub>A</sub> receptor blockers. This was
indeed the case when cells were recorded in the presence of picrotoxin (PTX, 100 µM; Fig. 3C; 98±2% of the CV$_{\text{ISI}}$ measured in PTX; U(16)=32, Z=0.0, p=0.50).

These results suggest that the improvement of spike-train precision following sustained burst discharges required a reduction in spontaneous GABAergic activity under the control of endocannabinoids. We addressed this directly by dynamically injecting random inhibitory postsynaptic conductance waveforms (spontaneous-IPSGs; SP-IPSGs) into CA1 pyramidal cells recorded in the presence of kynurenate (2mM) and picrotoxin (100 µM, Fig. 3D) in order to evaluate the consequence of spontaneous GABAergic activity on excitability and spike precision. For the purpose of these dynamic-clamp experiments the GABA$_A$ reversal potential was set at -75mV. Changes in excitability were normalized for each individual cell by the average change in spike number at each DC step when SP-IPSGs were present. We found that the rate of spontaneous GABAergic activity predicted both changes in excitability and CV$_{\text{ISI}}$; although GABAergic activity affected spike-train precision (CV$_{\text{ISI}}$) more than excitability (Fig. 3E). The level of spontaneous GABAergic activity correlated with the change in CV$_{\text{ISI}}$ (Fig. 3F): in the presence of AM251 (5 µM), pyramidal cells received a higher level of spontaneous GABAergic activity (148±23% of the mean drug-free GABAergic charge; U(18)=21, Z=1.69, p=0.046); in the presence of the GABA$_A$ receptor blocker picrotoxin (PTX, 100 µM) CV$_{\text{ISI}}$ was reduced relative to the drug-free condition (57±5% of control, U(18)=14.5, Z=2.27, p=0.012). When comparing CV$_{\text{ISI}}$ measured in different pharmacological or dynamic-clamp conditions, we found that CV$_{\text{ISI}}$ in drug-free conditions before and during DSI corresponded to spontaneous dynamic-clamp GABAergic activity of 33 events / s (U(15)=15, Z=1.22, p=0.11) and 10 events / s (U(15)=18, Z=0.86, p=0.19), respectively. Moreover, the CV$_{\text{ISI}}$ measured in AM251 (5 µM) was comparable to the CV$_{\text{ISI}}$ measured with a spontaneous dynamic-clamp GABAergic activity of 100 events / s (U(13)=18, Z=0.29, p=0.38). These results demonstrate that spontaneous GABAergic activity set the initial level of spike-train precision and conditioned, under the control of endocannabinoid signaling, the improvement of spike-train precision following sustained neuronal discharge.

**Tuning of EPSP-Spike coupling by spontaneous and feed-forward inhibition**

Next we addressed how DSI affected the tuning of neuronal discharge in response to excitatory postsynaptic potentials (EPSPs), a phenomenon referred to as EPSP-Spike coupling. In order to avoid the trial to trial fluctuation of EPSP amplitude, we dynamically elicited EPSPs by injecting Excitatory Postsynaptic Conductance waveforms (EPSGs) to CA1 neurons together with randomly
injected Inhibitory Postsynaptic Conductance waveforms (IPSGs, Fig. 4A and B, see methods). Dynamic DSI (dDSI) was simulated by an initial 10% reduction of IPSG rate followed by a 30s exponential recovery. Following dDSI, EPSP-Spike latency remained stable (92±1% of control latency, U(8)=4, Z=1.15, p=0.171, Fig. 4C) but EPSP-Spike jitter decreased (61±5% of control jitter, U(8)=0, Z=2.31, p=0.014, Fig. 4D), showing that spontaneous GABAergic activity received by CA1 neurons correlated with their spike-time precision.

In the hippocampus endocannabinoid CB1 receptors are predominantly expressed on the axon terminals of CCK/CB1-expressing interneurons that partially mediate the feed-forward inhibition (FFI) of CA1 neurons (Glickfeld and Scanziani, 2006; Katona et al., 1999; Tsou et al., 1999). We next aimed to evaluate how a transient suppression of FFI might tune EPSP-Spike coupling during DSI. We first placed an extracellular stimulation electrode in the stratum radiatum 200-400 µm from the recorded CA1 pyramidal cell body (Fig. 5A), in order to stimulate Schaffer collaterals, evoke both a monosynaptic EPSC and a disynaptic IPSC (Pouille and Scanziani, 2001) and evaluate the consequence of a depolarization on both amplitude responses (Fig. 5B). We observed that a 1s depolarization to 0 mV reliably elicited within 5 seconds a transient reduction of the GABAergic response (Fig. 5C) explained by a reduced disynaptic IPSCs (Fig. 5D; elIPSC; 76±2 %, U(8)=0, Z=2.31, p=0.014) while the monosynaptic EPSC remained unaffected (Fig. 5D; eEPSC; 105±6 %; U(8)=6, Z=0.58, p=0.34). Thus, the depolarization of CA1 pyramidal cells transiently decreased FFI while the excitatory drive from the Schaffer collaterals remained constant. In order to evaluate how this change in excitatory/inhibitory balance affected spike-timing of CA1 pyramidal cells, neurons were next driven by dynamic EPSGs followed with a 2 ms delay by feed-forward IPSGs, referred to as FF-IPSGs. In this case both EPSP-Spike latency and jitter could be evaluated for the full range of EPSP-Spike coupling probabilities (see Methods). We found that increased FF-IPSG amplitude reduced EPSP-Spike coupling probability (Fig. 5E, F). In contrast we observed a bell-shaped response curve of EPSP-Spike latency as a function of IPSG amplitude (Fig. 5G). A similar bell-shaped relationship was observed between the EPSP-Spike precision (as measured by the spike jitter) and the spike coupling probability in all recorded cells, with the maximum jitter for a mean coupling probability of 0.50±0.06 (Fig. 5H; n=4).

These results imply that the integration time-window for Schaffer collateral EPSPs depended on the recruitment of GABAergic interneurons mediating disynaptic inhibition and followed a biphasic tuning conditioned to the initial EPSP-Spike coupling probability. Since a depolarization-induced
suppression of FFI was observed in CA1 pyramidal neurons, spike-time precision changed according
to the initial contribution of FFI to EPSP-Spike coupling.

**Theoretical contribution of spontaneous and feed-forward inhibition to EPSP-Spike coupling**

The coexistence of correlated (evoked) and uncorrelated (spontaneous) GABAergic inhibitions
implies that the integration time-window of excitatory inputs by CA1 pyramidal neurons may
fluctuate, depending on the relative contribution of both types of inhibition before and after place cell
discharge activity. In order to evaluate how spontaneous and feed-forward inhibition tune EPSP-
Spike coupling we used a “Leaky Integrate and Fire” with “Random action potential Threshold” (RT-
LIF) model that focuses on subthreshold membrane properties while excluding the mechanisms
underlying action potential generation (Holden, 1976; Manwani et al., 2002). Such a model also fairly
reproduces the spike-time jitter attributed to the stochastic properties of voltage-dependent ion
channels (Caillard, 2011).

In a first series of simulations the model received excitatory inputs represented by EPSGs of
different amplitudes [0-100 nS], combined or not with spontaneous GABAergic activity, referred to as
SP-IPSG [0-250 Events/s] (Fig. 6A-D). In the absence of inhibition, the ESPG amplitude determined
both EPSP-Spike coupling probability and spike-time precision. Once spontaneous GABAergic
activity was added to the model both EPSP-Spike latency and jitter (as expressed by SD of latency,
Fig 6B) increased with SP-IPSG rate. Thus spontaneous GABAergic activity reduces EPSP-Spike
coupling probability (Fig. 6C) and increases spike latency and jitter at any given EPSG amplitude
(Fig. 6D).

In the second series of simulations, the same range of EPSGs was injected into the model in
association with feed-forward IPSGs – referred to as FF-IPSG of various amplitudes [0-100 nS] (Fig.
6E-H). Both spike-time latency and jitter (Fig 6F) increased with the amplitude of FF-IPSG until
EPSP-Spike probability dropped to 0.5 (Fig 6F and Fig 6G); however a further increase of FF-IPSG
then decreased these two key features of EPSP-Spike coupling.

To identify the mechanism underlying this biphasic tuning of EPSP-Spike coupling, we next
determined how FFI affected EPSP amplitude and kinetics, and correlated these parameters with
spike coupling. In the absence of FFI (Fig. 6E, left panels), EPSPs crossed the spike threshold
rapidly (Fig. 6I; 0 nS, red trace) and as a consequence both the mean spike latency and jitter were
small (Fig. 6J; 2.6±0.1 ms). Increasing FFI reduced EPSP amplitude and shortened its decay time
constant (Fig. 6I). With a FFI of 39 nS EPSP-Spike coupling probability fell to 0.5 (Fig 6E, middle
panels). Because EPSP peak remained in the lower part of the Gaussian noise distribution of spike threshold, spike latency was distributed in the widest range (Fig. 6J; 3.1±0.4 ms). For stronger FFI (60 nS) EPSP peak amplitude was low with respect to spike threshold and the fast repolarization shortened the time window during which EPSP could elicit an action potential (Fig. 6I, 60 nS; green trace). As a consequence spikes were rarely fired (Fig 6E, right panels), and occurred within a short and narrow latency range (Fig. 6J; 2.6±0.3 ms). Therefore, both the amplitude and the repolarization rate of the EPSP/IPSP sequence determined the broadness of the integration time window, with respect to the fluctuating spike threshold for action potential generation. With these mechanisms, the RT-LIF model matched the experimental data showing that FFI can tune EPSP-Spike time precision, and that it correlated with EPSP-Spike coupling probability.

Finally, we determined how the simultaneous presence of spontaneous and FFI affected EPSP-Spike coupling (Fig. 7). We first found that increasing spontaneous activity [range 0-100 SP-IPSG events/s] while maintaining FFI constant [39nS FF-IPSG] reduced EPSP-Spike coupling probability below its 0.5 initial value but had no effect on spike-time latency and precision (Fig. 7A and B). Thus, a DSI of spontaneous GABAergic activity would not be associated with any change in EPSP-spike time precision if FFI is present and insensitive to DSI. However, increasing FFI [range 0-70nS FF-IPSGs] while maintaining constant the spontaneous inhibition rate (50 SP-IPSG events/s) reduced the EPSP-Spike coupling probability below its 0.5 initial value as previously but decreased EPSP-Spike jitter (Fig. 7C and D). In contrast to the simulation performed without spontaneous inhibition (Fig. 6F), FFI was only associated with an improvement of spike-time precision as the biphasic tuning of EPSP-Spike timing was not observed (Fig. 7C and 7D). Thus, a DSI of FFI would be associated with a disruption in EPSP-spike time precision if spontaneous GABAergic activity is present and insensitive to DSI. Last, when both feed-forward and spontaneous inhibition were simultaneously present and varied with a constant SP-IPSG/FF-IPSG ratio, they bipherasicaly tuned EPSP-Spike latencies and jitter responses (Fig. 7 E and F). The largest spike-time jitter was observed at a spike-coupling probability of 0.5. Thus tuning of spike-time precision was governed by the relative contribution of the two combined forms of inhibition during DSI: DSI of FFI imposed a biphasic response of spike time jitter, unless spontaneous inhibition was sufficiently high to prevent this biphasic time code (Fig. 7G and H).
Discussion

In this study we established that firing patterns of place cells recorded in vivo induce DSI in CA1 pyramidal cells in vitro. This DSI is mediated by the activation of CB1 receptors. A major consequence of the eCB-mediated transient suppression of GABAergic inputs is the fine tuning of spike-timing.

GABAergic contribution to spike-time precision

DSI is the most widely studied form of eCB-mediated short-term plasticity, but whether it can be induced by physiological firing patterns remained to be determined. Here we successfully induced DSI injecting waking patterns of CA1 place cells discharge recorded during a spatial task (Dupret et al., 2010). CB1 receptors are expressed in axons and terminals of CCK-expressing GABAergic interneurons (Katona et al., 1999; Tsou et al., 1999; Katona et al., 2000; Nyiri et al., 2005; Pan et al., 2011). CCK-interneurons are a heterogeneous family of interneurons that innervate pyramidal cells on their somatic and dendritic domains (for reviews, see Klausberger and Somogyi, 2008; Klausberger, 2009). Spontaneous GABAergic activity recorded in pyramidal cells may arise from CCK-inhibitory inputs targeting both subcellular domains (Cope et al., 2002). Nevertheless, because Schaffer collateral-associated dendritic GABAergic synapses are less sensitive to DSI than basket cell synapses (Lee et al., 2010) and as demonstrated with extracellular stimulations of dendritic GABAergic inputs (Morishita and Alger, 2001), it is likely that the CB1-sensitive component of spontaneous GABAergic inhibition originates primarily from perisomatic synapses. Our experiments revealed that a transient reduction in CB1R-dependent spontaneous GABAergic activity improved spike-time precision of discharging pyramidal cells. This self-tuned improvement of spike-time precision was achieved: 1) during DSI; 2) after pharmacological blockade of GABA_A receptors; 3) when the rate of dynamic GABA_A events was reduced. However, DSI was not associated with an increase in pyramidal cell firing. Although carbachol (5 µM, applied in the bath) reduced Ca^{2+}-dependent K^+ conductances activated during DSI (Pitler and Alger, 1992), we observed transient shifts in the holding current. Such shifts likely reflected the incomplete blockade of these conductances known to control excitability after repetitive discharges in a feed-back manner (Madison and Nicoll, 1984). It is likely that DSI counterbalanced the reduction in intrinsic excitability. In fact, dynamic injection of IPSPs at various rates demonstrated that spontaneous GABAergic activity was associated with a reduction of excitability.
In order to evaluate the impact of DSI on EPSP-Spike coupling, we have considered time, frequency and amplitude variations in GABAergic conductance for constant excitatory conductance. A consensual correlation exists between the transience of an excitatory signal and its ability to precisely time a spike, when the excitatory input signal amplitude is scaled up or down in order to maintain a constant spike coupling probability (Mainen and Sejnowski, 1995; Jaeger and Bower, 1999; Pouille and Scanziani, 2001; Axmacher and Miles, 2004; Mittmann et al., 2005; Cudmore et al., 2010; Gastrein et al., 2011). Measuring simultaneously Schaffer collateral EPSCs and disynaptic IPSCs showed no significant changes in EPSC amplitude during DSI. The transient changes in EPSC amplitude observed in a subset of cells were likely due to the temporal overlap of the reduced IPSC (Wagner and Alger, 1996). In hippocampal cell cultures, both excitatory and inhibitory synapses can be depressed by exogenous cannabinoid agonists or postsynaptic depolarization, but the cannabinoid sensitivity of excitatory synapses appeared lower than GABAergic synapses (Ohno-Shosaku et al., 2002; Xu et al., 2010), such that the physiological conditions for eCB-mediated transient reduction in excitatory synaptic transmission may be reached but only under conditions allowing a large increase in postsynaptic calcium concentration (Nakamura et al., 1999).

When GABAergic and glutamatergic activities were uncorrelated, i.e. in the presence of spontaneous activity, the disruption of spike-time jitter was proportional to the rate of spontaneous GABAergic activity, as represented by our modeling study (Fig. 6). Therefore, during DSI a transient improvement in spike-time precision was observed either when the cell integrated phasic glutamatergic inputs or when it integrated summed uncorrelated inputs, giving rise to a repetitive discharge of action potentials. In contrast, when excitatory and inhibitory inputs correlated in time through the disynaptic recruitment of presynaptic interneurons, the relationship between the amplitude of the IPSP and EPSP-Spike parameters were more complex and depended on the initial EPSP-Spike coupling probability (Fig. 5 and 6). In our model, voltage fluctuations before spike initiation explained how modulation of EPSP amplitude and decay kinetics by disynaptic inhibition affected EPSP-Spike timing in a biphasic manner. During DSI, the reduction in disynaptic inhibition, together with the initial EPSP-Spike coupling probability were critical for defining qualitatively and quantitatively the impact on EPSP-Spike timing. When feed-forward inhibition was present and reduced coupling probability to at least 50%, spike-timing was exclusively controlled by disynaptic inhibition (Fig. 7B). As feed-forward inhibition is only partly mediated by CB1R-expressing interneurons (Glickfeld et al., 2008; Glickfeld and Scanziani, 2006), this earlier component of disynaptic inhibition should be relatively insensitive to DSI, when compared to feedback inhibition.
Enhanced firing probability during DSI would therefore be mainly supported by uncorrelated inhibition. Nevertheless, as observed in our recordings, coincident reduction in both uncorrelated and correlated inhibition during DSI should affect spike-time precision in a biphasic manner (Fig. 7F).

**Place cell activity, DSI firing requirement and endocannabinoids**

We confirm here that spike train duration determine the magnitude of DSI (Pitler and Alger, 1992). However the broad time-window observed for the build-up of DSI contrasted with the firing requirements, especially at low (<20 Hz) firing frequencies, that were previously reported in the hippocampus (Hampson et al., 2003; Zhuang et al., 2005) but not in the neocortex (Fortin et al., 2004). The DSI observed at low (10Hz) firing frequencies, together with the slow time course for DSI recovery, suggest that underlying postsynaptic calcium transients are large in amplitude and display slow kinetics (Lenz and Alger, 1999; Myoga et al., 2009; Wang and Zucker, 2001).

Carbachol enhances both the frequency of spontaneous IPSCs (Pitler and Alger, 1992) and the magnitude of DSI in CA1 pyramidal neurons (Pitler and Alger, 1994; Martin and Alger, 1999; Martin et al., 2001; Kim et al., 2002) through the activation of postsynaptic M1 and M3 receptors (Ohno-Shosaku et al., 2003). The number of action potentials required for DSI induction may thus be linked to endogenous release of acetylcholine. Indeed, endogenous release of acetylcholine affected GABA release and DSI magnitude (Pitler and Alger, 1994; Martin et al., 2001; Nagode et al., 2011). Moreover, activation of the cholinergic system in the hippocampus appears relevant when studying synaptic transmission and plasticity in vitro (Isaac et al., 2009). In fact, microdialysis measurements of acetylcholine in the hippocampus have revealed marked increases in the basal levels during locomotion, exploration and spatial learning (for review see Pepeu and Giovannini, 2004).

Different reasons can account for the low activity threshold for DSI induction reported here, compared to a previous report (Hampson et al. (2003). Not only magnitude but also recovery time course increases with the duration of postsynaptic depolarization or action potential number during discharge (Pitler and Alger, 1992; Zhuang et al., 2005; Fortin et al., 2004). In the present study we have measured DSI in the 4 seconds that followed action potential discharge. Therefore the firing discharge required for successfully inducing a significant DSI will appear to be lower than expected from previous studies where DSI magnitude was evaluated later following DSI induction (4-7 seconds after the end of induction protocol; Hampson et al. 2003). Next, the age of the animals is critical for DSI induction. In immature animals DSI has been reported to be weak (Zhuang et al., 2005; Zhu and Lovinger, 2010). The place cell activity may not be sufficient to induce DSI in a one
week old animal (Hampson et al., 2003), but adequate later in development (Langston et al., 2010; Wills et al., 2010; Scott et al., 2011).

CCK-expressing interneurons preferentially fire on the ascending phase of theta oscillations, at times when CA1 place cells with place fields in front of the animal also discharge (O'keefe and Recce, 1993; Klausberger et al., 2005; Dragoi and Buzsaki, 2006; Huxter et al., 2008; Klausberger and Somogyi, 2008). Moreover, place cells also exhibit a gradual phase shift relative to theta rhythm as the animal passes through the place field (phase precession, O'keefe and Recce, 1993). Therefore, the activity-dependent transient suppression of CCK-expressing cell outputs by eCB released following place cell infield discharge can improve spike-time precision on consecutive theta cycles, support the segregation of spatially-selective pyramidal cells forming distinct assemblies, and contribute to theta-paced dynamic flickering of hippocampal place-cell maps (Jezek et al., 2011; Dupret et al., 2013). Many place cells fire at goal locations during goal-oriented tasks, indicating that salient locations are represented in the hippocampal code (Markus et al., 1995; Hollup et al., 2001; Hok et al., 2007; McKenzie et al., 2013). Place cells encoding such locations showed increased firing synchronization during waking sharp-wave/ripple (SWR, 150-250Hz) events (O'Neill et al., 2006; Dupret et al., 2010). Therefore coincident firing of place cells related to goal locations may provide additional eCB levels and DSI. During SWRs, individual CCK-expressing interneurons exhibited highly variable firing responses, in contrast to the reliable increased firing of PV-expressing basket cells for instance (Klausberger et al., 2005). Moreover, during repetitive discharges, CCK interneurons display an asynchronous mode of GABA release (Losonczy et al., 2004; Hefft and Jonas, 2005; Foldy et al., 2006; Karson et al., 2008; Ali and Todorova, 2010; Lee et al., 2010). Because asynchronous GABA release from CCK-expressing outputs outlasts ripple episodes (Karson et al., 2009), DSI may provide an efficient retrograde presynaptic mechanism to shutdown asynchronous GABA release from CCK-expressing cells. As suggested by Ali and Todorova (2010), decreasing asynchronous release would certainly reduce spike jitter. Nevertheless spike jitter cannot be directly correlated to asynchronous release because, as shown here with feed-forward inhibition in dynamic–clamp and modeling experiments, a delayed inhibition, even if perfectly synchronized, can tune up and down spike jitter according to EPSP-spike coupling probability.

In summary, our data show that place-related firing activity observed during spatial learning can drive an eCB-mediated transient reduction of synaptic inhibition received by pyramidal cells in vitro. This depolarization-induced suppression of inhibition not only affected pyramidal cell excitability but also spike-time precision in such way that self-tuning of CB1R-dependent GABAergic inputs
following place cell activity may be physiologically relevant for both spike-timing coordination and network oscillations in the hippocampus.
Acknowledgements

This work was supported by the Institut National de la Santé et de la Recherche Médicale (INSERM), the Centre National pour la Recherche Scientifique (CNRS), and by a grant from the Fondation Française pour la Recherche sur l’Epilepsie (FFRE). Part of the equipment used in this study were funded by European Community (LSHM-CT-2004-511995), Agence Nationale pour la Recherche (ANR 06-Neuro-014-01) and région PACA (APO ‘Plexin’) grants attributed to D. Debanne. Salary support of F. Dubruc was provided by région PACA (APO ‘Plexin’) doctoral studentship and Neuroservice (B. Buisson). D. Dupret was supported by a MRC Intramural Programme Grant (U138197111). We thank J. Csicsvari for sharing spike-time occurrence of place cells during in vivo recordings of behavioral tasks, M. Manko for her constructive comments on a previous version of the manuscript, L.S. Milescu for help in installing and tuning QuB dynamic clamp system, L. Fronzaroli-Molinieres and C. Gomez for technical assistance, the members of INSERM UMR_S 1072 for helpful comments and suggestions during completion of this work, D. Debanne, K. Lamsa and M. Seagar for critical reading of the manuscript.
References


Figure Captions

**Fig. 1. In vivo firing patterns induce DSI in vitro.**

(A) Up: Scheme representing the position at which action potentials were emitted by a CA1 place cell during 3 consecutive trials when evaluating spatial memory on a cheeseboard maze (Dupret et al., 2010). During learning, the animal is given consecutive trials to locate 3 hidden food rewards (blue circles). The startbox is opened for each trial and the animal harvests the 3 rewards before returning to the startbox and collects a 4th pellet. Each dot corresponds to the location at which place cell emitted an individual spike and is superimposed on the animal’s path. Down: The bars represent the time at which action potentials were emitted by a CA1 place cell during the first, second and third trial. (B) Voltage-clamp recordings (V\text{hold}=−80 mV) of spontaneous GABAergic activity interrupted by transient current-clamp switches during which brief DC injections were performed in order to replay the whole sequence of action potentials that were recorded in vivo (including the time spent in the startbox). Above and with a similar time scale are depicted the time spent in the start box and the cheeseboard maze during the 3 consecutive trials, together with the numbers of action potentials emitted during the crossing of the place field at a time depicted by an arrow. (C) Same experiment as in C except that the cell was dialyzed with THL (1 µM). (D) Time course of spontaneous GABAergic charge during the replay of the whole action potential sequence depicted in B and C in control (black, n=3) and THL (1 µM, white, n=4). The time spent in the place field is denoted in grey. (E) Color-coded rate map of simultaneously recorded place cells for the last 20 trials starting from those depicted in B for Cluster C56 (up) and C71 (down). C56 is the same place cell as the one depicted in A. (F) Voltage-clamp recordings (V\text{hold}=−80 mV) of spontaneous GABAergic activity interrupted by transient current-clamp switches during which brief DC injections were performed in order to replay a sequence of action potentials that were recorded in vivo during a single trial in two different place cells (C56 and C71). The insets represent the sequence of action potentials that was evoked in the in vitro recorded cells (G) mean time course of spontaneous GABAergic charge before and after the replay of the different action potential sequences depicted in F (C56, n=6; C71,n=4). (H) DSI Magnitude measured in the next 4 seconds following action potential discharge for the cells depicted in G.
Fig. 2. Pyramidal cell firing induces endocannabinoid-mediated suppression of GABAergic activity.

(A) Voltage-clamp recordings of spontaneous GABAergic activity (V_{hold}=-80 mV). After a control period the cell is transiently switched to current-clamp mode in order to allow the firing of action potentials either by a series of brief DC injections in order to replay a sequence of action potentials that were recorded in vivo during a single trial in two different place cells (upper and lower left, same patterns as in Fig. 1) or a 1s DC step injection (upper and lower right). (B) DSI magnitude as a function of the number of spikes evoked during a 1s DC step injection in control (black, n=20), in the presence of AM251 (5µM grey, n=8) or THL (1µM, white, n=3). The average change was measured between 1 and 5 s after the end of neuronal discharge. (C) Voltage-clamp recordings (V_{hold}=-80 mV) of spontaneous GABAergic activity interrupted by transient current-clamp switches during which brief DC injections were performed in order to evoke a series of action potentials at various rates and durations. Insets represent voltage fluctuations during each paradigm. (D) DSI magnitude measured at various spike frequency rates and number of action potentials. The number of spikes was either 1 (white) 5 (grey) or 30 (black). (E1) Voltage-clamp recordings of spontaneous GABAergic activity (V_{hold}=-50 mV), interrupted by episodic current-clamp switches, during which 1s duration DC injections were performed in order to evoke a series of action potentials at various rates (insets). The last but one series of action potentials was evoked at a precise time to replay a place cell discharge. (E2) Spike rate vs DC injection for the cell depicted in E1. For DC injection evoked every 30s (black) or 5 sec after the last DC injection of the series (white). (E3) Coefficient of Variation of the Inter Spike Interval (CV_{ISI}) vs spike rate for the cell depicted in A1. (F) Pooled normalized changes in excitability observed during DSI induced by an in vivo spike pattern (n=4 black circles) or a large DC step (n=6 grey circles). (G) Pooled normalized changes in CV_{ISI} observed during DSI. The control CV_{ISI} was interpolated from the spike rate measured during DSI (n=4 and 6 as for F).

Fig. 3. Endocannabinoid-mediated self-tuning of spike-timing in pyramidal neurons.

(A1) Voltage-clamp recordings (V_{hold}=-80 mV) of spontaneous GABAergic activity, interrupted by episodic current-clamp switches, during which 1s duration DC injections were performed to evoke a series of action potentials (AP) at various rates (upper insets). The last series of AP was performed 4 s after the previous one in order to evaluate spike timing during DSI. (A2) Coefficient of Variation
of the Inter Spike Interval (CV$_{ISI}$) vs. spike rate for the cell depicted in A1 in control (black) and
during DSI (white). (A3) Summary of changes in CV$_{ISI}$ for the discharge rate during DSI vs. an
equivalent rate of discharge during the control period (n=10). (B) Same experiments as in (A) but in
the presence of AM251 (5 µM, n=8). (C) Same experiments as in (A) but in the presence of PTX
(100 µM, n=8). (D2) CV$_{ISI}$ vs. spike firing rate and (D3) firing rate vs. DC step in control (blue), in the
presence of SP-IPSGs randomly injected at a rate of 10 (green), 33 (orange) or 100 (red) events/s.
(E) Changes in excitability (black) and CV$_{ISI}$ (white) when neurons received different rates of SP-
IPSGs. (F) Summary of changes in CV$_{ISI}$ for a discharge rate of 20 spikes/s in control conditions
(CTL, n=10), after DSI, in the presence of AM251 (5 µM, n=8), in the presence of PTX and SP-
IPSGs injected dynamically (Dyn) at a rate of 0 (n=8), 10, 33 and 100 events per second (n=5).

Fig. 4. Pyramidal cell firing tunes spike-timing in CA1 pyramidal Neurons.

(A) Current-clamp recording of a cell receiving fluctuating GABAergic dynamic inputs mimicking DSI
after the replay of an in vivo place cell firing pattern and a series of EPSG before (control) during
dDSI and during recovery from DSI. (B) 5 superimposed Vm fluctuations in response to an EPSG
recorded in the presence of spontaneous dynamic-clamp IPSGs, in control conditions, during DSI
and recovery (same cell as in A). (C) EPSP-Spike latencies for the cell depicted in F in control,
during DSI and recovery. (D) Summary for EPSP-Spike jitter measured in different cells for the
same protocol as the one depicted in F (n=4). The filled circles correspond to the mean spike jitter.

Fig. 5. Biphasic tuning of EPSP-Spike timing by Feed-Forward inhibition.

(A) Schema of the stimulating and recording configuration for evoking feed-forward inhibition. (B)
Superimposed current responses recorded in a CA1 pyramidal cell following the extracellular
stimulation of the Schaffer collateral pathway in control, 1 and 30s after DSI. stimulation intensity
was set to record a sequence of inward and outward currents mediated by glutamatergic and
GABAergic synapses respectively, while recording in a voltage-clamp configuration at -50mV with a
low intracellular chloride concentration (C) Fluctuations (grey circles) and average (filled circles)
eIPSC amplitudes over time before and after a 1 s depolarization to 0 mV for 5 successive DSI
protocols. Same cell as the one depicted in B. IPSC amplitude was normalized to 1 during the
control period. (D) Average fluctuations of normalized eEPSC (red) and eIPSC amplitudes (blue)
over time before and after a 1 s depolarization to 0 mV (n=4). (E) From upper to lower superimposed
Vm fluctuations in response to an EPSG (peak amplitude 6.25 nS) followed after 2 ms by FF-IPSGs of various amplitudes (black, 1nS; light grey, 2nS and strong grey, 2.5 nS). Vm fluctuations associated with a spike emission failure are also displayed. The lower traces correspond to the EPSG and IPSG fluctuations over time for the 3 different conditions. (F) EPSP-Spike coupling probability vs the amplitude of feed-forward IPSG. The black and grey circles correspond to conditions depicted in (E). (G) Individual EPSP-Spike latencies (red) superimposed with the mean EPSP-Spike latency +/- jitter vs the amplitude of feed-forward IPSG for the cell depicted in (E). The black and grey circles correspond to conditions depicted in (E). The black and red traces correspond to third order polynomial fits of mean latency and mean latency ± jitter, respectively. (H) Third order polynomial fits of EPSP-Spike jitter vs the probability of EPSP-Spike coupling measured in different cells.

Fig. 6. Modeling Tuning of EPSP-Spike coupling by spontaneous and Feed-forward GABAergic activity.

(A) From left to right superimposed (25) Vm fluctuations in response to a constant EPSG (80nS) in the absence of spontaneous GABAergic activity, in the presence of SP-IPSGs occurring at a rate of 50 and 200 events /s. On the top of each group of traces is represented the mean latency ± jitter (n=250). The EPSP-Spike coupling probability and latency +/- jitter (ms) are written under Vm. Only Vm traces where a spike was successfully emitted are displayed. SP-IPSG fluctuations associated with a spike emission success or failure are shown in blue and grey, respectively (25 traces each). The EPSG is colored in black. (B) EPSP-Spike coupling probability (red) and latency +/- jitter (black) vs. the rate of randomly occurring GABA_A events/s. EPSG amplitude was set at 80nS (n=250). (C) EPSP-Spike coupling probability displayed on a pseudocolor scale vs. SP-IPSG rate and EPSG amplitude. The dashed line corresponds to the conditions displayed in A and B. (D) EPSP-Spike coupling jitter displayed on a pseudocolor scale vs SP-IPSG rate and EPSG amplitude. The dashed line corresponds to the conditions displayed in A and B. (E) From left to right superimposed (25) Vm fluctuations in response to a constant EPSG (80nS) in the absence of FF-IPSG, with FF-IPSG amplitude of 38.5 and 60 nS. On the top of each group of traces is represented the mean latency ± jitter (n=250). The EPSP-Spike coupling probability and latency +/- jitter (ms) are written under Vm. The EPSG (black) and FF-IPSG (red) are displayed and aligned with Vm. (F) EPSP-Spike coupling probability (red) and latency +/- jitter (black) vs. the amplitude of the FF-IPSG. EPSG amplitude was
set at 80 nS (n=250). (G) EPSP-Spike coupling probability displayed on a pseudocolor scale vs. FF-IPSG and EPSG amplitudes. The dashed line corresponds to the conditions displayed on E and F.

(H) EPSP-Spike coupling jitter displayed on a pseudocolor scale vs. FF-IPSG and EPSG amplitudes. The dashed line corresponds to the conditions displayed in E and F. (I) Left Vm fluctuations for a constant EPSG (80 nS) in the absence of FF-IPSG (red), with FF-IPSG amplitude of 39 (blue) and 60 nS (green). In grey is represented the fluctuating threshold. The dashed lines represent the upper and lower limits for spike threshold, when considering its Gaussian distribution and its SD value (1 mV). The firing was cancelled in this simulation in order to see the integration of conductances overtime in the absence of firing. Right traces show Vm fluctuations around spike threshold on an expanded scale. The superimposed bold traces represent the distribution of the time and Vm at which action potential generation were generated in each conditions. (J) Cumulative histograms for action potential latencies for the three conditions depicted in (I). Same colour code as in (I).

Fig. 7. Modeling tuning of EPSP-Spike coupling by combined spontaneous and feed-forward inhibition.

(A) From left to right superimposed (25) Vm fluctuations in the presence of constant FF-IPSG of 39nS and various rates of SP-IPSGs (0-30-65 events/s). The EPSG amplitude was set at 80nS. Only Vm traces where a spike was successfully emitted are displayed. On the top of each group of traces is represented the mean latency ± jitter (n=250). The EPSP-Spike coupling probability and latency +/- jitter are written under Vm. The EPSG is colored in black; FF-IPSG in red; SP-IPSG fluctuations in blue and grey for spike success and failures, respectively. (B) EPSP-Spike coupling probability (red) and latency ± jitter (black) vs. rate of spontaneous GABAergic activity. The EPSG amplitude was set at 80nS. FF-IPSG was set at 39 nS (n=250). (C) Same conditions as in (A) except that the model receive a SP-IPSG rate of 50 events/s and FF-IPSGs of various amplitudes (0-34-44 nS). (D) Same graph as in (B) except that the model receive a SP-IPSG rate of 50 events/s and FF-IPSGs of various amplitudes (n=250). (E) Same conditions as in (A) except that the model receive SP-IPSG rates and FF-IPSGs at constant ratio (7.7 events/s and 6 nS; 28.2 events/s and 22 nS; 50 events/s and 39 nS for SP-IPSG rate and FF-IPSGs, respectively). (F) EPSP-Spike coupling probability (red) and latency ± jitter (black) vs. the percentage of inhibition. 100% inhibition was set when SP-IPSG rate and FF-IPSGs were 50 events/s and 39 nS, respectively. SP-IPSG rates and FF-IPSGs were changed in similar proportions from 0 to 120% of control. (G) EPSP-Spike coupling
probability displayed on a pseudocolor scale vs. FF-IPSG amplitude and SP-IPSG rates. The horizontal, vertical and diagonal lines correspond to the conditions displayed on A-B, C-D and E-F, respectively. (H) EPSP-Spike coupling jitter displayed on a pseudocolor scale vs. FF-IPSG amplitude and SP-IPSG rates. The horizontal, vertical and diagonal lines correspond to the conditions displayed on A-B, C-D and E-F, respectively.
**Control dDSI Recovery**

**Spike Latency (ms)**
- CTL
- dDSI
- Recovery

**Spike Jitter (ms)**
- CTL
- dDSI
- Recovery

**in vivo firing pattern (stim)**
A

STIM
CA1
REC

B

STIM
-50 mV

C

1s @ 0mV

D

1s @ 0mV

E

P=1
Latency = 6.6 +/- 0.7ms

F

Spike Latency (ms)

G

Spike Jitter (ms)

H

Spike Probability

IPSG Amplitude (nS)

IPSG Amplitude (nS)

IPSG Amplitude (nS)