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Kaoutsar Nasrallah, Rebecca A. Piskorowski and Vivien Chevaleyre

CNRS UMR8118, Team Synaptic Plasticity and Neural Networks, FR3636, Université Paris Descartes, Sorbonne Paris Cité, 45 rue des Saints-Pères, 75006 Paris, France

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Correspondence should be addressed to: Vivien Chevaleyre, vivien.chevaleyre@parisdescartes.fr

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3. Authors names: Kaoutsar Nasrallah, Rebecca A. Piskorowski and Vivien Chevaleyre

Affiliation : CNRS UMR8118, Team Synaptic Plasticity and Neural Networks, FR3636, Université Paris Descartes, Sorbonne Paris Cité, 45 rue des Saints-Pères, 75006 Paris, France

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5. Correspondence should be addressed to : Vivien Chevaleyre (vivien.chevaleyre@parisdescartes.fr)

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ABSTRACT

Area CA2 is emerging as an important region for hippocampal memory formation. However, how CA2 pyramidal neurons (PNs) are engaged by intra-hippocampal inputs remains unclear. Excitatory transmission between CA3 and CA2 is strongly inhibited and is not plastic. We show in mice that different patterns of activity can in fact increase the excitatory drive between CA3 and CA2. We provide evidence that this effect is mediated by a long-term depression at inhibitory synapses (iLTD), as it is evoked by the same protocols and shares the same pharmacology. In addition, we show that the net excitatory drive of distal inputs is also increased following iLTD induction. The dis-inhibitory increase in excitatory drive is sufficient to allow CA3 inputs to evoke action potential firing in CA2 PNs. Thus, these data reveal that the output of CA2 PNs can be gated by the unique activity-dependent plasticity of inhibitory neurons in area CA2.

SIGNIFICANCE STATEMENT

Long overlooked, recent work has demonstrated that area CA2 of the hippocampus is a critical region for social memory and aggression. How area CA2 integrates in the hippocampal circuit is not understood. While CA2 pyramidal neurons (PNs) receive excitatory input from CA3 PNs, these inputs cannot drive action potentials in CA2 PNs because of a large feed-forward inhibition. Furthermore, CA2 PNs do not express long-term potentiation, so it is unclear if CA2 PNs can be engaged by CA3. We demonstrate that a unique activity-dependent plasticity of CA2 interneurons can increase the excitatory/inhibitory balance between CA3 and CA2 PNs and allow the recruitment of CA2 PNs. Therefore, our results reveal a mechanism by witch CA2 PNs can be engaged by CA3.

INTRODUCTION

Recent findings have revealed that hippocampal area CA2 plays an important role in learning and memory. Area CA2 has been shown to be necessary for social memory formation (Hitti and Siegelbaum, 2014; Stevenson and Caldwell, 2014) and may act to detect discrepancies between stored memories and current sensory information (Wintzer et al., 2014). It has recently been found that the receptive fields of CA2 pyramidal neuron (PN) place cells change rapidly with time, indicating that this region is playing a role in hippocampal learning that may be separate from spatial orientation (Mankin et al., 2015). Neurons in area CA2 seem poised to modulate hippocampal activity, given the striking number of receptors that are enriched or uniquely expressed in this area (Jones and McHugh, 2011). A clearer understanding of the connectivity in area CA2 has recently evolved (Chevaleyre and Siegelbaum, 2010; Cui et al., 2012; Rowland et al., 2013; Kohara et al., 2014); however, a better understanding of the plasticity of these connections is critical for understanding how this region contributes to hippocampal learning.

Axons from CA3 PNs, i.e. the Schaffer collaterals (SC), connect both CA2 and CA1 PNs. However, unlike the well-described SC-CA1 synapses, the SC-CA2 connection does not display activity-dependent long term potentiation (LTP) (Zhao et al., 2007; Chevaleyre and Siegelbaum, 2010) due to the unique expression of calcium buffering proteins (Simons et al., 2009) and of the regulator of G-protein signaling RGS14 (Lee et al., 2010). Furthermore, there is a higher density of interneurons in area CA2 as compared to CA1 (Benes et al., 1998; Andrioli et al., 2007; Piskorowski and Chevaleyre, 2013; Botcher et al., 2014). While inhibition is known to control the strength and plasticity of excitatory transmission in the hippocampus (Chevaleyre and Piskorowski, 2014), the control exerted by inhibition in area CA2 is extensive. When inhibitory transmission is intact, electrical (Chevaleyre and

Siegelbaum, 2010) or selective optogenetic (Kohara et al., 2014) stimulation of the SC inputs results in a very small depolarizing post-synaptic potential (PSP) comprised of both excitatory and inhibitory potentials (EPSPs, IPSPs). The depolarizing EPSP is abruptly truncated by a large hyperpolarizing component due to inhibitory currents from feed-forward inhibition. This very large feed-forward inhibition completely prevents CA3 excitatory inputs from evoking action potentials (APs) in CA2 PNs (Chevaleyre and Siegelbaum, 2010). Following pharmacological block of inhibition, the amplitude of the PSP becomes ~5 fold larger and is sufficient to drive action potentials in CA2 PNs (Chevaleyre and Siegelbaum, 2010; Piskorowski and Chevaleyre, 2013).

Inhibitory transmission from Parvalbumin expressing (PV+) interneurons in area CA2 undergoes a long-term depression (iLTD) mediated by Delta opioid receptor (DOR) activation (Piskorowski and Chevaleyre, 2013). However, it is unknown whether the synapses that undergo iLTD are playing any role in controlling the net strength of SC-CA2 transmission. We show that the balance between excitation and inhibition between CA3 and CA2 is persistently altered following the induction of DOR-mediated iLTD. Furthermore, induction of iLTD by SC stimulation also permits a net increase in the excitation/inhibition ratio at distal CA2 inputs. Finally, this dis-inhibitory mechanism is sufficient to allow SC inputs to drive AP firing in CA2 PNs, thereby engaging CA2 in the tri-synaptic circuit.

MATERIALS AND METHODS

Slice preparation. All animal procedures were performed in accordance with the Author University animal care committee's regulations. 400 μM transverse hippocampal slices were prepared from 6- to 9-week-old C57BL6 male mice. Animals were euthanized under

anesthesia with isofluorane. Hippocampi were removed and placed upright into an agar mold and cut with a vibratome (Leica VT1200S, Germany) in ice-cold extracellular solution containing (in mM): 10 NaCl, 195 sucrose, 2.5 KCl, 15 glucose, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 1 $CaCl_2$ and 2 $MgCl_2$). A cut was made between CA3 and CA2 with a scalpel in some of the slices before being transferred to 30°C ACSF (in mM: 125 NaCl, 2.5 KCl, 10 glucose, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 2 Na Pyruvate, 2 $CaCl_2$ and 1 $MgCl_2$) for 30 min and kept at room temperature for at least 1.5 hr before recording. All experiments were performed at 33°C. Cutting and recording solutions were both saturated with 95% O2 and 5% CO2 (pH 7.4).

Electrophysiological recordings and analysis. Field recordings of PSPs were performed in current clamp mode with a recording patch pipette (3–5 MΩ) containing 1M of NaCl and positioned in the middle of SR or *stratum pyramidale* (SP) in CA1 or CA2. Whole-cell recordings were obtained from CA2 PNs in current clamp mode held at -73 mV with a patch pipette (3–5 MΩ) containing (in mM): 135 KMethylSulfate, 5 KCl, 0.1 EGTA-Na, 10 HEPES, 2 NaCl, 5 ATP, 0.4 GTP, 10 phosphocreatine and 5 µM biocytin (pH 7.2; 280–290 mOsm). Inhibitory currents were recorded with pipette solution containing 135 CsMethylSulfate instead of KMethylSulfate. The liquid junction potential was ~1.2 mV and membrane potentials were corrected for this junction potential. Some recordings were performed with the perforated patch technique. For these experiments, 75 µg/ml gramicidin was added to the intracellular solution along with an additional 4 mM calcium to ensure recordings were acquired in perforated configuration only. Series resistance (typically 12–18 MΩ for whole cell recordings and 65.5 ± 2 MΩ for perforated patch recordings) was monitored throughout each experiment; cells with more than 15% change in series resistance were excluded from analysis. Synaptic potentials were evoked by monopolar stimulation with a patch pipette filled with ACSF and positioned in the middle of CA1 SR or SLM. When

evoking PSPs in both SR and SLM in the same experiment, we tested the independence of the inputs prior to the experiment. Using the same stimulation intensity used for the experiment, we compared the PSP amplitude evoked by SR stimulation with the PSP amplitude evoked by SR stimulation 100 ms after SLM stimulation. We also performed the reverse measurement, comparing the PSP amplitude evoked by SLM stimulation to a PSP evoked with SLM stimulation following SR stimulation by 100 ms. The stimulation of the SR and SLM inputs were considered independent if a preceding evoked PSP in the separate pathway had no effect on the amplitude of the second evoked PSP. When axons of CA2 pyramidal neurons were directly recruited by the stimulation pipette, as observed with a back-propagating AP in the recorded CA2 neuron, the stimulating pipette was moved until the direct activation of the axon disappeared.

A HFS (100 pulses at 100Hz repeated twice), a 10Hz stimulation (100 pulses at 10Hz repeated twice) or DPDPE ([D-Pen^{2,5}]Enkephalin) was applied following 15 – 20 min of stable baseline. Before beginning whole cell experiments, we identified the CA2 PNs by somatic location and size. Furthermore, the cell type was confirmed by several electrophysiological properties (input resistance, membrane capacitance, resting membrane potential, sag amplitude, action potential amplitude and duration). For several experiments, particularly when Cs^+ was in the pipette solution, the slices were fixed following the recording with 4% paraformaldehyde and the neurons were identified by biocytin-streptavidin labeling and immunohistochemistry to label the CA2-specific protein RGS-14.

The amplitudes of the PSPs were normalized to the baseline amplitude. The magnitude of plasticity was estimated by comparing averaged responses at 30-40 min for whole cell and at 50-60 min for extracellular recordings after the induction protocol with baseline-averaged responses 0–10 min before the induction protocol. All drugs were bath-applied following

dilution into the external solution from concentrated stock solutions. We used Axograph X software for data acquisition and Origin Pro for data analysis. Student's t-test was performed for statistical comparisons and results are reported as mean ± SEM.

RESULTS

Inhibition in area CA2 has been shown to play a very prominent role in controlling the size of the depolarizing component of the compound EPSP-IPSP following stimulation of the SC (Piskorowski and Chevaleyre, 2013). Furthermore, inhibition has been shown to be highly plastic, undergoing an iLTD mediated by DORs (Piskorowski and Chevaleyre, 2013). We asked whether this plasticity of inhibitory transmission might be sufficient to modulate the level of excitatory drive at SC-CA2 synapses. To address this question, we first recorded extracellular post-synaptic potentials (fPSPs) in CA2 *stratum radiatum* (SR) in response to electrical stimulation of SC fibers. These fPSPs are a compound readout of both the local excitatory and inhibitory post-synaptic potentials. After a stable baseline, we applied either a high frequency stimulation (HFS) protocol (100 pulses at 100 Hz repeated twice) or a 10 Hz protocol (100 pulses at 10 Hz repeated twice). These two protocols efficiently induce iLTD of inhibitory inputs in area CA2 (Piskorowski and Chevaleyre, 2013). We found that both the HFS and 10 Hz protocol evoked a lasting increase in the amplitude of the compound fPSP (Figure 1A; with 100Hz: 160.5 ± 4.2% of fPSP amplitude, $p < 0.00001$ $n = 10$; with 10 Hz: Figure 1B; 144.8 ± 9.9% of fPSP amplitude, $p = 0.0034$ $n = 8$). We also observed a significant but smaller increase in the compound fPSP when measuring the slope (136.2 ± 6.4% of fPSP slope, $p = 0.0009$ $n = 8$). We expected a smaller change in the fPSP when measuring the slope, as most of the inhibition evoked by the stimulation is recruited by the SC. The extra synaptic delay of this feed-forward inhibition onto CA2 PNs (compared to the direct SC

transmission) will result in a larger control of the peak rather than of the slope of the fPSP. Therefore, the amplitude of the PSP was used for the analysis of the subsequent experiments.

To address whether a change in excitability of CA3 PNs was responsible for the increase in fEPSP in CA2, we measured the amplitude of the fiber volley (a measure of the number of axons firing an action potential). As shown in figure 1A and B, we did not detect any significant increase in the fiber volley amplitude after HFS (112.0 ± 5.3%, $p = 0.06$) or after 10 Hz stimulation (105.8 ± 4.1, $p = 0.19$). In addition, we also applied HFS in slices that had a detached CA3. In this condition, we found a similar increase of the compound fPSP amplitude (Figure 1A, 158.9 ± 6.2% of fPSP amplitude, $n = 4$, $p = 0.0027$ $p = 0.84$ with uncut slices). These data indicate that the long-term increase in the amplitude of the compound fPSP in area CA2 is not due to recurrent activation of CA3 neurons.

We also tested the effect of HFS and 10 Hz stimulation in whole cell current clamp recordings of CA2 PNs to ascertain that the increased amplitude of the fPSP was a result of an increased excitatory/inhibitory ratio onto CA2 PNs. We found that both the HFS and 10 Hz protocols triggered a large increase in the PSP recorded in this condition (Figure 1 C,D, 223.4 ± 14.1% after HFS, $n = 9$; 195.5 ± 26.3% after 10 Hz, $n = 6$). The larger increase in PSP amplitude observed in whole cell as compared to extracellular recording is not surprising as DORs have been reported to be expressed in interneurons that target PN soma and proximal dendrites (Erbs et al., 2012). A potential iLTD at somatic inputs will likely have a minor contribution in regulating the size of fPSP amplitude recorded in the dendritic area, but will have a large impact on PSP amplitude measured by whole-cell recordings. Consistent with this idea, we found that fPSPs monitored in the somatic region show a larger increase after HFS compared to fPSP monitored simultaneously in the dendritic area (fPSP in soma: 222.3 ± 28.4 % vs

fPSP in dendrite: 164.3 ± 7.1 %, n = 5, p =0.047). Thus, our data show different patterns of activity can increase the excitatory drive between CA3 and CA2.

Inhibitory transmission is mandatory for the HFS-induced long-term increase of the SC-CA2 PSP.

We hypothesize that the increase in the amplitude of the PSP we measured is the consequence of the previously described DOR-mediated iLTD at inhibitory synapses (Piskorowski and Chevaleyre, 2013). That is, this increase in amplitude is due to a dis-inhibitory mechanism, and not a result of a potentiation of excitatory transmission. If this prediction is true, then the lasting increase in the compound PSP amplitude can only occur if inhibitory transmission is left intact.

To test this hypothesis, we recorded extracellular compound fPSPs in areas CA1 and CA2 in response to HFS of SC inputs either in presence or in the absence of $GABA_A$ and $GABA_B$ receptor antagonists (1µM SR 95531 and 2µM CGP 55845). In area CA1, the HFS induced a small increase in the fPSP amplitude when inhibition was intact (Figure 2A; 128.2 ± 4.1% of fPSP amplitude, p = 0.0020, n = 5). As expected, this increase was significantly enhanced when the recordings were performed in the continuous presence of GABA receptor antagonists (Figure 2A; 167.9 ± 13.4%, p = 0.0017 and p = 0.0447 with intact inhibition, n = 8). In area CA2, a robust and lasting increase of the fPSP was induced with inhibition intact (Figure 2B; 160.5 ± 4.2% of PSP basal amplitude, p < 0.00001 n = 10). Furthermore, unlike what was observed in area CA1, the HFS did not evoke any lasting change in the fPSP amplitude when inhibitory transmission was blocked (Figure 2B; 104.9± 2.3% of EPSP amplitude, p = 0.09 n = 10 and p < 0.0001 with intact inhibition).

We also performed the same experiment using whole-cell current clamp recordings of CA2 PNs. Consistent with the extracellular recordings, the HFS induced an increase in the amplitude of the depolarizing response when inhibition was intact (Figure 2C; 207.4 ± 12.6% of PSP amplitude, $p < 0.00001$, $n = 10$), but not in presence of GABA receptor antagonists (Figure 2C; 103.1 ± 5.7% of EPSP amplitude, $p = 0.6997$ and $p = 0.000006$ with intact inhibition, $n = 7$). Altogether, these results show that in area CA2, intact inhibitory transmission is a requirement for the long-term increase of the compound PSP amplitude.

If the increase in the PSP after HFS indeed results from a decrease in inhibition, then it is possible to make the following prediction: pharmacological block of inhibition should increase the amplitude of the PSP, and part of this increase should be occluded by a previous HFS. In order to test this, we performed current clamp recordings of CA2 PNs and applied GABA receptor blockers following a HFS of SC inputs, or in control experiments with no tetanus. We found that when the GABA receptor blockers were applied without the HFS, the amplitude of the PSP amplitude was strongly increased (Figure 3A; 472.8 ± 85.5% of the PSP amplitude, $p = 0.0026$, $n = 6$), confirming that GABAergic transmission exerts a strong negative control on the PSP amplitude in CA2 PNs (Piskorowski and Chevaleyre, 2013). When the GABA receptor blockers were applied after the HFS, the increase in the PSP amplitude was smaller than the effect of the blockers applied without the HFS. (Figure 3A; 185.8 ± 7.8% of increase of the PSP amplitude, $p< 0.00001$ and $p = 0.0074$ compared to blockers without HFS, $n = 6$). However, the final PSP amplitude after the HFS and GABA blocker application was identical to the amplitude of the PSP after GABA blocker application alone (Figure 3A, $p = 0.7$, $n = 6$). Altogether, these results indicate that the HFS-induced increase of the PSP amplitude is mediated by a decrease in GABAergic transmission.

It was previously reported that the SC-CA2 synapse does not express LTP when inhibition is pharmacologically blocked, but also when inhibition is kept intact (Zhao et al., 2007). One potential explanation for the apparent discrepancy with our results could be the difference of chloride used in our internal solution and that of the previous study. The intracellular [Cl^-] of our pipette solution is 7 mM, making an E_{Cl} of -77 mV. The previous study used 16 mM (Zhao et al., 2007), with an E_{Cl} of -55 mV, which is more depolarized than the resting membrane potential of the cell. With the higher [Cl^-] in the intracellular solution, a decrease in GABAergic transmission would likely not increase the compound PSP amplitude. To test whether the difference in E_{Cl} could explain the discrepancy between our results and those of Zhao *et al*., we performed whole-cell current clamp recordings of CA2 PNs with an internal solution containing 16 mM Cl^-. We found that a HFS did not evoke an increase in the amplitude of the PSP in CA2 PNs when using high concentration of chloride in the pipette even with intact GABAergic transmission (Figure 3B; 111.0 ± 16.9% of PSP basal amplitude, $p = 0.4103$ and $p = 0.006$ with control slices $n = 5$). Finally, in order to directly monitor the compound EPSP/IPSP in pyramidal neurons without affecting intracellular [Cl^-], we performed perforated patch recordings with gramicidin in the pipette. In these conditions, we found that HFS evoked a large increase in the amplitude of the PSP that was not different than the one observed with whole cell recordings (Figure 3C; 200.8 ± 19.9% of baseline, $n = 5$, $p = 0.008$ with baseline, $p = 0.7$ compared to whole cell experiments). Altogether, these data strongly indicate that the dis-inhibitory increase in PSP amplitude can occur during physiological conditions.

The long-term increase in the CA2 compound PSP amplitude requires the activation of delta-opioid receptors

Our results show that the potentiation of the PSP amplitude in area CA2 induced by a HFS results from a decrease in inhibition, rather than a direct potentiation of excitatory inputs. If iLTD in area CA2 indeed mediates the increase in the compound PSP, both iLTD and the increase in PSP amplitude should share similar pharmacology. Because the induction of iLTD in area CA2 requires the activation of DORs (Piskorowski and Chevaleyre, 2013), we tested whether HFS-mediated increase in PSP amplitude is also dependent on activation of these receptors.

First we performed extracellular and whole cell current clamp recordings of CA2 PNs in response to SC stimulation and applied a HFS in the presence of a DOR competitive antagonist, ICI 74864. We found that the HFS did not significantly change the CA2 PN PSP amplitude if DORs were not activated during the tetanus (Figure 4A; ICI 74864: 119.6 ± 12.9% of the PSP amplitude, n = 7, p = 0.135 vs. interleaved control: 215.5 ± 20.6% of the PSP amplitude, p = 0.005, n = 5). Similarly, using extracellular recordings, we found no lasting change in the amplitude of the fPSPs following HFS in the presence of DOR antagonists ICI 74864 or Naltrindol (Figure 4B; 108.7±4.6% of the fPSP basal amplitude, p = 0.0822 and p = 0.0006 with absence of DOR antagonist, n = 8). For these experiments, we used two structurally distinct DOR antagonists (ICI 74864 and Naltrindol). Because the increase in PSP amplitude was blocked with both antagonists, data were pooled together.

Together, these data show that DOR activation is needed for induction of the lasting increase in CA2 PN PSP amplitude. We then asked whether activation of DORs would be sufficient to mediate this plasticity. To address this question, we performed extracellular recordings in the SR of area CA2 and applied the specific DOR agonist DPDPE for 15 minutes. We found that the application of DPDPE induced a long-term increase of the fPSP amplitude (Figure 4C, 162.2 ± 17.7% of baseline, p = 0.02, n = 5). To make sure that this long-term potentiation is

due to a DOR-dependent decrease in inhibition and not to a potential direct increase in SC-CA2 excitatory transmission, we performed the same experiment in the presence of GABA receptor blockers. We found that when inhibitory transmission was blocked, DOR activation did not induce any lasting increase in the PSP amplitude at SC-CA2 synapses (Figure 4C; 99.8 ± 4.2% of baseline, $p = 0.71$ $n = 6$). Altogether, these data show that DOR activation is necessary and sufficient to trigger a lasting increase in the amplitude of the PSP between SC and CA2 PNs.

iLTD evoked by SC input stimulation also alters the excitatory/inhibitory balance at distal inputs of CA2 PNs

CA2 PNs are strongly activated by distal inputs in *stratum lacunosum molecualare* (SLM). Thus, we wondered whether stimulation of proximal SC-inputs, via a dis-inhibitory mechanism, could also influence distal inputs in SLM. We used two stimulating electrodes to evoke PSPs in SC and SLM inputs, as diagramed in figure 5A, and checked for the independence of the two pathways before starting experiments (see methods). We found that tetanic stimulation in SR not only led to a large increase in the PSP amplitude of SC inputs, but also increased the amplitude of PSPs from SLM inputs (Figure 5B; 125.9 ± 5.9% of baseline, $p = 0.0017$ with baseline, $n = 10$). In contrast to SC inputs, SLM inputs have been described to express a LTP that is independent of inhibitory transmission (Chevaleyre and Siegelbaum, 2010). Thus, we wondered whether our observed increase in SLM PSP is a result of a direct LTP at these inputs or from a dis-inhibitory mechanism similar to what we have found at SC-CA2 inputs. We repeated the experiment in the presence of GABA receptor blockers and found that the tetanic stimulation in SR did not result in any increase of SLM-evoked PSPs (Figure 5C; 94.6 ± 7.9% of baseline, $p = 0.52$ with baseline, $n = 8$), consistent with a dis-inhibitory mechanism. We then tested if this dis-inhibitory mechanism at the SLM

pathway also depends on DOR activation by applying the SR tetanus in the presence of Naltrindol. We found that the increase in the PSP amplitude of SLM input was completely blocked under these conditions (Figure 5C; 103.9 ± 3.4% of baseline, p = 0.31 with baseline, n = 6).

We postulate that this increase in the strength of SLM inputs is a result of the same DOR-dependent dis-inhibition that increases the strength of SC inputs. To test this hypothesis, we directly monitored IPSCs evoked by stimulation in SR and SLM. We found that a tetanus in SR evokes a lasting and significant iLTD in both SC and SLM inputs onto CA2 PNs (Figure 5D; 77.7 ± 4.8% of baseline, p = 0.005 for SR IPSCs and 87.0 ± 2.5% of baseline, p = 0.003, for SLM IPSCs, n = 6).

The dis-inhibition at SC-CA2 allows SC inputs to drive firing in CA2 PNs

The large feed-forward inhibition recruited by SC stimulation completely prevents action potential firing in CA2 PNs. However, when inhibition is pharmacologically blocked, SC inputs are able to drive action potential firing in CA2 PNs (Chevaleyre and Siegelbaum, 2010). Therefore, we asked whether the decrease in inhibition following iLTD, and the consequent increase in compound PSP amplitude would sufficiently increase the net excitatory drive between CA3 and CA2 PNs to allow SC to drive firing in CA2 PNs.

To test this hypothesis, we monitored the magnitude of the population spike (PS) in the somatic layer of area CA2 before and after HFS. We found that before HFS, there was no PS or a PS of very small amplitude measurable only at the highest intensity stimulation (30 V). This observation confirms that the stimulation of CA3 SC axons was not capable of driving the firing of PNs in the CA2 region. Interestingly, a large PS was revealed in CA2 after HFS (Figure 6A, B, C; with 20V stimulation: PS amplitude increases from 0.035 ± 0.024 mV to

0.37 ± 0.11mV after HFS, p = 0.01, n = 5; with 30V stimulation: PS amplitude increases from 0.09 ± 0.04 mV to 0.59 ± 0.14 mV after HFS, p = 0.006, n = 5). This result shows that the plasticity induced by the HFS is sufficient to allow CA3 inputs to evoke firing of cells in CA2.

To determine if the pyramidal cells in area CA2 experience an increased firing probability following HFS of SC, we performed whole-cell current clamp recordings of CA2 PNs and measured the probability of AP firing in response to a series of 5 pulses at 100 Hz at different stimulus intensities before and after HFS. Before HFS, no APs were evoked in CA2 PNs. However, after HFS, CA2 PNs were able to generate APs in response to SC stimulation. Both the number of APs per train and the proportion of cell firing at least one AP during the train were significantly increased after HFS (Figure 6D, E, F; with 30 V stimulation: from 0 to 1.15 ± 0.3 AP per train after HFS, p = 0.012, n = 6 and from 0 to 80% of cell firing APs after HFS). To ensure that this increase in firing results from the DOR-mediated plasticity at inhibitory synapses evoked by HFS, we performed the same experiment in presence of the DOR antagonist ICI 174864 (2 μM). When HFS was applied in presence of the drug, no significant change in action potential firing was evoked by HFS (Figure 6G, H, I; with 30V stimulation: from 0 to 0.15 ± 0.15 AP per train after HFS, p = 0.37, n = 5 and from 0 to 20% of cell firing APs after HFS). These results show that the DOR-dependent dis-inhibitory increase in PSP is sufficient to allow CA3 inputs to drive AP firing in CA2 PNs.

DISCUSSION

In this study, we have shown that the net excitatory drive between CA3 and CA2 can be persistently increased in an activity dependent manner, even though the CA3-CA2 excitatory transmission does not express a direct LTP. Our results show that the increase in the excitatory drive between CA3 and CA2 is dependent on inhibition and results from a DOR-

mediated iLTD at inhibitory synapses. Furthermore, we show that this dis-inhibition also increases the excitatory drive between SLM inputs and CA2. Finally, this plasticity sufficiently increases the net excitatory drive between CA3 and CA2 to allow SC inputs to drive action potential firing in CA2 PNs.

Activity induces a long-term increase of CA3-CA2 transmission via a dis-inhibitory mechanism.

There are several examples of dis-inhibitory plasticity in the hippocampus. For instance, a pairing protocol induces a shift in GABA reversal potential, thereby increasing synaptic strength at CA3-CA1 synapses (Ormond and Woodin, 2009). Similarly, a pairing protocol between distal and proximal inputs strongly facilitates CA3-CA1 synapses, an effect dependent on a decrease in feed-forward inhibition recruited by SC synapses (Basu et al., 2013). In both cases, the decrease in inhibition facilitates a plasticity that can occur independently of a change in inhibition. In our study, the increase in excitatory/inhibitory balance between CA3 and CA2 is entirely dependent on a dis-inhibition mechanism. Indeed, SC-CA2 excitatory synapses do not express LTP after HFS or other induction protocols (Zhao et al., 2007; Simons et al., 2009; Chevaleyre and Siegelbaum, 2010; Lee et al., 2010). However, we found that HFS or 10Hz stimulation could trigger a lasting increase in the net excitatory drive between CA3 and CA2. Several experiments indicate that this increase is dependent on GABAergic transmission and results from a dis-inhibitory mechanism. First, the lasting increase in PSP amplitude was absent when inhibitory transmission was pharmacologically blocked. Second, increasing the reversal potential for Cl^- in the recorded cell also prevented induction of this plasticity. Third, the increase in the amplitude of the PSP after application of GABA receptor blockers was partially occluded by previous HFS. Fourth, the activity-dependent increase in PSP shares the same pharmacology as the iLTD, i.e., a

strict dependence on DOR activation. And finally, application of a DOR agonist, which induces a lasting decrease in inhibition but does not alter isolated excitatory transmission, was sufficient to evoke the lasting increase in PSP amplitude.

Our results are in agreement with several studies showing a lack of plasticity at SC-CA2 synapses when inhibition was blocked. Nonetheless, they seem to differ from a previous study where no plasticity was evoked at SC synapses with inhibitory transmission left intact (Zhao et al., 2007). We believe that the recording conditions likely account for this apparent discrepancy. While the previous study used a high concentration of Cl^- in the recording solution (16 mM), we used 7 mM of Cl^-. In accordance with this idea, we found no increase in PSP amplitude with 16 mM Cl^- in the recording pipette. The precise physiological concentration of intracellular Cl^- in CA2 PNs is unknown. However, the fact that we observe an increase in PSP amplitude when intracellular Cl^- is not perturbed (using gramicidin perforated patch recordings) and when both intracellular ionic composition and resting membrane potential are not affected (using extracellular recordings), strongly indicates that the dis-inhibitory-mediated increase in PSP amplitude can occur in an intact system. Furthermore, we are confident that the fPSP we record in CA2 is not contaminated by PSP in CA1 because we do not observe LTP in CA2 in presence of GABA blockers, while a large potentiation is observed with simultaneous fPSP recordings in CA1.

The dis-inhibitory increase in CA3-CA2 transmission is dependent on DOR

Our results strongly indicate that the DOR-mediated iLTD recently described (Piskorowski and Chevaleyre, 2013) is involved in the dis-inhibitory increase in PSP between CA3 and CA2. First, the dis-inhibitory potentiation was abolished in presence of two different selective antagonists of DORs (ICI 74864 and Naltrindol). Second, direct activation of DORs with the selective agonist DPDPE was sufficient to trigger a lasting increase in the PSP amplitude.

Therefore, these data show that DORs are necessary and sufficient to increase the PSP amplitude between CA3 and CA2.

Opioids have long been known to increase excitability in the hippocampus and to facilitate the induction of plasticity at excitatory synapses. For instance, activation of mu opioid receptors (MORs) and DORs is required for LTP induction at the perforant path – granual cell synapse in the dentate gyrus (Bramham and Sarvey, 1996) and activation of MORs facilitates LTD induction at the SC-CA1 PN synapse (Wagner et al., 2001). In both cases, the observed increase in excitatory drive is believed to result from a transient decrease in inhibition during the induction protocol, hence facilitating the induction of the plasticity at excitatory inputs.

Our results differ in several ways from the previously reported action of opioids. First, in contrast to the transient effect of DORs on GABAergic transmission in CA1, activation of DORs was shown to induce a lasting depression of inhibition in area CA2 (Piskorowski and Chevaleyre, 2013). In addition, in contrast to the well-described facilitatory action of opioids on plasticity at excitatory synapses, our results show that DOR activation is mandatory for the change in excitatory/inhibitory balance between CA3 and CA2.

Our results show that stimulation in SR increases synaptic strength of both proximal and distal inputs. The increase in the strength of excitatory transmission was dependent on GABAergic transmission. There are several reports of hetero-synaptic plasticity between proximal and distal inputs onto hippocampal PNs. For example, the potentiation of SC inputs onto CA1 PNs following theta-burst stimulation of distal dendritic inputs (Han and Heinemann, 2013), and potentiation of SC inputs and depression of distal dendritic inputs following low frequency stimulation of distal inputs (Wöhrl et al., 2007). To our knowledge, there is no report of a lasting increase in synaptic strength at distal dendritic input that is induced by stimulation in the proximal dendritic region. The simplest explanation for our

results is that stimulation in SR evokes iLTD, and both SC and SLM stimulation recruit the depressed inhibitory inputs. In agreement with this idea are the findings that PV+ basket cells in area CA2 have dendrites that traverse SR and SLM (Mercer et al., 2012; Tukker et al., 2013). Furthermore, it was recently shown that synaptic transmission from PV+ interneurons in area CA2 is depressed during DOR-mediated iLTD (Piskorowski and Chevaleyre, 2013). Together, our results highlight a new mechanism by which activity in SR can enhance the net excitatory drive of synapses in SLM. This interplay is likely to have important consequences on information transfer by extrahippocampal (SLM) and intrahippocampal (SC) inputs onto CA2 PNs.

Physiological and pathological consequences of the dis-inhibitory action of DOR in CA2

When inhibition is intact, SC EPSPs are very small and fail to induce AP in CA2 PNs. Our results show that following the induction of DOR-mediated iLTD, stimulation of SC inputs can evoke APs in CA2 PNs. This was observed both in whole-cell and extracellular recordings, indicating that it can occur when the resting potential of CA2 PNs is not affected. This result is consistent with a previous study using voltage sensitive dyes to study information propagation through the hippocampus that reported that dentate gyrus stimulation resulted in either a fast propagation of activity between CA3 and CA1, or a slower propagation successively recruiting CA3, CA2 and CA1 PNs (Sekino et al., 1997). Interestingly, blockade of GABA receptors allowed recruitment of CA2 from slices in which activation of this region was initially not detected. Our results provide a mechanism for the result observed in this previous study and show that the dis-inhibitory-dependent recruitment of CA2 can be evoked in an activity-dependent manner. While HFS (100 Hz) might not be very physiological, the activity-dependent increase in PSP amplitude can also be evoked with

452 a 10 Hz stimulation, a frequency that falls within the range of theta oscillations. Furthermore,
453 it was previously shown that the depression of inhibitory transmission underlying the increase
454 in PSP can be evoked with 100 and 10 Hz, but also with a theta burst stimulation protocol.
455 Therefore, we think it is likely that physiological patterns of activity will be efficient to
456 trigger the increase in excitatory drive between CA3 and CA2, thus allowing CA2 to be
457 recruited by CA3. We propose that inhibition in area CA2 is acting as a gate to control
458 information flow between CA3 and CA2, and DOR activation can be considered as one of the
459 keys to open this gate.

460 While our results suggest that an activity-dependent decrease in inhibition could be
461 relevant for information transfer by allowing SC inputs to activate CA2 PNs, it is likely that a
462 more global decrease in inhibition in CA2 is detrimental for the hippocampus. For instance, a
463 decrease in the density of PV+ interneurons has been reported to occur uniquely in area CA2
464 during schizophrenia (Knable et al., 2004). Similarly, the density of PV+ cells is considerably
465 reduced in CA2 during epilepsy (Andrioli et al., 2007) and physiological recordings in the
466 hippocampi of human epileptic patients revealed an important decrease (Wittner et al., 2009)
467 or a complete loss of inhibition onto CA2 PNs (Williamson and Spencer, 1994). Our results
468 provide a potential explanation for why a persistent decrease in the inhibitory gate in CA2
469 could alter the tri-synaptic circuit during schizophrenia (Benes, 1999), and why CA2 might be
470 the locus of epileptiform activity generation both in rodents (Knowles et al., 1987) and in
471 humans (Wittner et al., 2009).

472 In summary, our results show that while there is no LTP at the SC-CA2 PN synapse, the
473 excitatory/inhibitory balance can be persistently shifted toward excitation in an activity-
474 dependent manner through a dis-inhibition mechanism. Interestingly, this dis-inhibition
475 results in an increase in net excitatory drive at both SC inputs as well as distal dendritic

476 inputs. Furthermore, our results reveal that the decrease in inhibitory transmission sufficiently
477 increases the excitatory drive from SC inputs to allow recruitment of CA2 by CA3 PNs. Thus,
478 our data add complexity to the hippocampal circuitry and reveal how CA2 PNs can be
479 engaged by intra-hippocampal inputs following activity.

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482 **REFENCES**

483 Andrioli A, Alonso-Nanclares L, Arellano J, DeFelipe J (2007) Quantitative analysis of
484 parvalbumin-immunoreactive cells in the human epileptic hippocampus. Neuroscience
485 149:131–143.

486 Basu J, Srinivas KV, Cheung SK, Taniguchi H, Huang ZJ, Siegelbaum SA (2013) A cortico-
487 hippocampal learning rule shapes inhibitory microcircuit activity to enhance hippocampal
488 information flow. Neuron 79:1208–1221.

489 Benes FM (1999) Evidence for altered trisynaptic circuitry in schizophrenic hippocampus.
490 Biol Psychiatry 46:589–599.

491 Benes FM, Kwok EW, Vincent SL, Todtenkopf MS (1998) A reduction of nonpyramidal cells
492 in sector CA2 of schizophrenics and manic depressives. Biol Psychiatry 44:88–97.

493 Botcher NA, Falck JE, Thomson AM, Mercer A (2014) Distribution of interneurons in the
494 CA2 region of the rat hippocampus. Front Neuroanat 8:104.

495 Bramham CR, Sarvey JM (1996) Endogenous activation of mu and delta-1 opioid receptors is
496 required for long-term potentiation induction in the lateral perforant path: dependence on

GABAergic inhibition. J Neurosci 16:8123–8131.

Chevaleyre V, Piskorowski RA (2014) Modulating excitation through plasticity at inhibitory synapses. Front Cell Neurosci 8.

Chevaleyre V, Siegelbaum SA (2010) Strong CA2 pyramidal neuron synapses define a powerful disynaptic cortico-hippocampal loop. Neuron 66:560–572.

Cui Z, Gerfen CR, Young WS (2012) Hypothalamic and other connections with the dorsal CA2 area of the mouse hippocampus. J Comp Neurol.

Erbs E, Faget L, Scherrer G, Kessler P, Hentsch D, Vonesch J-L, Matifas A, Kieffer BL, Massotte D (2012) Distribution of delta opioid receptor-expressing neurons in the mouse hippocampus. Neuroscience 221:203–213.

Han EB, Heinemann SF (2013) Distal dendritic inputs control neuronal activity by heterosynaptic potentiation of proximal inputs. Journal of Neuroscience 33:1314–1325.

Hitti FL, Siegelbaum SA (2014) The hippocampal CA2 region is essential for social memory. Nature 508:88–92.

Jones MW, McHugh TJ (2011) Updating hippocampal representations: CA2 joins the circuit. Trends Neurosci 34:526–535.

Knable MB, Barci BM, Webster MJ, Meador-Woodruff J, Torrey EF, Stanley Neuropathology Consortium (2004) Molecular abnormalities of the hippocampus in severe psychiatric illness: postmortem findings from the Stanley Neuropathology Consortium. Mol Psychiatry 9:609–20–544.

Knowles WD, Traub RD, Strowbridge BW (1987) The initiation and spread of epileptiform

518 bursts in the in vitro hippocampal slice. Neuroscience 21:441–455.

519 Kohara K, Pignatelli M, Rivest AJ, Jung H-Y, Kitamura T, Suh J, Frank D, Kajikawa K, Mise
520 N, Obata Y, Wickersham IR, Tonegawa S (2014) Cell type-specific genetic and
521 optogenetic tools reveal hippocampal CA2 circuits. Nature Neuroscience 17:269–279.

522 Lee SE, Simons SB, Heldt SA, Zhao M, Schroeder JP, Vellano CP, Cowan DP, Ramineni S,
523 Yates CK, Feng Y, Smith Y, Sweatt JD, Weinshenker D, Ressler KJ, Dudek SM, Hepler
524 JR (2010) RGS14 is a natural suppressor of both synaptic plasticity in CA2 neurons and
525 hippocampal-based learning and memory. Proc Natl Acad Sci U S A 107:16994–16998.

526 Mankin EA, Diehl GW, Sparks FT, Leutgeb S, Leutgeb JK (2015) Hippocampal CA2
527 Activity Patterns Change over Time to a Larger Extent than between Spatial Contexts.
528 Neuron 85:190–201.

529 Mercer A, Eastlake K, Trigg HL, Thomson AM (2012) Local circuitry involving
530 parvalbumin-positive basket cells in the CA2 region of the hippocampus. Hippocampus
531 22:43–56.

532 Ormond J, Woodin MA (2009) Disinhibition mediates a form of hippocampal long-term
533 potentiation in area CA1. Tell F, ed. PLoS One 4:e7224.

534 Piskorowski RA, Chevaleyre V (2013) Delta-Opioid Receptors Mediate Unique Plasticity
535 onto Parvalbumin-Expressing Interneurons in Area CA2 of the Hippocampus. Journal of
536 Neuroscience 33:14567–14578.

537 Rowland DC, Weible AP, Wickersham IR, Wu H, Mayford M, Witter MP, Kentros CG
538 (2013) Transgenically Targeted Rabies Virus Demonstrates a Major Monosynaptic
539 Projection from Hippocampal Area CA2 to Medial Entorhinal Layer II Neurons. Journal

of Neuroscience 33:14889–14898.

Sekino Y, Obata K, Tanifuji M, Mizuno M, Murayama J (1997) Delayed signal propagation via CA2 in rat hippocampal slices revealed by optical recording. Journal of Neurophysiology 78:1662–1668.

Simons SB, Escobedo Y, Yasuda R, Dudek SM (2009) Regional differences in hippocampal calcium handling provide a cellular mechanism for limiting plasticity. Proc Natl Acad Sci USA 106:14080–14084.

Stevenson EL, Caldwell HK (2014) Lesions to the CA2 region of the hippocampus impair social memory in mice. Eur J Neurosci.

Tukker JJ, Lasztóczi B, Katona L, Roberts JDB, Pissadaki EK, Dalezios Y, Márton L, Zhang L, Klausberger T, Somogyi P (2013) Distinct dendritic arborization and in vivo firing patterns of parvalbumin-expressing basket cells in the hippocampal area CA3. Journal of Neuroscience 33:6809–6825.

Wagner JJ, Etemad LR, Thompson AM (2001) Opioid-mediated facilitation of long-term depression in rat hippocampus. J Pharmacol Exp Ther 296:776–781.

Williamson A, Spencer DD (1994) Electrophysiological characterization of CA2 pyramidal cells from epileptic humans. Hippocampus 4:226–237.

Wintzer ME, Boehringer R, Polygalov D, McHugh TJ (2014) The Hippocampal CA2 Ensemble Is Sensitive to Contextual Change. Journal of Neuroscience 34:3056–3066.

Wittner L, Huberfeld G, Clémenceau S, Eross L, Dezamis E, Entz L, Ulbert I, Baulac M, Freund TF, Maglóczky Z, Miles R (2009) The epileptic human hippocampal cornu

ammonis 2 region generates spontaneous interictal-like activity in vitro. Brain 132:3032–3046.

Wöhrl R, Haebler von D, Heinemann U (2007) Low-frequency stimulation of the direct cortical input to area CA1 induces homosynaptic LTD and heterosynaptic LTP in the rat hippocampal-entorhinal cortex slice preparation. Eur J Neurosci 25:251–258.

Zhao M, Choi Y-S, Obrietan K, Dudek SM (2007) Synaptic plasticity (and the lack thereof) in hippocampal CA2 neurons. J Neurosci 27:12025–12032.

FIGURE LEGENDS

Figure 1: HFS and 10 Hz stimulations induce a long-term increase of SC-CA2 PSP amplitude.

Time course of the average normalized PSP amplitude obtained by extracellular recording **(A, B)** in CA2 SR or whole-cell current clamp recording **(C, D)** of CA2 PNs, in response to SC stimulation. Both a HFS protocol (two sets of 100 pulses at 100 Hz; A: $p < 0.00001$, $n = 10$; C: $p = 0.00018$, $n = 9$) and a 10 Hz protocol (two sets of 100 pulses at 10 Hz; B: $p = 0.0009$, $n = 8$; D: $p = 0.01596$, $n = 6$) induce a long-term increase in the SC PSP amplitude in CA2. The fiber volley (FV, a measure of the number of axons firing an action potential) was not significantly increased after HFS (A; $p = 0.06$) or 10Hz stimulation (B; $p = 0.19$). Panel A also shows that making a cut between CA3 and CA2 does not affect the magnitude of the potentiation evoked by HFS ($p = 0.59$ with uncut slices, $n = 5$). Upper right-hand corner in all panels: averaged PSP traces of a representative experiment corresponding to time points before (a) and 60 minutes (b) (A, B) or 40 minutes (C, D) after the stimulation protocol. Error

584 bars show SEM in all panels.

585

586 **Figure 2: The HFS-induced long-term potentiation of the PSP in CA2 is dependent on**
587 **GABAergic transmission.**

588 **(A)** Time course of average normalized fPSP amplitude recorded in CA1 SR in response to
589 SC stimulation, showing that the HFS-induced increase in PSP amplitude in control
590 conditions (open circles, $p = 0.0020$, $n = 5$) was facilitated in continuous presence of the
591 GABA A and B receptor antagonists, 1μM SR 95531 and 2μM CGP 55845 (filled circles, $p =$
592 0.0017, $n = 8$). **(B, C)** In CA2, HFS does not trigger long-lasting increase in the SC PSP
593 amplitude in continuous presence of GABA receptor antagonists (filled circles, B:
594 extracellular recordings, $p = 0.09$, $n = 10$; C: whole cell recording, $p = 0.6997$, $n = 10$), but
595 evokes a large and lasting increase in the PSP amplitude in control experiments (open circles,
596 B: extracellular recordings, $p < 0.00001$, $n = 10$; C: whole cell recording, $p < 0.00001$, $n =$
597 10). In all panels, averaged PSP traces corresponding to the time points before (a) and after
598 (b) HFS are shown on the right. Error bars show SEM in all panels.

599

600 **Figure 3: HFS induces a long-term increase of the PSP amplitude in CA2 via a dis-**
601 **inhibition mechanism.**

602 **(A)** Two representative examples of normalized PSP time course from CA2 PN whole-cell
603 recordings illustrating how a HFS partially occludes the effect of GABAA and GABAB
604 receptor blocker application on PSP amplitude. Top right: PSP traces corresponding to the
605 time points before (a), after HFS (b) and after application of GABA receptor blockers (c),
606 with or without HFS. Bottom right: summary histograms showing the percentage of increase
607 in the PSP amplitude induced by HFS applied alone (1), GABA receptor blocker application

after HFS (2), HFS plus GABA blockers (1+2) and GABA blockers applied without previous HFS (3). **(B)** Normalized CA2 PN SC PSPs recorded with either 7 mM (open circles) or 16 mM (closed circles) Cl- in the pipette solution. A HFS (arrow at time 0) fails to induce a long-lasting increase PSP amplitude when high concentration of chloride is used in the pipette solution (filled circled, $p = 0.4103$, $n = 5$) but evokes normal long-term potentiation in control experiments (open circles, $p = 0.0047$, $n = 5$). (C) Summary graph of experiments performed using the gramicidin-perforated patch-recording configuration. HFS triggers an increase in PSP amplitude ($p = 0.008$, $n = 5$) similar to the one observed using whole-cell recording configuration.

Figure 4: The increase in SC PSP amplitude in CA2 PNs is dependent on DOR activation.

(A) A HFS does not trigger a long-term increase of the PSP amplitude recorded in CA2 PNs in the presence of 2 µM of the DOR competitive antagonist, ICI 174864 (filled circles, $n = 7$, $p = 0.135$) but induces a normal long-term increase in the PSP magnitude in interleaved control experiments (open circles, $p = 0.005$, $n = 5$). **(B)** Time course of normalized fPSP amplitude recorded in CA2 SR showing how the application of a DOR antagonist (ICI 174864 or Naltrindol 0.1µM) during HFS (filled circles, $p = 0.0822$ and $p = 0.0006$ with absence of ICI 74864, $n = 8$) prevented the induction of a lasting increase of the fPSP amplitude observed in absence of drug application (open circle). Averaged PSP traces corresponding to the time points before (a) and after (b) HFS performed in control conditions (top) or in the presence of ICI 174864 (bottom) are shown on the right. **(C)** Application of 0.5 µM of a DOR-selective agonist (DPDPE) is sufficient to induce a long lasting increase in the fPSP amplitude recorded in SR of CA2 in the absence (open circles, $p = 0.02$, $n = 5$) but not in the presence (filled circles, $p = 0.55679$, $n = 6$) of GABAA and GABAB receptor blockers.

Right, example fPSP traces corresponding to the time points before (a) and after (b) application of DPDPE in absence (top) or in continuous presence (bottom) of the GABAA and GABAB receptor blockers. Error bars show SEM in all panels.

Figure 5: Stimulation in SR induces a heterosynaptic iLTD and increases distal excitatory drive onto CA2 PN.

(A) Cartoon illustrating the arrangement of the stimulating recording electrodes in SR and SLM. **(B)** Average PSP amplitudes of SR (open circles) and SLM (closed circles) inputs following HFS stimulation in SR. Note that both SR and SLM inputs are potentiated following the HFS ($p = 0.00035$ for SR inputs, $p = 0.0017$ for SLM inputs, $n = 10$), but only SR inputs show a rapid post-tetanic increase in amplitude. Top, averaged PSP traces corresponding to the time points before (a) and after (b) HFS. **(C)** The increase in distally evoked PSP following stimulation in SR was blocked by GABAA and GABAB receptor blockers (open circles, $p = 0.52$, $n = 8$) and by the DOR antagonist naltrindol (grey circles, $p = 0.31$, $n = 6$). **(D)** Average amplitude of IPSCs evoked by stimulation in SR and SLM following HFS in SR. Note that both inputs express an inhibitory LTD following HFS in SR ($p = 0.006$ for SR inputs, $p = 0.003$ for SLM inputs, $n = 6$).

Figure 6: HFS in SR allows CA3 inputs to evoke action potential firing in CA2 PNs.

(A) Traces of extracellular recordings in the CA2 pyramidal layer in response to a 20V stimulation of SC inputs, before (grey traces) and after a HFS (black traces), illustrating how HFS induces an increase in the population spike (PS) amplitude (negative peak). **(B)** Average PS amplitude as a function of stimulation intensity before (open circle) and after (filled circle) HFS (with 20V stimulation: $p = 0.01$, $n = 5$; with 30V stimulation: $p = 0.006$, $n = 5$). **(C)**

Time course of average normalized PS amplitude recorded in CA2 pyramidal layer in response to a 20V stimulation of SC inputs, showing a long lasting increase in PS amplitude after HFS (p=0.01, n=5). **(D)** Traces of whole-cell current clamp recordings in a CA2 pyramidal cell in response to a train of stimulations (5 pulses at 100Hz) of SC inputs, illustrating how APs can be evoked in CA2 neurons after (black traces) but not before HFS (grey traces). **(E)** Average number of APs per train at different stimulation intensities, showing how a 5 pulse train at 100Hz of the SC inputs does not trigger APs in CA2 PNs before HFS (open circles) but induces APs after HFS (filled circles, with 30 V stimulation: from 0 to 1.15 ± 0.3 AP per train after HFS, p = 0.012, n = 6). **(F)** A HFS increases the percentage of CA2 PNs firing at least one AP during the train (with 30V stimulation: from 0% to 80% of cell firing APs). **(G)** Traces of whole-cell current clamp recordings in a CA2 PN in response to a stimulation train (5 pulses at 100Hz) of SC inputs in the presence of the DOR antagonist ICI 174864 (2μM), illustrating how application of ICI 174864 prevents the induction of APs in CA2 PNs after HFS (black traces). **(H)** Average number of APs per train at different stimulation intensities in the presence of the DOR antagonist before (open circles, with 0 to 30V stimulations: 0 AP per train) and after HFS (filled circles, with 30 V stimulation: 0.15 ± 0.15 AP per train after HFS, p = 0.37, n = 5). **(I)** In presence of ICI 174864 HFS does not induce a large increase of the percentage of CA2 PNs firing at least one AP during the train (with10V stimulation: 0% of cell firing APs before and after HFS; with 20V and 30V stimulation: from 0% to 20% of cell firing APs). Error bars show SEM in all panels.