Bi-directional interplay between proximal and distal inputs to CA2 pyramidal neurons

Kaoutsar Nasrallah, Rebecca Ann Piskorowski, 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

A R T I C L E   I N   P R E S S

Hippocampal area CA2 is emerging as a critical region for memory formation. Excitatory Schaffer collateral (SC) inputs from CA3 do not express activity-dependent plasticity at SC-CA2 synapses, and are governed by a large feed-forward inhibition that prevents them from engaging CA2 pyramidal neurons. However, long-term depression at inhibitory synapses evoked by stimulation of SC inputs highly increases the excitatory/inhibitory balance coming from CA3 and allows the recruitment of CA2 pyramidal neurons. In contrast, distal excitatory inputs in stratum lacunosum moleculare (SLM) can drive action potential firing in CA2 pyramidal neurons and also express a long-term potentiation. However, it is unknown whether stimulation of distal inputs can also evoke plasticity at inhibitory synapses and if so, whether this plasticity can control the strength of excitatory inputs. Here we show that stimulation in SLM evokes a long-term depression at inhibitory synapses. This plasticity strongly increases the excitatory drive of both proximal and distal inputs and allows CA3 to recruit CA2 pyramidal neurons. These data reveal a bi-directional interplay between proximal and distal inputs to CA2 pyramidal neurons that is likely to play an important role in information transfer through the hippocampus.

© 2016 Elsevier Inc. All rights reserved.

1. Introduction

Activity-dependent changes in synaptic efficacy, such as long-term potentiation and depression (LTP and LTD), are crucial to experience-driven refinement of neural connections in the mammalian brain and are an important component of the cellular mechanisms underlying learning and memory formation (Martin, Grimwood, & Morris, 2000; Mayford, Siegelbaum, & Kandel, 2012). Such forms of synaptic plasticity have been extensively studied in the hippocampus, a brain region critically involved in memory formation. In the hippocampus, the pyramidal cells in areas CA1, CA2 and CA3 receive distinct sets of inputs at discrete regions in their dendritic arbor. Much less is known about how synaptic plasticity at one set of synaptic inputs can modulate the synapses at a separate set of inputs that converge onto the same postsynaptic neuron. Such hetero-synaptic forms of plasticity are of great interest because they enable different neuronal circuits to perform a wide range of mnemonic processing (Abbott & Regehr, 2004; Spruston, 2008).

Area CA2 of the hippocampus has been shown to play a crucial role in social memory (Hitti & Siegelbaum, 2014; Stevenson & Caldwell, 2014) and aggressive behavior (Pagani et al., 2015). Furthermore, this region has recently been reported to be the basis of a hippocampal network that encodes location during immobility and sleep (Kay et al., 2016). Given the importance of these newly-discovered roles, a better understanding of the physiology of the often-overlooked area CA2 is necessary. CA2 pyramidal neurons (PNs) have distinct biophysical, molecular and anatomical properties that clearly distinguish them from their CA1 and CA3 neighbors (recently reviewed in (Dudek, Alexander, & Farris, 2016)). For instance, they are more hyperpolarized, have lower input resistance, larger membrane capacitance less of a sag in response to hyperpolarizing current. At a molecular level, they uniquely express numerous proteins such as vasopressin 1b receptors, adenosine receptors and the regulator of G-protein signaling protein, RGS14. CA2 PNs receive glutamatergic inputs from CA3 PNs via the Schaffer collateral (SC) pathway onto their proximal apical dendrites located in stratum radiatum (SR) and from the entorhinal cortex onto their distal apical dendrites located in stratum lacunosum-moleculare (SLM) (Kohara et al., 2013). Unlike SC-CA1 excitatory synapses, the SC-CA2 glutamatergic synapses do not express activity-dependent long-term potentiation (LTP) (Zhao, Choi, Obrietan, & Dudek, 2007), potentially because of the unique hippocampal expression of postsynaptic signaling molecules and potential calcium binding proteins (Lee et al., 2016).
2010; Simons, Escobedo, Yasuda, & Dudek, 2009). Moreover, the Schaffer collateral (SC) input to CA2 PNs is dominated by a powerful feed-forward inhibition (FFI) that tightly controls the excitatory postsynaptic potential (EPSP) and prevents action potential firing of CA2 PNs, while SC inputs strongly excite CA1 PNs (Chevaleyre & Siegelbaum, 2010; Piskorowski & Chevaleyre, 2013). In contrast to SC-CA2 excitatory synapses, inhibitory transmission from parvalbumin-expressing (PV+) interneurons in area CA2 is highly plastic, undergoing a long-term depression (iLTD) that is mediated by delta opioid receptor (DOR) activation following stimulation of SC inputs or activation of DORs with agonist application (Piskorowski & Chevaleyre, 2013). This DOR-mediated iLTD allows a lasting increase of both proximal and distal input excitatory drive onto CA2 PNs (Nasrallah et al., 2015). Therefore, we wondered whether the crosstalk between proximal and distal inputs could be bi-directional, i.e. whether stimulation of distal inputs could also trigger an iLTD of inhibitory transmission and a dis-inhibitory increase in proximal and distal excitatory inputs.

Using electrophysiology and selective pharmacology in adult mouse hippocampal slices, we show that high frequency stimulation (HFS) of distal inputs induces both homo-synaptic and heterosynaptic iLTD in area CA2. We found that this iLTD is potentiated by a NMDA receptor antagonist (D-APV) (Chevaleyre & Siegelbaum, 2010), we applied D-APV (50 μM) to block NMDA receptors during the HFS (10 min before to 5 min after HFS). We found that EPSPs recorded in presence of GABAA and GABAB receptor antagonists are not potentiated by a HFS when NMDA receptors were blocked (Fig. 2A: filled circles: 103.9 ± 11.3%, p = 0.75, n = 5), confirming that distal inputs to CA2 PNs do not express LTP when NMDA receptors are blocked. We then performed the same experiment with inhibitory transmission intact. In these conditions we found that a HFS induced a lasting increase in the PSP amplitude (Fig. 2A: open circles: 127.5 ± 10.0%, p = 0.033, n = 7). To ensure that this change in PSP amplitude was not an artifact of directly stimulating interneurons with the stimulating electrode, we performed the same experiment with the stimulating electrode in CA1 SLM far from CA2. In these conditions, HFS application in SLM still induced a long-term increase in the PSP amplitude when inhibitory transmission was intact (Fig. 2B: white circles: 170.8 ± 21.9%, p = 0.032, n = 5) but not in the continuous presence of GABAA and GABAB receptor antagonists (Fig. 2B: filled circles: 106.3 ± 8.6%, p = 0.65, n = 5, p = 0.026 with the interleaved controls).

In order to test whether the HFS-induced increase in PSP amplitude occurs when both intracellular [Ca2+] and membrane potential are unaltered by the intracellular recording solution, and to determine whether inhibitory synapses that express iLTD exert a local control in CA2 SLM, we performed extracellular recordings of field PSPs (fPSP) by placing a recording pipette in CA2 SLM. The fPSPs were evoked with a stimulating pipette placed in CA1 SLM far from CA2. We found that HFS induced a lasting increase in the fPSP amplitude in presence of GABAA and GABAB receptor antagonists (Fig. 2C: filled circles: 100.4 ± 8.4%, p = 0.043, n = 5, p = 0.002 with the interleaved controls). Altogether, these data indicate that HFS of distal inputs is capable of increasing distal PSP amplitude via a dis-inhibitory mechanism, and part of this dis-inhibition occurs locally in SLM.

2.2. Stimulation in SLM induces a hetero-synaptic iLTD and increases proximal excitatory drive onto CA2 PNs

In area CA2, a HFS of proximal SC inputs can trigger a hetero-synaptic iLTD of distally evoked IPSCs and a hetero-synaptic dis-inhibition of distal excitatory inputs (Nasrallah et al., 2015). Could this interplay be bi-directional? Can a HFS in SLM also trigger iLTD and a dis-inhibition of proximal excitatory inputs? To answer this question, we first recorded IPSCs in CA2 PNs evoked by stimulation in SR in the continuous presence of NBQX (10 μM) and D-APV (50 μM). After a stable baseline period, we applied a HFS in SLM with a stimulation pipette near CA2. We found that this resulted
in a lasting decrease in the SR-evoked IPSC amplitude (Fig. 3A: 85.9 ± 3.5%, p = 0.016, n = 5). This iLTD was also accompanied by a significant increase in the PPR (Fig. 3A, from 0.695 ± 0.044 to 0.738 ± 0.037; p = 0.038). We then wondered 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 performed whole-cell current clamp recordings of CA2 neurons in response to SC stimulation in the presence of N-APV (50 μM). We monitored the PSP amplitude and applied a HFS in CA1 SLM either near or further away from area CA2. We found that both configurations induced a large and lasting increase in SR PSP amplitude (Fig. 3B: white circles, 232.4 ± 20.0%, p = 0.0002, n = 5; Naltrindole: filled circles, 101.4 ± 15.9%, p = 0.93, n = 5; p = 0.001 with interleaved controls). To confirm that the increase in proximal PSP is a result of a disinhibition and not a direct LTP of excitatory synapses, we repeated the experiment in the presence of GABA receptor blockers. We observed that the HFS in SLM did not result in an increase in the amplitude of SR-evoked EPSPs (Fig. 3B: filled circles, 110.3 ± 6.0%, p = 0.13, n = 10). These data show that a HFS of distal inputs can trigger a hetero-synaptic iLTD of proximal inputs that is sufficient to increase the PSP amplitude of SC-CA2 inputs.

2.3. DOR activation is required for the long-term decrease in IPSC and increase in PSP in both proximal and distal inputs to CA2 neurons

It has been shown that both iLTD and the dis-inhibitory increase in PSP amplitude evoked by SR stimulation are mediated by DORs (Nasrallah et al., 2015; Piskorowski & Chevaleyre, 2013). Therefore, we tested whether the iLTD and the dis-inhibitory mechanism evoked by a HFS at the SLM pathway also depends on DOR activation. First, we performed whole-cell voltage clamp recordings of CA2 neurons in response to SC stimulation in the presence of NBQX (10 μM) and D-APV (50 μM). (B: p = 0.023, n = 5). Right, example IPSC traces corresponding to time points before (a) and after (b) HFS. If the traces are normalized to the peak of the first IPSC, the increase in the PPR is apparent (bottom traces). (C) Change in PPR for the individual experiments before and after HFS. (D) Cartoon illustrating the arrangement of the recording and stimulating electrodes in CA1 SLM far from CA2. (E, F) HFS in CA1 SLM far from CA2 induce a long-term decrease in the IPSC amplitude in absence (E: p = 0.007, n = 6) but not in presence of NBQX and D-APV (D: p = 0.50, n = 6). Averaged PSP traces corresponding to the time points before (a) and after (b) HFS are shown above. Error bars indicate the SEM in all panels.
controls). Altogether, these results show that following distal HFS, both iLTD and the dis-inhibitory increase in PSP amplitude at proximal and distal inputs to CA2 PNs is mediated by DOR activation.

2.4. HFS of distal inputs increased the firing of CA2 PNs in response to both proximal and distal excitatory inputs

SLM inputs have been shown to strongly excite CA2 PNs even though they target the distal region of CA2 PN apical dendrites, (Chevaleyre & Siegelbaum, 2010). Moreover, it has been reported that blocking inhibitory transmission greatly increases the amplitude of the population spike (PS) evoked by SLM input stimulation in CA2, reflecting an increase CA2 neuron firing (Chevaleyre & Siegelbaum, 2010). We asked whether the decrease in inhibition following HFS-induced iLTD is sufficient to increase the firing of CA2 PNs in response to distal input stimulation. To test this hypothesis, we performed extracellular recordings in the somatic layer of area CA2 and stimulated SLM input in presence of D-APV (50 μM). We monitored the magnitude of the PS before and after a HFS of distal input. We found that a HFS induced a large increase

Fig. 2. HFS of distal inputs induces a lasting increase of the PSP amplitude in GABA-dependent and NMDA-independent manner. Cartoons illustrating the arrangement of the recording (R) and stimulating (S) electrodes in CA1 SLM near (A) or far (B, C) from CA2 are shown on the left. Time course of average normalized PSP amplitude obtained from whole-cell current clamp recordings of CA2 PNs (A, B) or extracellular recordings in CA2 SLM (C) in response to SLM stimulation. HFS induces a lasting increase in PSP amplitude in control conditions (open circles, A: \( p = 0.033, n = 7 \); B: \( p = 0.032, n = 5 \); C: \( p = 0.007, n = 5 \)) but not in the continuous presence of the GABA<sub>A</sub> and GABA<sub>B</sub> receptor antagonists (1 μM SR 95531 and 2 μM CGP 55845; filled circles, A: \( p = 0.75, n = 5 \); B: \( p = 0.65, n = 5 \); C: \( p = 0.43, n = 5 \)). In all panels, averaged PSP traces corresponding to the time points before (a) and after (b) HFS are shown on the right and error bars indicate the SEM.
in PS amplitude in control conditions (Fig. 5A–C; ANOVA two-way RM for HFS: F(1,7) = 14.9 p = 0.006, Tukey test p = 0.0005). However, in the presence of a DOR antagonist, no increase in PS was observed, indicating that it is entirely mediated by the DOR-dependent dis-inhibition (ANOVA two-way RM for HFS: F(1,5) = 1.25 p = 0.3; ANOVA two-way RM for DOR antagonist: F(1,5) = 8.33 p = 0.03, Tukey test p = 0.004).

We then asked whether the decrease in inhibition after HFS in SLM would be sufficient to increase the E/I ratio between CA3 and CA2 PNs to allow SC to drive firing in CA2 PNs. To address this question, we monitored the PS evoked by proximal input stimulation before and after a HFS in SLM. We found that a HFS induced a large increase in PS amplitude in control conditions (Fig. 5D–F; ANOVA two-way RM for HFS: F(1,7) = 20.1 p = 0.003, Tukey test p = 0.0003) but not in the presence of a DOR antagonist (ANOVA two-way RM for HFS: F(1,5) = 9.2 p = 0.03, Tukey test p = 0.49). These results demonstrate that the DOR-mediated iLTD induced by the HFS in SLM increases the firing of CA2 neurons in response to both distal and proximal input stimulation.

3. Discussion

In this study, we examined whether a stimulation of CA2 PN distal inputs could induce activity-dependent changes in inhibitory transmission and how such plasticity could impact the different converging inputs onto CA2 PNs. First, we have shown that HFS of distal inputs evoked iLTD on both proximal and distal inhibitory synapses in area CA2. Second, we demonstrated that this plasticity of inhibition increases the net excitatory drive between SLM inputs and CA2 PNs and also between CA3 inputs and CA2 PNs. Third, we found that these activity-dependent changes are sufficiently large to increase CA2 PN firing in response to both proximal and distal input activation, allowing SC inputs to drive action potential firing in CA2 PNs. Our results show that the increase in the E/I ratio at both SLM-CA2 and SC-CA2 synapses are dependent on inhibition and DOR activity.

3.1. Activity in distal inputs induces a long-term increase of SLM–CA2 transmission via a DOR-dependent dis-inhibitory mechanism that is sufficient to increase CA2 PN firing

In addition to the NMDA-dependent presynaptic LTP at distal excitatory synapses in CA2 (Chevaleyre & Siegelbaum, 2010), our results highlight a DOR-mediated dis-inhibitory mechanism by which activity can enhance the net excitatory drive at distal inputs. Indeed, DOR activation can increase the excitatory/inhibitory balance at distal inputs by reducing inhibitory transmission while NMDA receptor activation directly increases the strength of excitation (Chevaleyre & Siegelbaum, 2010). Our data indicate that this increase is dependent on GABAergic transmission and results from a dis-inhibitory mechanism. First, the plasticity was evoked in the presence of NMDA receptor antagonist D-APV avoiding any contribution of the excitatory LTP. Second, HFS-induced increase in distally-evoked PSP amplitude was completely prevented in
presence of GABA_{A} and GABA_{B} receptor antagonists. Indeed, blockade of inhibitory transmission increased the amplitude of the excitatory synaptic potentials and occludes further potentiation by HFS. Third, we found that HFS induced a decrease in inhibitory transmission by directly monitoring IPSC amplitude. The fact that we also found an increase in PSP amplitude using extracellular recordings, a condition with both intracellular ionic composition and resting membrane potential left unaltered strongly indicates that the dis-inhibitory increase in PSP amplitude can occur in an intact system. Finally, HFS fails to evoke iLTD and to increase the PSP amplitude in the presence of a DOR antagonist.

We found that the activity-dependent dis-inhibitory plasticity is sufficient to increase or reveal action potential firing in CA2 PNs in response to distal input stimulation. The HFS-induced increase in population spike magnitude was found in the presence of NMDA receptor antagonist excluding any contribution of the excitatory LTP, but was abolished in the presence of DOR antagonist. It was reported that a complete block of inhibition with...
GABA<sub>A</sub> and GABA<sub>B</sub> receptor antagonists increases CA2 neuron firing in response to distal input stimulation (Chevaleyre & Siegelbaum, 2010). Here we show that a more physiological, activity-dependent, decrease in inhibition is also sufficient to increase action potential firing of CA2 PNs in response to distal input stimulation. Interestingly, previous reports have shown that the distal inputs provide a strong excitatory drive onto CA2 PNs that can be enhanced by a direct LTP (Chevaleyre & Siegelbaum, 2010). Here we show that a more physiological, activity-dependent, decrease in inhibition is also sufficient to increase action potential firing of CA2 PNs in response to distal input stimulation. Interestingly, previous reports have shown that the distal inputs provide a strong excitatory drive onto CA2 PNs that can be enhanced by a direct LTP (Chevaleyre & Siegelbaum, 2010). We show that the strength of distal excitatory inputs can also be increased by a dis-inhibitory mechanism.

3.2. Activity of distal input to CA2 opens the gate between CA3 and CA2

Our data are consistent with the fact that SC excitatory inputs in area CA2 are strongly and negatively controlled by inhibition (Chevaleyre & Siegelbaum, 2010; Kohara et al., 2014; Piskorowski & Chevaleyre, 2013). Furthermore, it has been recently shown that stimulation of CA2 proximal inputs increases synaptic strength of both proximal and distal inputs via a similar DOR-dependent dis-inhibitory mechanism (Nasrallah et al., 2015). Similarly, our results show that stimulation in SLM increases the synaptic strength of both proximal and distal inputs via a GABA- and DOR-dependent mechanism, indicating a bidirectional interaction between proximal and distal excitatory inputs in CA2 via inhibitory transmission. Indeed, we confirm directly that inhibitory transmission evoked either proximally or distally was decreased following stimulation in the distal part of the dendrite and this iLTD was dependent on activation of DORs. We believe that the DOR-dependent iLTD could play an important role in controlling the flow of information from excitatory inputs. We have shown that the amplitude of evoked excitatory potentials from both proximal and distal inputs is strongly increased after iLTD induction. Given the high density of PV+ interneurons in CA2, it is also very likely that they will contribute to spontaneous inhibitory potentials in CA2 PNs. Therefore, change in GABA release from PV+ interneurons might also have consequences on tonic inhibition and potentially on the level of depolarization of PNs.

Although the exact nature of the excitatory/inhibitory microcircuit remains to be determined, our current results and those from a previous study (Piskorowski & Chevaleyre, 2013) allow us to make some predictions. A HFS in either SR or in SLM triggers iLTD (and dis-inhibition of excitatory inputs) of both proximal and distal inputs. Thus, the simplest explanation is that interneurons that undergo iLTD are connected by both proximal and distal excitatory inputs. This hypothesis is consistent with the fact that (i) iLTD is expressed by PV+ interneurons and (ii) anatomical data show that in area CA2, PV+ interneurons have dendrites that span throughout SR and SLM (Mercer, Botcher, Eastlake, & Thomson, 2012; Tukker et al., 2013). Similarly, we postulate that the cells that are releasing enkephalin are also connected by both proximal and distal inputs even though the origin and mechanism of enkephalin release is unknown. It has been reported that enkephalin can be detected in the mossy fibers and axons originating from the entorhinal cortex (Simmons & Chavkin, 1996) but also in a small subpopulation of interneurons that target other interneurons in the hippocampus (Blasco-Ibáñez, Martínez-Guijarro, & Freund, 1998; Fuentebalba et al., 2008). In area CA1, enkephalin-containing interneurons preferentially project to PV+ interneurons but not to somatostatin-, calbindin-, or CCK-expressing interneurons (Fuentebalba et al., 2008). Therefore, it is possible that these enkephalin-containing interneurons...
interneurons are connected by both distal and proximal inputs and are the main trigger of the plasticity we observe. We cannot discard the fact that the cells releasing enkephalin upon stimulation of proximal and distal inputs could be distinct populations. However, because there is a local increase in field PSP in both the SR (Nasrallah et al., 2015) and the SLM (current study), this would imply that the distinct populations release enkephalin in both the proximal and distal compartments of area CA2, or that enkephalin released locally in one compartment can diffuse.

Interestingly, in vivo recordings during network activity have shown that the enkephalin-expressing interneurons were phase modulated throughout theta oscillations, but silenced during sharp-wave/ripple episodes and exhibited rebound activity of high-frequency spike bursts (Fuentealba et al., 2008). Therefore, we think that enkephalin-containing neurons display patterns of activity that are consistent with the stimulation that we use to evoke iLTD. This suggests that the increase in excitatory drive between CA3 and CA2 and the recruitment of CA2 PNs by CA3 could occur in vivo during physiological conditions.

There are several reports of hetero-synaptic plasticity between proximal and distal inputs onto hippocampal PNs. Theta-burst stimulation of distal CA1 input induces a LTD of proximal input without affecting distal inputs, while low frequency stimulation of distal inputs induces LTD at distal inputs and LTP at proximal inputs (Han & Heinemann, 2013; Wohrer, Haebler, & Heinemann, 2007). Furthermore, in area CA2 we have shown that HFS of proximal input induces LTD allowing a lasting increase in both the E/I ratio and the firing of CA2 neurons in response to both proximal and distal excitatory input stimulation (Nasrallah et al., 2015; Piskorowski & Chevaleyre, 2013). Here, we found similar results with a HFS of distal inputs, revealing a bi-directional interplay between proximal and distal excitatory inputs to CA2. Therefore, we can postulate that paired stimulation between proximal and distal inputs, as used in CA1 to evoke a cannabinoid-dependent dis-inhibition of SC inputs (Basu et al., 2013), will also be efficient to evoke DOR-dependent dis-inhibition in CA2. Thus, our data confirm that the plasticity of inhibitory transmission in area CA2 is playing a critical role in controlling excitatory drive onto CA2 PNs, and they reveal an interesting interaction between converging inputs onto CA2 PNs that is likely to have important consequences on information transfer in the hippocampus.

4. Material and methods

4.1. Slice preparation

All animal procedures were performed in accordance with the regulations of the animal care committee of the Université Paris Descartes. 400 μm transverse hippocampal slices were prepared from 5- to 12-week-old C57BL/6 male mice as previously described (Nasrallah et al., 2015). Animals were anesthetized with isoflurane and killed in accordance with institutional regulations. Animals were anesthetized with NaCl, 195 sucrose, 2.5 KCl, 15 glucose, 26 NaHCO₃, 1.25 NaH₂PO₄, 1 CaCl₂, and 2 MgCl₂. For mice older than 6 weeks, we used a cutting solution with 95% O₂ and 5% CO₂, pH 7.4. All experiments were performed at 33 °C.

4.2. Electrophysiological recordings and analysis

Field recordings of postsynaptic potentials (PSPs) were performed in current-clamp mode with a recording patch pipette (3–5 MΩ) containing 1 M NaCl and positioned in the middle of stratum lacunosum moleculare (SLM) or stratum pyramidale (SP) in CA2.

PSPs (post-synaptic potentials) were obtained with whole-cell recordings from pyramidal neurons in current-clamp mode. Current was injected to keep the resting membrane potential at −73 mV. Patch pipettes (2.5–4 MΩ) contained the following (in mM): 135 K methyl sulfate, 5 KCl, 0.1 EGTA-Na, 10 HEPES, 2 NaCl, 5 ATP, 0.4 GTP, and 10 phosphocreatine (pH 7.2; 286–295 mOsm) and biocytin (4 mg/mL). Membrane potentials were corrected for liquid junction potential, which was measured to be −1.2 mV. The bridge was balanced and monitored throughout the recording.

Whole-cell recordings of IPSCs (inhibitory post-synaptic current) were performed in voltage-clamp mode with the cells held at +10 mV and in the absence or presence of 10 μM NBQX and 50 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) with an internal solution containing Cs methyl sulfate in place of K methyl sulfate. Series resistance (typically 9–18 MΩ) was monitored throughout each experiment; cells with >15% change in series resistance were excluded from analysis.

Identification of recorded neurons: Before beginning whole-cell experiments, we identified the CA2 PNs by their somatic location and size. Furthermore, the cell type was confirmed by several electrophysiological properties as previously described (Chevaleyre & Siegelbaum, 2010) (input resistance: 27.7 ± 1.8 MΩ, membrane capacitance: 290.4 ± 11.8 pF, resting membrane potential −78.2 ± 0.54 mV, sag amplitude: 2.3 ± 0.2 mV, action potential amplitude: 82.8 ± 1.2 mV, and duration: 1.00 ± 0.02 ms). For all experiments monitoring IPSCs, the properties of the cell were recorded immediately after break-in before the Cs⁺ ion sufficiently diffused into the cell. For several experiments, particularly when Cs⁺ was in the pipette solution, the slices were fixed after the recording with 4% paraformaldehyde, and the neurons were identified post-hoc by biocytin-streptavidin labeling. This allowed us to confirm that the recorded cells were CA2 pyramidal neurons by the location of their soma and shape of the dendritic arbor. Briefly, the apical dendrite bifurcates close to the soma into 2 or 3 main branches from which emerge few oblique branches in SR but numerous branches in SLM.

Synaptic potentials/currents were evoked by monopolar stimulation with a patch pipette filled with ACSF and positioned in the middle of CA1 SR or SLM. The duration of the stimulating pulse was 0.1 ms. 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 after SR stimulation by 100 ms. The stimulation of the SR and SLM inputs was considered to be 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. High-frequency stimulation (HFS; 100 pulses at 100 Hz repeated twice) was applied after a stable baseline of 10–20 min duration.
The amplitudes of both the PSPs and the IPSCs were normalized to the baseline amplitude. The magnitude of long-term plasticity was estimated by comparing averaged responses at 30–40 min after the induction protocol with baseline-averaged responses 0–10 min before the induction protocol. Statistical comparisons were performed using Student’s t test or two-way ANOVA with repeated measure (RM) and post-hoc Tukey test. Results are reported as mean ± SEM. All drugs were bath applied after dilution into the external solution from concentrated stock solutions. We used Axograph X software for data acquisition, and Origin Pro for data analysis.

Acknowledgements

This work was supported by the CNRS ATIP-Avenir (VC), Agence Nationale de la Recherche ANR-12-BSV4-0021-01 (VC), ANR-13-JSV4-0002-01 (RAP), the Ville de Paris programme Emergences (RAP).

References


Please cite this article in press as: Nasrallah, K., et al. Bi-directional interplay between proximal and distal inputs to CA2 pyramidal neurons. Neurobiology of Learning and Memory (2016), http://dx.doi.org/10.1016/j.nlm.2016.06.024.