Firing Induced Suppression of Synaptic Inhibition in the Stratum Lacunosum Moleculare of the Hippocampus

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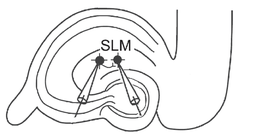
1. Introduction & Aim

Little is known on how neuron firing recorded *in vivo* retrogradely influences synaptic strength. We injected the firing of hippocampal neurogliaform cells (NGFCs), a widely expressed hippocampal GABAergic neuron type, detected *in vivo* during theta rhythm (Fuentealba et al., J Neurosci 30, 1595-1609, 2010.) into NGFCs recorded *in vitro*. We found that the *in vivo* NGFC firing elicited a depolarization-induced suppression of inhibition (DSI)-like phenomenon. We termed this event as firing induced suppression of inhibition (FSI). We studied FSI phenomenology, mechanisms and possible physiological role.

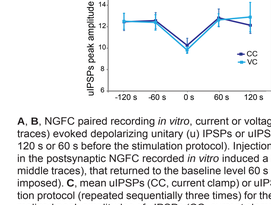
AIM: to investigate retrograde signalling induced by *in vivo* firing during theta oscillations

2. Methods



Slice preparation and electrophysiology

Juvenile rats (P14 – P22) or mice (NOs-Cre-tdTomato, P31 - P38) were anesthetised with isoflurane and decapitated. Horizontal sections (thickness: 350 μm) were prepared consisting of the dorsal hippocampus and attached entorhinal cortex using a vibratome. Whole-cell paired recording was performed using an EPC10 amplifier (HEKA) in current or voltage clamp from neurons with the soma in the stratum lacunosum moleculare (SLM) of the hippocampus. Recording electrodes (resistance ~5-6 MΩ) were filled with a solution containing (in mM): 42 KGluconate, 84 KCl, 4 ATP-Mg, 0.3 GTP-Na2, 127 Na_2-phosphocreatine, 10 HEPES, pH 7.3 (290 mOsm). Electrophysiological data were analyzed offline using custom made MATLAB software.

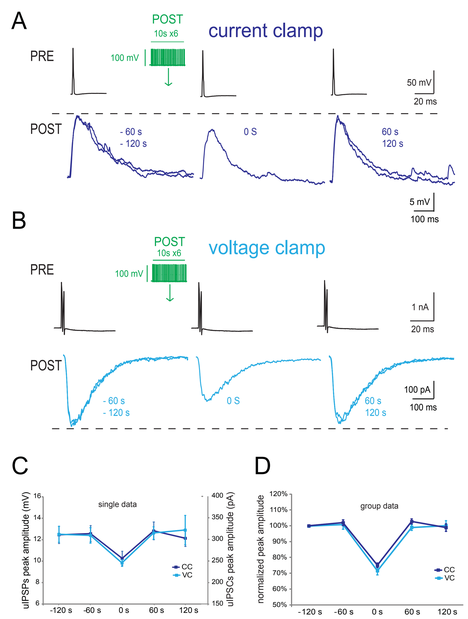


Voltage sensitive dye (VSD) and Calcium imaging

In voltage sensitive dye (VSD) and Ca^{2+} imaging experiments, a low chloride internal solution containing (in mM): 140 K-gluconate, 10 HEPES, 10 Na_2Phosphocreatine, 4 Mg-ATP, 0.3 Na-GTP, pH 7.3 with KOH was used. In voltage imaging experiments, the VSD JPW1114 (0.25-0.5mg/ml) was loaded into the cell by using the whole cell patch clamp recording pipette of the cell soma as shown on the left and described in detail previously (Canepari et al., Biophysical J., 98, 2032-2040, 2010). In calcium imaging experiments, the low-affinity indicators Oregon green 5N (OG5N) or Mag-Fura-2 (MF2) were added at 0.5 mM concentration to the internal solution. Ca^{2+} fluorescence was excited with an OptoFlash (Cairn Research Ltd., Faversham, UK) using either a 470 nm LED (for OG5N) or a 385 nm LED (for MF2), mounted on the epifluorescence port of the microscope. The excitation light, either from the laser or the LED, was directed to a water immersion objective (Olympus 60x/1.1 NA). Fluorescent images, de-magnified by 0.25x or 0.38x, were visualised with a high speed CCD camera NeuroCCD-SM (RedShirtImaging LLC, Decatur, GA, USA) at a frame rate ranging between 125 Hz and 5 kHz. The corresponding electrophysiological signals from the soma were recorded at a frequency ranging between 8 kHz and 20 kHz.

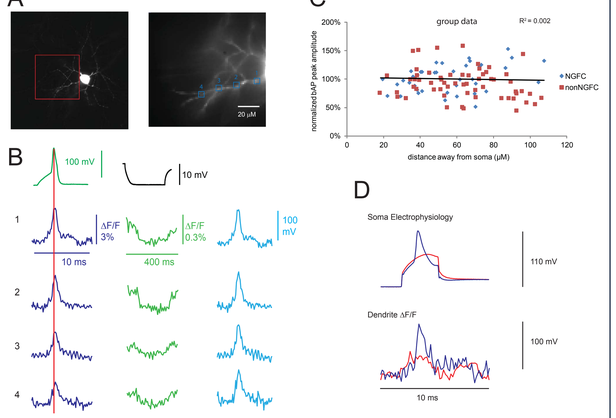
Both VSD and Ca^{2+} imaging data were analysed with dedicated software written in MATLAB. Optical signals were initially expressed as fractional changes of fluorescence (ΔF/F) averaged from 4x4 (16 pixels) regions of interest (ROIs) and obtained from averages of four trials unless otherwise mentioned.

3. *in vivo* NGFC firing pattern induces FSI



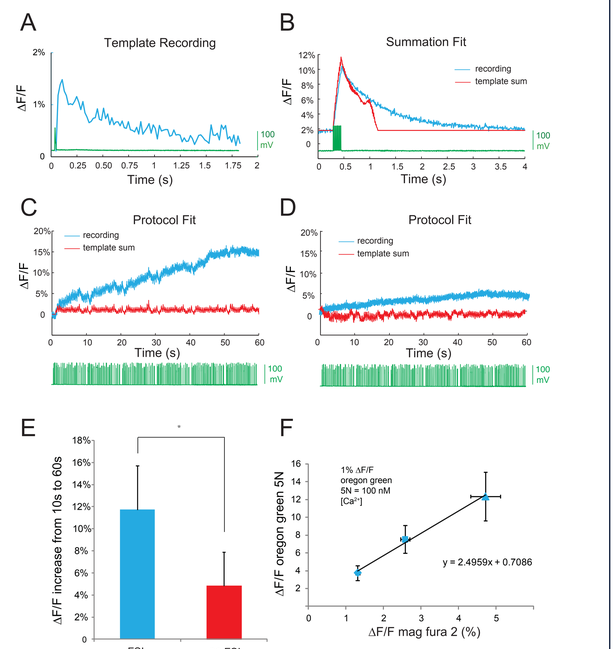
A, **B**, NGFC paired recording *in vitro*, current or voltage clamp mode, presynaptic action potentials or currents (PRE, black traces) evoked depolarizing unitary (u) IPSPs or uIPSCs in postsynaptic cells (POST, blue traces superimposed recorded 120 s or 60 s before the stimulation protocol). Injection of firing recorded *in vivo* from a NGFC for 60 s (stimulation protocol) in the postsynaptic NGFC recorded *in vitro* induced a transient depression of the amplitude of the uIPSP or uIPSC (POST, middle traces), that returned to the baseline level 60 s and 120 s after the end of the stimulation protocol (right traces superimposed). **C**, mean uIPSPs (CC, current clamp) or uIPSCs (VC, voltage clamp) peak amplitude before and after the stimulation protocol (repeated sequentially three times) for the cell pairs shown in A and B; error bars are SEM. **D**, summary of normalized peak amplitudes of uIPSPs (CC, current clamp) or uIPSCs (VC, voltage clamp) before and after the stimulation protocol in all pairs showing FSI (34 out of 96), error bars are SEM ($p < 0.001$, paired t-test, n = 23 for CC data, n = 11 for VC data).

4. What causes FSI in the postsynaptic neuron?



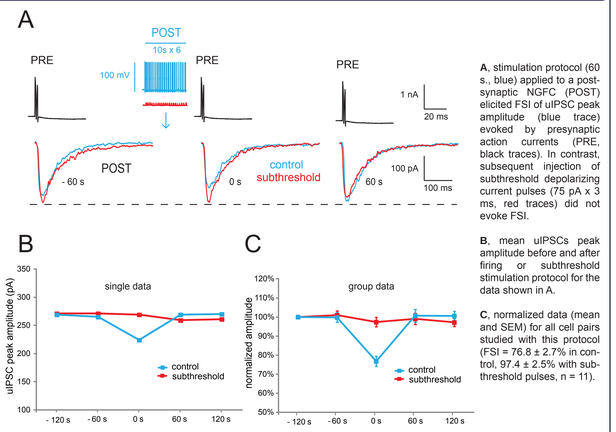
FSI was initiated by the postsynaptic neuron firing suggesting that the postsynaptic neuron delivers a chemical messenger from the dendritic site to the presynaptic terminals. According to this idea, AP back-propagation (bAP) should occur along the postsynaptic dendrites in order to trigger this event. To test this hypothesis, we visualized bAPs in interneurons of the SLM by using single cell voltage imaging as described in the Methods. **A**, Image of a NGFC (after biocytin-streptoavidin-Cy3 reaction) showing characteristic stellate dendrites (left picture, objective x20). This neuron was loaded with JPW-1114 and voltage imaging was performed from dendritic sites included in the red box; in particular, ΔF/F signals at the sites indicated (1-4) were analyzed. **B**, **left**, a depolarizing current pulse induced a somatic action potential (AP) recorded in current clamp (green trace) and the corresponding ΔF/F signals. **B**, **middle**, ΔF/F evoked by hyperpolarizing current pulse (average of 9 traces) injected into the soma and recorded from sites 1-4 of the dendrites; somatic current clamp recording is illustrated on the top. **B**, **right**, ΔF/F of the bAPs normalized to the correspondent ΔF/F induced by the hyperpolarizing current pulse. **C**, group data of normalized peak amplitudes of optically recorded bAPs along dendrites at different distances from the soma (n = 24). **D**, TTX (1 μM, red traces) blocked electrical (top traces) and optical APs (bottom traces).

5. Dendritic calcium triggers FSI



Next, we tested the hypothesis that the postsynaptic bAP elicited an increase in the dendritic Ca^{2+} concentration leading to the release of a retrograde messenger. **A**, ΔF/F Ca^{2+} signal from a NGFC filled with OG5N (blue trace) associated with an AP (green trace). **B**, Same as A (in the same neuron) but for a train of ten APs at 100 Hz; note the increased decay time compared to single AP Ca^{2+} signal; the red trace is the summation of 10 template ΔF/F Ca^{2+} signals associated with an AP (note the faster decay time). **C**, **D**, ΔF/F Ca^{2+} signals (blue traces) associated with the stimulation protocol (green traces) from the dendrites of two NGFC cells, one exhibiting FSI (cell in C) and the other not showing FSI (cell in D); the red traces are the summation of template ΔF/F Ca^{2+} signals associated with an AP along the firing protocol of stimulation; note the larger supralinear summation of ΔF/F Ca^{2+} signals in the cell exhibiting FSI. **E**, ΔF/F dendritic Ca^{2+} signal changes during stimulation protocol in postsynaptic neurons with FSI (blue column) and without FSI (red column, n = 5, $p < 0.05$). **F**, calibration of OG5N ΔF/F Ca^{2+} signal: plot of OG5N ΔF/F peak vs MF2 ΔF/F peak associated with different protocols (spade, 5 APs at 100 Hz; square, 10 APs at 100 Hz; triangle, 20 APs at 100 Hz, n = 6) in the same cells. The linear fit gives a conversion factor of 2.5; given that 1% for MF2 corresponds to ~250 nM, 1% for OG5N corresponds to ~100 nM.

6. Subthreshold stimulation does not elicit FSI

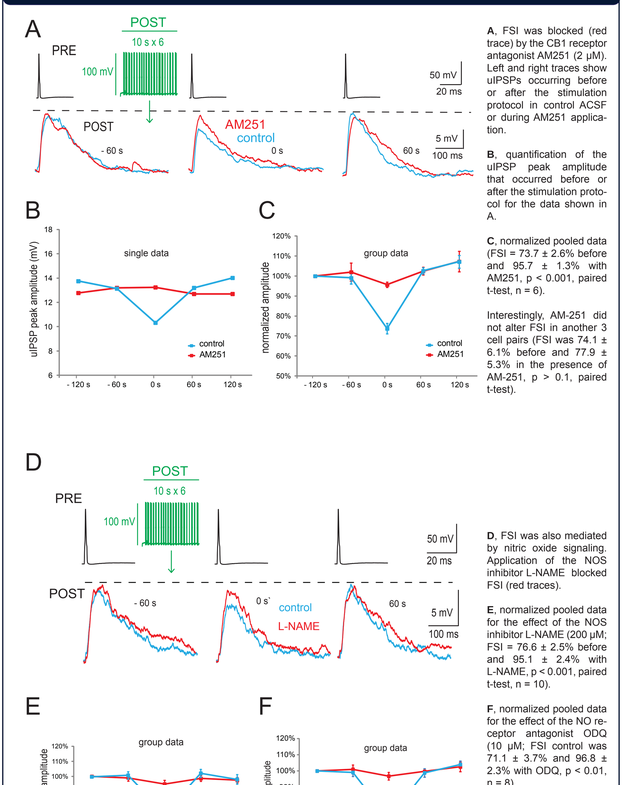


A, stimulation protocol (60 s., blue) applied to a postsynaptic NGFC (POST) elicited FSI of uIPSC peak amplitude (blue trace) evoked by presynaptic action currents (PRE, black traces). In contrast, subsequent injection of subthreshold depolarizing current pulses (75 pA x 3 ms, red traces) did not evoke FSI.

B, mean uIPSCs peak amplitude before and after firing or subthreshold stimulation protocol for the data shown in A.

C, normalized data (mean and SEM) for all cell pairs studied with this protocol (FSI = 76.8 ± 2.7% in control, 97.4 ± 2.5% with subthreshold pulses, n = 11).

7. What retrograde signals mediate FSI?



A, FSI was blocked (red trace) by the CB1 receptor antagonist AM251 (2 μM). Left and right traces show uIPSPs occurring before or after the stimulation protocol in control ACSF or during AM251 application.

B, quantification of the uIPSP peak amplitude that occurred before or after the stimulation protocol for the data shown in A.

C, normalized pooled data (FSI = 73.7 ± 2.6% before and 95.7 ± 1.3% with AM251, $p < 0.001$, paired t-test, n = 6).

Interestingly, AM-251 did not alter FSI in another 3 cell pairs (FSI was 74.1 ± 6.1% before and 77.9 ± 5.3% in the presence of AM-251, $p > 0.1$, paired t-test).

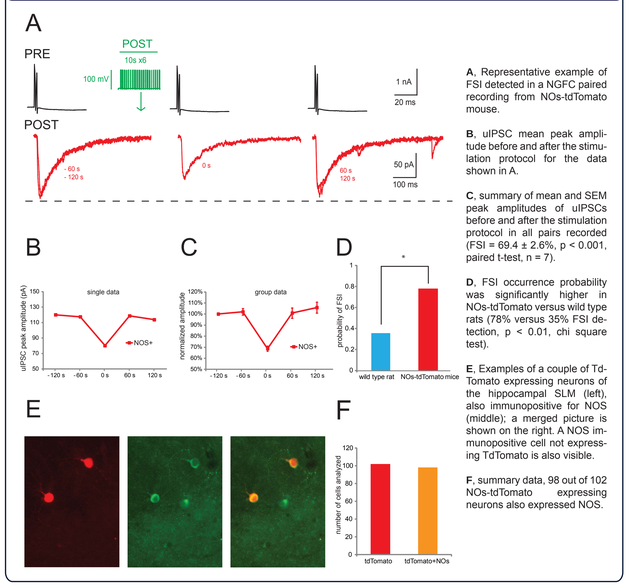
D, FSI was also mediated by nitric oxide signaling. Application of the NOS inhibitor L-NAME blocked FSI (red traces).

E, normalized pooled data for the effect of the NOS inhibitor L-NAME (200 μM; FSI = 76.6 ± 2.5% before and 95.1 ± 2.4% with L-NAME, $p < 0.001$, paired t-test, n = 10).

F, normalized pooled data for the effect of the NO receptor antagonist ODQ (10 μM; FSI control was 71.1 ± 3.7% and 96.8 ± 2.3% with ODQ, $p < 0.01$, n = 8).

Together, these data indicate that FSI is triggered by the retrograde release of endocannabinoid (eCB) and/or NO from postsynaptic dendrites to activate CB_1 and/or NOs-GC receptors on presynaptic axons.

8. FSI occurs more frequently in pairs of NOS-expressing neurons



A, Representative example of FSI detected in a NGFC paired recording from NOs-tdTomato mouse.

B, uIPSC mean peak amplitude before and after the stimulation protocol for the data shown in A.

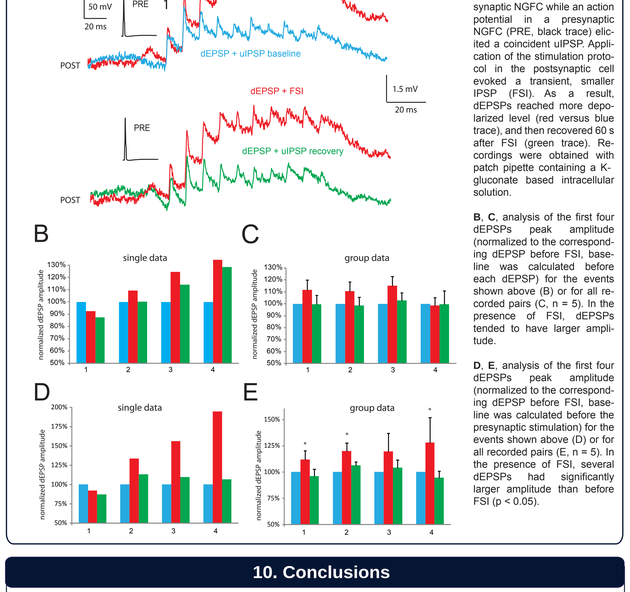
C, summary of mean and SEM peak amplitudes of uIPSCs before and after the stimulation protocol in all pairs recorded (FSI = 69.4 ± 2.6%, $p < 0.001$, paired t-test, n = 7).

D, FSI occurrence probability was significantly higher in NOs-tdTomato versus wild type rats (78% versus 35% FSI detection, $p < 0.01$, chi square test).

E, Examples of a couple of Td-Tomato expressing neurons of the hippocampal SLM (left), also immunopositive for NOS (middle); a merged picture is shown on the right. A NOS immunopositive cell not expressing TdTomato is also visible.

F, summary data, 98 out of 102 NOs-tdTomato expressing neurons also expressed NOS.

9. What is the physiological role of FSI?



A, train of EPSPs were injected as synaptic conductance by using dynamic clamp (POST, dEPSPs, blue trace) in a postsynaptic NGFC while an action potential in a presynaptic NGFC (PRE, black trace) elicited a coincident uIPSP. Application of the stimulation protocol in the postsynaptic cell evoked a transient, smaller IPSP (FSI). As a result, dEPSPs reached more depolarized level (red versus blue trace), and then recovered 60 s after FSI (green trace). Recordings were obtained with patch pipette containing a K-gluconate based intracellular solution.

B, **C**, analysis of the first four dEPSPs peak amplitude (normalized to the corresponding dEPSP before FSI, baseline was calculated before each dEPSP) for the events shown above (B) or for all recorded pairs (C, n = 5). In the presence of FSI, dEPSPs tended to have larger amplitude.

D, **E**, analysis of the first four dEPSPs peak amplitude (normalized to the corresponding dEPSP before FSI, baseline was calculated before the presynaptic stimulation) for the events shown above (D) or for all recorded pairs (E, n = 5). In the presence of FSI, several dEPSPs had significantly larger amplitude than before FSI ($p < 0.05$).

10. Conclusions

- **FSI is a novel phenomenon present between interneurons of the SLM hippocampus**
- **bAP in interneurons of the SLM elevate Ca^{2+} and trigger NO and/or eCB release to induce FSI**
- **Novel role for NO expressed by sub-population of NGFCs**

11. Acknowledgements

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Prof. Klausberger & Somogyi for the use of firing patterns recorded *in vivo* in hippocampal NGFCs and published in Fuentealba P, Klausberger T, Karayannis T, Suen WY, Huck J, Tomioka R, Rockland K, Capogna M, Studer M, Morales M, Somogyi P, J Neurosci 30, 1595-1609, 2010.