DENDRITIC SIGNAL INTEGRATION IN HIPPOCAMPAL INTERNEURONS

Doctoral thesis

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1. Introduction

The cellular structure of neuronal cells has long intrigued scientists dealing with the nervous system. As first, Ramon y Cajal noticed that axons and dendrites – despite being greatly intertwined – are separate cellular processes and do not fuse in anastomotic structures, and he established the law of *dynamic polarization*, describing that the direction of information flow is from the axons towards the dendrites through synapses. Our view on the communication between neurons has since expanded, as not just axo-dendritic, but axo-axonic and dendro-dendritic connections have also been discovered. Further on, the computational properties that affect action potential generation have also been deeply studied in the recent decades.

My aim is to point out what role the smallest computational subunits situated on the dendrites might play in the output of the neurons. I will give a brief description of the currently known passive and active dendritic processing capabilities. Finally, since my work involves many technical aspects, I will introduce the novel imaging methods currently available for fine-scale or large-scale neuroscience research.

1.1. Hippocampal interneurons

The hippocampal formation is a set of structures situated in the medial temporal lobe, and is one of the most widely studied brain structures, owing to its major role in memory consolidation and spatial mapping tasks. Though they only constitute ~10% of all neuronal cells, interneurons are considered to be the controlling units of this functionally important structure, by feed-back and feed-forward inhibition. The classification of these GABAergic cells has been done using their electrophysiological properties, immunohistochemical markers and based on the target of their innervation or projection. My work involved the regular-spiking interneurons of the hippocampal CA1 stratum radiatum.

1.2 Dendritic Characteristics

The dendrites are extensions of the cell body specialized for receiving and processing synaptic inputs. Their electrical properties resemble that of a simple electric cable – hence the name Cable Theory by Rall for the passive propagation of electric signals along the dendrites. According to the theory, multiple inputs add up arithmetically and travel passively along the dendrites and the net effect of local inhibition and excitation drives the neurons to a depolarized state, so whenever excitatory inputs overcome the inhibitory ones, the cell gets more depolarized. The passive cables show the leaking of synaptic currents and membrane capacitance acts as a low-pass filter, where the attenuating factor is even more aggravated by the distance-dependent tapering of the dendrites.

Though synaptic scaling can compensate for the signal loss, it has long been proposed that dendritic voltage-gated ion channels can aide the active amplification of the conducted signals. Here, a major role for voltage-gated calcium channels (VGCCs), sodium channels, N-methyl-D-aspartate receptors (NMDAR) and nicotionic acetylcholine receptors (nAChRs) has been shown. Their density affects the amplification of backpropagating action potentials (bAPs).

1.3 Dendritic Signal Integration

As proposed by Cable Theory, the linear summation of inputs would cut off distant synapses from affecting the output of the cell. Is there a way to overcome this? Compartmentalization is a key component in pyramidal cells, where dendritic spines show supralinear signal integration for synchronous excitatory inputs and backpropagating action potentials (bAPs). Though interneurons are mostly aspiny, evidence for the functional compartmentalization of responses in interneuron dendrites gave the possibility of similar integrational properties to appear locally as in pyramidal neurons.

1.4 Dendritic spikes

Theoretically, the arrival of multiple inputs to the same dendritic segment can increase the local membrane potential to above the

threshold levels for the locally present, voltage-dependent ion channels. These local regenerative events have been named dendritic spikes according to their main voltage-gated channel component: dendritic Sodium spikes, Calcium spikes or NMDA spikes.

1.4.1 Dendritic Sodium spikes

The voltage-gated Sodium channels (Na_V) have first been shown as fast prepotentials that preceded the somatic action potentials, with fast rise times, dubbed a 'spikelet', sitting on top of the fast AMPA-receptor mediated excitatory responses. The number of synchronously activated synaptic inputs that resulted in the appearance of a dendritic Sodium spike was found to be ~ 6, and the spikelet could be blocked by TTX.

The functional significance of dendritic Sodium spikes has been suggested in in long-term potentiation (LTP) of synaptic inputs, since branches of hippocampal CA1 pyramidal neurons that showed linear summation produced dendritic Sodium spikes after a evoking synaptic LTP. In addition, dendritic Sodium spikes help in lowering the threshold of action potentials.

1.4.2 Dendritic Calcium spikes

Dendritic Calcium spikes can be:

1) fast, burts-like events that follow short synaptic events that reach high enough amplitudes to exceed the threshold for voltage-gated Calcium channel (VGCC) activation, but decay swiftly;

2) Calcium plateau potentials, which can travel below the low-pass filtering threshold.

They have been shown to provide a boost to firing frequency, but in a distance-dependent manner from the soma and they can drive bursts of Sodium action potentials at the soma. Dendritic Calcium spikes can induce LTP on pyramidal cells, and their role in network functions has been demonstrated by the *in vivo* recording of dendritic Calcium spikes on pyramidal cells during sharp-wave oscillations in the hippocampus.

Dendritic NMDA spikes

NMDA receptors are ligand-gated ion channels with an influx of Sodium and Calcium ions and efflux of Potassium ions. They function as voltage-gated ion channels, since the relief of Mg-block is linked to the increase in membrane voltage. NMDA spikes are local events that arise by the synchronous activation of ~15 inputs and propagate passively when other Na_V or VGCCs are unavailable. Dendritic NMDA spikes can be complex spikes, since they are coupled to the activation of AMPA receptors along with Na_V and VGCCs, but can appear as pure NMDA-spikes, too. In addition, a threshold of ~8–10 nS NMDA conductance concentrated within a 25 μm dendritic region is required for their induction, but AMPA synaptic input is not necessary.

Dendritic NMDA spikes affect dendritic integration. The summation of inputs raises the Calcium influx by NMDA spikes, which in turn helps the local synaptic plasticity processes to take place, driving both long-term and short-term plasticity or modifying intracellular signalling cascades. The 20-50 μm spatial extent of the NMDA-spikes along a single dendritic branch that can reach even 100 μm suggests that multiple, dynamic functional subunits might exist within one dendritic branch, which seems to be the case with interneurons. Nevertheless, the activation of the same number of inputs in a clustered or a distributed fashion yielded similar results on hippocampal oblique dendrites, both evoking dendritic spikes, which supports the 'one branch – one computational subunit' hypothesis.

1.5 Two-Photon Microscopy for Neuroscience

Two-photon excitation occurs when two low-energy photons together excite a fluorescent molecule, which then decays back to its fundamental state by emission of a photon of somewhat lower energy than the sum of the two exciting ones. This has several advantages:

1. Deeper imaging with infrared light.
2. Inherent „confocality" with excitation spots of z = ~1.5 μm.
3. Less photobleaching and photodamage.
4. Higher signal-to-noise ratio.

Recent advances in two-photon microscopy extended observation limits from simple raster scanning and line scanning to freehand line scans, multiple line scanning and free region-of-interest scanning. Since living tissue contains elements or points that lie scattered in space, the improvement of three-dimensional (3D) methods was necessary. Two such 3D techniques emerged:

1. Moving objective with piezo scanning: the objective is moved quickly in the z-axis, which is followed by the galvanoscanners. The methods requires less technical modifications, but heavy software support, and provides limited z-range with limited scanning speed.

2. Static objective with acousto-optic (AO) scanning: allows random-access scanning of points. Multiple AO-deflectors can extend scanning to 3D. Points can be addressed with ~≤50kHz speed in greater depths. Needs extensive technical and software support.

2. Aims

I. Our previous results showed that interneurons have dendritic functional compartments that appear as a result of synaptic stimulation. Consequently, my first aims were to characterize their interactions:

1. Is there linear or supralinear summation for their joint activation locally on the dendrites and globally, on the soma?
2. Is there a time window for these interactions that is critical for coincidence detection?
3. Is there a distance dependence of any interactions?

II. Next, combined electrophysiology and 3D two-photon imaging and uncaging was used to characterize patterns of excitatory inputs on CA1 interneuron dendrites:

1. Do interneuron dendrites show similar supralinear responses to pyramidal cells for different patterns of dendritic inputs?
2. What is the input-putput function for the synchronous activation of different numbers of clustered inputs?
3. By using a custom-made recording chamber, can we attain spontaneous responses of similar nature to the evoked ones?

III. Since our experiments so far suggested that dendritic spikes are present on interneuron dendrites, I set out to identify its properties:

1. What ion channels play a role in the generation of dSpikes?
2. The appearance of the voltage waveform suggested an NMDA-spike. Can we confirm that using pharmacology?
3. Do VGCCs and Na_V play a role in its generation?
4. How do NMDA-spikes propagate in interneuron dendrites?
5. How do they relate to the bAPs?

IV. Finally, as a separate study, we wished to examine the role nAChRs play on bAPs and how do they affect LTP on interneurons.

3. Methods

3.1 Slice preparation and electrophysiology

Acute hippocampal brain slices were prepared from 16-20 day old Wistar rats stored at room temperature in artificial cerebrospinal fluid (ACSF) (in mM: 126 NaCl, 2.5 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$ and 10 glucose). Hippocampal CA1 interneurons were visualized using 900 nm infrared lateral illumination. Current-clamp recordings were made at 23 °C and 33 °C with a MultiClamp 700B amplifier and digitized by Digidata 1440. For whole cell current-clamp recordings a glass electrodes (6–9 MΩ) were used filled with (in mM): 125 K-gluconate, 20 KCl, 10 HEPES, 10 Di-Tris-salt phosphocreatine, 0.3 Na-GTP, 4 Mg-ATP, 10 NaCl, and 0.1 Oregon Green BAPTA-1 (OGB-1). In fast propagation speed measurements 0.2 Fluo-5F pentapotassium salt (Fluo-5F) and 0.2 Alexa 594 was used. Focal synaptic stimulation was with 6-9 MΩ glass electrodes filled with ACSF were placed at a distance of 10-15 µm from the dendrite (stimulation: 0.1 ms, 10-50 V, 10 ms pulse interval; 1-3 stimuli). Backpropagating APs were induced by somatic current injections (200–400 pA, 5 ms; 1-5 bAPs were evoked at 50 Hz). All evoked EPSPs were verified for synaptic delay.

3.2 Calcium imaging

Two-photon imaging started 15-20 min after attaining the whole-cell configuration on a two-photon laser-scanning system using femtosecond lasers (800-840 nm). The spatially normalized and projected Ca^{2+} response (defined as 3D Ca^{2+} response) was calculated from the raw 3D line-scan, F(d,t) by applying the $\Delta F/F=(F(d,t)-F_0(d))/F_0(d)$ formula where d and t denote distance along the curve and time respectively, and $F_0(d)$ denotes the average background-corrected prestimulus fluorescence as a function of distance along the curve. All 3D Ca^{2+} responses are color coded (colors from yellow to red show increasing Ca^{2+} responses, 0–63 % $\Delta F/F$), and projected as a function of d and t.

At the end of each experiment, a series of images across the depth of the volume encompassing the imaged neuron was taken. Measurement

control, real-time data acquisition and analysis were performed with a MATLAB based program and by a custom-written software. Statistical comparisons were performed by using Student's paired t test. If not otherwise indicated, data are presented as means ± SEM.

3.3 Calculating supralinearity

For the somatic recordings, we calculated the summation ratio in percentage (%SR) using the formula %SR=(2S-S1)/S2*100, where 2S is the two stimuli given within a certain interstimulus interval (ISI), while S1 and S2 are the responses after the first and second stimuli respectively. When imaging the Calcium signals, the sum of the two inputs was calculated at location L1 as the arithmetic sum of the values recorded after the first ($S1_{L1}$) and second stimulus ($S2_{L1}$) at location L1 ($SUM_{L1}=S1_{L1}+S2_{L1}$). This arithmetic sum would mean linear summation at L1. The integration values (Int) were calculated by comparing the arithmetic sum to the coupled activation of the two inputs (2S), as follows: $Int=2S_{L1}/SUM_{L1}$ or $\%Int=2S_{L1}/SUM_{L1}*100$. The calculations were done L1, L2 and the between regions, too.

3.3 Two-photon uncaging

Caged glutamate (4-Methoxy-7-Nitroindolinyl (MNI)-caged L-glutamate at 2.5 mM or (MNI)-caged L-glutamate trifluoroacetate at 2.5 mM) was added to the bath. Photolysis of caged glutamate was performed with 720 nm ultrafast, pulsed laser light. Photolysis of caged glutamate was performed either in "clustered" (0.8 ± 0.1 μm distance between inputs) or "distributed" (2.3±0.16 μm distance between inputs) patterns along the dendrite. Functional mapping of postsynaptic elements was carried out according to Matsuzaki et al., 2001. Color coded map was constructed of the peak of the EPSCs.

Pharmacological experiments were done with the selective NMDA-receptor antagonist D,L-AP5 (60 μM), the NaV blocker TTX (1 μM) and VGCC blockers (Mibefradil, 50 μM, Nimodipine, 20 μM, omega-Conotoxin GVIA 5 μM).

4. Results

4.1 Synaptic integration between dendritic computational subunits

According to the previous results in our laboratory, interneurons show dendritic functional subunits. My aim was to determine how these subunits interact. Two different techniques were utilized along with two-photon imaging and somatic patch clamp recordings to achieve this: focal synaptic stimulation and two-photon photoactivation of glutamate (uncaging). While focal synaptic stimulation induces synaptic release of vesicle and therefore is considered more physiological, there is a chance of unspecific stimulation. Glutamate uncaging does not involve presynaptic mechanisms, but it is definitely local, without exciting any aspecific axons. We evoked excitatory postsynaptic potentials (EPSPs) on interneuron dendrites at two separate regions. The responses were analysed at the somatic level using the electrophysiological recordings and locally, using the two-photon Calcium imaging data.

The results were similar using the two techniques, as follows:

- Calcium imaging on dendrites for both stimulation techniques confirmed that responses appear at **distinct compartments** for single evoked EPSPs at the dendritic level on interneurons, furher supporting the theory of functional compartmentalization without morphological compartments such as spines on pyramidal cells
- somatic recordings for both stimulation techniques showed that the simultaneous activation of two separate compartment yields **supralinear signals**, with an added value in the range of 10 to 41 % when compared to linear summation
- the dendritic **Calcium traces showed supralineary** for both techniques at the simultaneous ativation of two compartments (increase in the range of 20 to 58 %)

These data show that the distinct dendritic compartments can interact and neighboring compartments can amplify each other's responses, both on the local and the the somatic scale.

4.2 Coincidence detection in interneuron dendrites

In the above mentioned studies, we stimulated the two separate input locations with a distinct, but negligible latency: 0.5 ms interstimulus interval (ISI), both for focal electrical stimulation and for 2P glutamate photoactivation. These latency values are interpreted by the dendrite as synchronous excitation with regards to signal integration. Nevertheless, the question arises: what is the coincidence detection window for differently timed dual input stimulation? To answer this question, I increased the delay between stimuli in the range of 1 to 100 ms (interstimulus interval in ms: ISI = 1; 2-7;10; 20; 30; 40; 50; 100). The uEPSPs recorded at the soma showed supralinearity in their amplitude only for the synchronous inputs with 1ms ISI, but supralinearity was present in a wider temporal window of 1-10ms for the uEPSP areas. Furthermore, the simultaneously imaged active areas showed supralinear increase for both the amplitude and area for their Ca^{2+}-traces.

4.3 Distance dependence of supralinear signal integration

Previous studies have pointed out that the activated input sites play a different role in signal processing, integration and propagation in the function of distance from the soma. The focal activation of inputs allowed the analysis of not just the somatic distance dependence of signal integration, but also the effect of intercluster distance and the distance from bifurcations. However, our results neither show any significant difference for the somatic distance of input sites, nor for the interlocation distances.

4.4 Spatial characteristics of dendritic signal integration

Here, our aim was to see how the spatial distribution of inputs affects signal integration at one given location. For this purpose, interneuron synapses were functionally mapped by glutamate uncaging as it has been shown previously for pyramidal cells.

The **clustered input patterns** (0.8 ± 0.1 μm/input) always induced 3D Ca^{2+} responses that increased in a **nonlinear** fashion as a function of input numbers with a sharp jump-like increase occurring at one

particular input number (threshold = 9.8 ± 1.4 inputs; range 5-18; n=12 cells), after which they continued to increase at a slower rate with each extra input. The corresponding somatic voltage response mirrored the Ca^{2+}-responses.

The nonlinear response pattern could well be followed on multiple cells. We plotted the normalized Ca^{2+}-responses aligned to the threshold of the nonlinear, step like increase for the clustered inputs, and a sigmoid-shaped input-output curve began to emerge. The same pattern could be observed for the somatically recorded EPSPs when plotting the responses in the function of stimulated site number. The EPSP responses could be aligned to the threshold values determined from the Ca^{2+}-traces, and produced a similar sigmoid input-output curve. The voltage traces showed a "depolarizing shoulder" – an increase in the decay after the peak of the voltage trace –, which is vastly similar to dendritic regenerative events – dendritic spikes (**dSpikes**) – shown previously in pyramidal cell dendrites.

The experiments were repeated with **distributed input patterns**, which resulted in a slowly increasing, more **linear** input-output relationship for both Ca^{2+}-transients and somatic voltage traces.

4.5 Active dendrites in interneurons

The above results led us to explore whether the nonlinear signal integration in dendrites of CA1 str. rad – LM interneurons peak in dendritic spikes. First, we applied pathway stimulation to induce EPSPs in RAD-LM interneuron dendrites and located the input sites with Calcium imaging while recording EPSPs at the soma. To compare the stimulation-evoked and spontaneous EPSPs, we took advantage of a newly developed submerged recording chamber with double superfusion for increased oxygenation of the sample, using 450- to 600-μm–thick brain slices.

We identified two types of responses:

1. Small amplitude (22 ± 2% ΔF/F; n = 9 cells), spatially narrow responses (5.2 ± 0.9 μm), accompanied by unitary EPSPs.
2. Larger amplitude (51 ± 8% ΔF/F; P = 0.005; n = 9 cells), broader spatial distribution (13.6 ± 2.4 μm; P = 0.003), in agreement with

properties characteristic of local dSpikes. The decay time of postsynaptic potentials underlying dSpikes was more prolonged compared with EPSPs (EPSP, 22.8 ± 5.8 ms; dSpike, 79.7 ± 23.8 ms; P = 0.02) and area increased by 314 ± 57% (P = 0.007; n = 9).

For further analysis, we wished to evoke dSpikes. Low stimulation intensities induced small amplitude 3D Ca^{2+} responses accompanied by EPSPs detailed earlier. Increased stimulation intensities occasionally evoked dSpike-like responses. We classified both the evoked and the spontaneous traces using cluster analysis, which again yielded two groups for both Calcium and voltage recordings: EPSPs and dSpikes.

4.6 Channels taking part in synaptic processing

Based on the waveforms of both somatic voltage traces and dendritic Ca^{2+}-transients, we presumed that the responses with a "depolarizing shoulder" and increased decay times were dendritic spikes. To characterize these dSpikes in a more detailed manner, we turned to pharmacology.

4.6.1 NMDA receptors

Based on previous data, we hypothesized that both features are due to the activation of n-methyl- D-aspartate (NMDA) receptors. To verify this, we used the NMDAR antagonist AP5 (60 μM) while applying focal electrical stimulation, which eradicated the depolarizing shoulder on somatic voltage traces, and led to a complete loss of Ca^{2+}-signals at the dendrites. Wash-out traces to control values. Next, we again turned to two-photon photoactivation of Glutamate and applied uncaging laser pulses at clustered points along the interneuron dendrite as a control measurement, and then we added AP5 (60 μM) in the bath. The comparison to distributed input patterns showed that the effect of AP5 was more prominent than the spatial unclustering of inputs. The same results could be attained for the somatically recorded voltage traces during glutamate uncaging.

When probing for the input-output function by gradually increasing the number of activated inputs in a cluster, blockade of NMDA-receptors reduced the 3D Ca^{2+}-transients produced by clustered inputs

by a factor of ~9 and switched the summation from sigmoid to linear. Corresponding EPSPs were more moderately (factor of ~2-3) reduced in amplitude and their decay time constants were shorter. The input-output function of EPSPs has also switched from sigmoid to linear.

Next, we used Mg^{2+}-free ACSF while applying focal electrical stimulation. Here, the Mg^{2+}-block of the receptors is naturally released as the wash-in of Mg^{2+}-free ACSF occurs, and the complete effect of the channels can be monitored. The removal of Mg^{2+} provided somatic voltage responses with an extremely elongated depolarizing shoulder ($p < 0.05$, t-test), while the corresponding Ca^{2+}-responses recorded on the dendrite showed increased area when compared to control ($p < 0.05$, t-test). Our pharmacological data suggest that the observed dendritic regenerative events are NMDA-spikes.

4.6.2 Voltage-gated Calcium Channels (VGCCs)

We used a cocktail of different blockers to affect all VGCC types that could modify signal integration properties in the dendrites of hippocampal CA1 interneurons. The blocking of the part of the Ca_V2 subfamily (N-, P/Q-type) was accomplished by Mibefradil (50 μM), the Ca_V1 subfamily of (L-type) VGCCs was blocked by Nimodipine (20 μM), while $Ca_V2.2$ (N-type) VGCCs were blocked by ω-Conotoxin GVIA (5 μM). Our results show that – in contrast to NMDA receptor blockage –, the cocktail of VGCC blockers shifted the threshold and decreased peak amplitudes only slightly above the threshold for both the dendritic Ca^{2+}-responses and the corresponding somatic voltage traces.

4.6.3 Voltage-dependent Sodium Channels (NaV)

Here, we wished to determine whether the observed dSpikes were purely NMDA-spikes or mixed spikes, and whether the active propagation of the signals was due to the activation and boosting provided by dendritic Na_V. We chose to again use two-photon glutamate uncaging in conjunction with pharmacology, using the Sodium channel blocker 1 μM TTX. In contrast to the NMDAR

blockage, TTX induced only minor changes in the input-output relationship of the cells.

4.7 Dendritic spike propagation

Our previous data showed that interneurons display dendritic NMDA-spikes. Next, our further aim was to characterize the exact nature of their propagation. Here, we used focal electrical stimulation to evoke EPSPs and dSpikes. Then, we determined the half maximal values for both EPSP-Ca^{2+} and dSpike-Ca^{2+}-responses, showing a marked difference in propagation values of the EPSPs and the dSpikes we observed. To receive the propagation speed, we plotted the latency for the onset of events in the function of distance. EPSP-Ca^{2+} propagation was well characterized by a parabolic fit, suggesting a determining role of diffusion, while dSpike latency showed an exponential increase, resulting in an average propagation speed approximately 10 fold higher for dSpikes than for EPSPs. Although increasing the temperature markedly increased the propagation speed of EPSP-Ca^{2+}, the difference between dSpike and EPSP propagation was still significant ($p < 0.05$).

4.8 Dendritic NMDA-spikes and bAPs in interneurons

Next we wished to examine whether there is any relation between the local regenerative activity and the global neuronal action potential, and then to study another characteristic of the global action potential in CA1 interneuron dendrites.

Previous studies have shown that dendritic segments can show highly or less responsive areas called hot or cold spots, respectively. We chose to examine the relation of local dSpikes to the bAP-induced Ca^{2+}-influx by focal electrical stimulation to induce EPSPs, dSpikes or somatic action potentials (APs) while recording dendritic Ca^{2+}-responses. Here, dSpikes induced localized 3D Ca^{2+} responses, while when combined with somatic APs, dSpike-induced 3D Ca^{2+} invaded the entire imaged segment of the dendrites.

To see whether the increase follows a linear characteristic when dSpikes are accompanied by a somatic spike, the spatial analyses

revealed that dSpike-Ca^{2+} exceeds bAP-Ca^{2+} (bAP-Ca^{2+}: 252±30 nM, dSpike: 620±134 nM, N = 6 cells). Moreover, bAPs in themselves show lower Ca^{2+}-responses than those of dSpikes followed by APs. Our data show that dSpikes and bAPs can summate supralinearly.

4.9 The effect of nAChRs on bAPs and LTP in interneurons

Since α7-nAChRs are abundant on interneuron dendrites, we determined that they have a functional role. Namely, bAPs are either facilitated when evoked synchronously with nAChR activation, or are depressed when they follow nAChR activation. Furthermore, nAChR agonists can boost the induction of LTP of the Schaffer collateral inputs.

5. Summary and Conclusions

Dendritic spikes are intensively studied events, since they may enhance the computational complexity of neurons. Although thoroughly investigated in principal cells, the occurrence of these events in interneurons has not been shown before our work. The work presented here clarifies some points regarding the computational properties of interneuron dendrites.

Firstly, I could confirm that aspiny dendrites can show functional instead of morphological compartmentalization. Furthermore, these compartments seemed to be dynamic clusters of inputs, and not the whole dendritic branch served as a computational subunit.

Secondly, my results show that the compartments can interact with each other, boosting the neighboring compartments' responses in a nonlinear fashion. In addition, the interaction between compartments can happen within a 10 ms time window, leaving ample time for coincidence detection.

Next, we have investigated the nature of these compartments by using newly developed 3D imaging techniques. The novel tools proved to be indispensable for following the tortuous dendritic processes and for mapping long dendrites during the localization of spontaneous events. The localized compartments showed a similar input-output curve as to the one observed for pyramidal neurons, where clusters of synchronously active inputs produced the amplification of synaptic responses, leading to dendritic spikes. The different stimulation techniques assured that these results stand on firm experimental grounds, while the imaging combined with electrophysiology proved that supralinearity exists for both local Calcium and more global, somatically recorded signals.

To clarify the exact mechanisms responsible for the appearance and nature of dendritic spikes, we turned to pharmacology. Here, the blockage of VGCCs or Na_V-channels had no significant impact on the appearance of dendritic spikes. On the other hand, the NMDA-receptor blocker AP5 clearly switched the sigmoidal input-output curve for both local Calcium and somatically recorded EPSPs to linear. This,

supported by the low-Mg^{2+} experiments, provided clear evidence that the observed phenomena on interneuron dendrites are dendritic NMDA-spikes.

We have also shown that NMDA-spike trigger zones can serve as hot spots on interneuron dendrites, since these locations showed supralinear increase when synaptic stimulation was combined with backpropagating action potentials.

Our final study provided evidence that nonsynaptic α7-nAChRs can also modify bAPs and LTP induction on interneuron dendrites.

In conclusion, our results provide insight on the dendritic computation of aspiny hippocampal interneurons. The gathered evidence suggests that interneurons show similar supralinear signal integration to pyramidal cell dendrites, and that the complexity of dendritic computations is a more general phenomenon in the nervous system. These dendritic regenerative events increase the computational power of neurons. Moreover, since our data showed that there are computational subunits below the dendritic branch level, this suggests that interneurons might be able to solve more elaborate computative tasks than we ever thought before.

Finally, the fact that dendritic spikes appeared spontaneously points to the direction that these regenerative events might play a more important role in network computations than previously imagined. Whether these dendritic spikes contribute significantly to network functions such as hippocampal oscillation remains to be identified, but recent data suggest that similar events are constantly shaping synaptic strenght and re-tuning neurons in vivo, in the intact neuronal network of the brain.

6. Publications

6.1 Publications related to thesis

Katona, G.*, A. Kaszas*, G. F. Turi, N. Hajos, G. Tamas, E. S. Vizi and B. Rozsa (2011) Roller Coaster Scanning reveals spontaneous triggering of dendritic spikes in CA1 interneurons. Proc Natl Acad Sci U S A 108(5): 2148-2153.
**These authors contributed equally to this work.*

Chiovini, B., G. F. Turi, G. Katona, A. Kaszas, F. Erdelyi, G. Szabo, H. Monyer, A. Csakanyi, E. S. Vizi and B. Rozsa (2010) Enhanced dendritic action potential backpropagation in parvalbumin-positive basket cells during sharp wave activity. Neurochem Res 35(12): 2086-2095.

Rozsa, B., G. Katona, A. Kaszas, R. Szipocs and E. S. Vizi (2008) Dendritic nicotinic receptors modulate backpropagating action potentials and long-term plasticity of interneurons. Eur J Neurosci 27(2): 364-377.

6.2 Patents related to the thesis

Rózsa B, Katona G, Vizi ES, Kaszás A, Turi GF
Method and measuring system for scanning multiple regions of interest (multiple free line scan). E08462011

Rózsa B, Katona G, Vizi ES, Kaszás A, Turi GF
Method and measuring system for scanning multiple regions of interest (multiple free line scan). Hivatali szám: 12/998,668

6.3 Publications independent from thesis

G. Katona*, G. Szalay*, P. Maák*, A. Kaszás*, Máté Veress, D. Hillier, B. Chiovini, E. S. Vizi, B. Roska, B. Rózsa (2012) Fast two-photon in vivo imaging with three-dimensional random access scanning in large tissue volumes. Nat Methods 9(2): 201-208.
**These authors contributed equally to this work.*

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Barabas, K., E. M. Szego, A. Kaszas, G. M. Nagy, G. D. Juhasz and I. M. Abraham (2006) Sex differences in oestrogen-induced p44/42 MAPK phosphorylation in the mouse brain in vivo. J Neuroendocrinol 18(8): 621-628.