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Ca^{2+} sparks and Ca^{2+} glows in superior cervical ganglion neurons[1]

Li-jun YAO[2,3,4], Gang WANG[3,4], Kun-fu OU-YANG[3], Chao-liang WEI[3], Xian-hua WANG[3], Shi-rong WANG[3], Wei YAO[3], Hong-ping HUANG[3], Jian-hong LUO[2], Cai-hong WU[3], Jie LIU[3], Zhuan ZHOU[3,5], He-ping CHENG[3,5]

[2]*Department of Neurobiology, Zhejiang University School of Medicine, Hangzhou 310031, China*; [3]*Institute of Molecular Medicine and State Key Laboratory of Biomembrane Engineering, Peking University, Beijing 100871, China*

Key words

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[4] The authors contribute equally to this work.
[5] Correspondence to Drs He-ping CHENG or Zhuan ZHOU.
Phn/Fax 86-10-6276-5957.
E-mail chengp@pku.edu.cn
zzhou@pku.edu.cn

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Abstract

Aim: Ca^{2+} release from the endoplasmic reticulum (ER) is an integral component of neuronal Ca^{2+} signaling. The present study is to investigate properties of local Ca^{2+} release events in superior cervical ganglion (SCG) neurons. **Methods:** Primary cultured SCG neurons were prepared from neonatal rats (P3–P7). Low concentration of caffeine was used to induce Ca^{2+} release from the ER Ca^{2+} store, and intracellular Ca^{2+} was recorded by high-resolution line scan confocal imaging and the Ca^{2+} indicator Fluo-4. **Results:** Two populations of local Ca^{2+} release events with distinct temporal characteristics were evoked by 1.5 mmol/L caffeine near the surface membrane in the soma and the neurites of SCG neurons. Brief events similar to classic Ca^{2+} sparks lasted a few hundreds of milliseconds, whereas long-lasting events displayed duration up to tens of seconds. Typical somatic and neurite sparks were of 0.3- and 0.52-fold increase in local Fluo-4 fluorescence, respectively. Typical Ca^{2+} glows were brighter ($\Delta F/F_0$ approximately 0.6), but were highly confined in space. The half maximum of full duration of neurite sparks was much longer than those in the soma (685 *vs* 381 ms). **Conclusion:** Co-existence of Ca^{2+} sparks and Ca^{2+} glows in SCG neurons indicates distinctive local regulation of Ca^{2+} release kinetics. The local Ca^{2+} signals of variable, site-specific temporal length may bear important implications in encoding a "memory" of the trigger signal.

Introduction

Ca^{2+} is an ubiquitous intracellular messenger and regulates a great variety of physiological processes including excitability, secretion, development, learning and memory[1]. In neurons, cytoplasmic Ca^{2+} elevation results primarily from two kinds of Ca^{2+} sources. One is the extracellular Ca^{2+} entry via voltage- and receptor-operated Ca^{2+} channels, the other is the Ca^{2+} release from intracellular Ca^{2+} stores via ryanodine receptors (RyR) or inositol 1,4,5-trisphosphate receptors ($InsP_3R$) in the endoplasmic reticulum (ER).

Ca^{2+} sparks as elementary Ca^{2+} release signals were first described in quiescent rat heart cells[2]. Since then, Ca^{2+} sparks have been found in many types of excitable and nonexcitable cells, such as skeletal and smooth muscle cells[3–5], hippocampal neurons[6], dorsal root ganglion (DRG) neurons[7], and hypothalamic neurons[8]. The high Ca^{2+} microdomains associated with Ca^{2+} sparks may stimulate high-threshold Ca^{2+}-dependent signaling processes in the vicinity of the release channels. As well, spatially and temporally coordinated activation of Ca^{2+} sparks gives rise to propagating Ca^{2+} waves or near synchronous Ca^{2+} transients throughout the cell.

While Ca^{2+} sparks have been extensively investigated in all three types of muscles, only limited information from a few types of neurons[6–8] is available as to the spatial and temporal architecture of intracellular Ca^{2+} signaling. Since space-time organization of Ca^{2+} signals is critical to the efficacy, specificity and diversity of Ca^{2+} signaling, much remains to be learnt about organization of intracellular Ca^{2+} signals in different types of neurons. In this regard, superior cervical ganglion (SCG) neuron has been extensively used in the study of various aspects of synaptic transmission

including synaptic plasticity (a cellular mechanism of "memory") in which cytosolic Ca^{2+} plays an important role[9]. In the present study, we investigated local Ca^{2+} release events in rat SCG neurons and analyzed their spatial and temporal properties with the aid of high resolution confocal microscopy.

Materials and methods

Cell culture SCG was removed from neonatal rats (P3-P7) and dissociated using previously described methods[10]. Briefly, ganglions were dissected and incubated in Ca^{2+} free solution containing collagenase (1.5 mg/mL) and trypsin (0.5 mg/mL) at 37 ℃ for 45 min. Then the dispersed cells were plated on poly-*L*-lysine coated glass culture dishes and primarily cultured with DMEM (Gibco) containing 10% fetal bovine serum (FBS), nerve growth factor (20 ng/mL; 2.5 s) and maintained at 37 ℃ in a 5% CO_2 incubator. The neurons of 3–10 cultured days were prepared for Ca^{2+} imaging experiments.

Ca^{2+} free solution contained (mmol/L): 109 NaCl, 5.4 KCl, 23.8 $NaHCO_3$, 10 NaH_2PO_4, 7.28 Na-Hepes, 17.72 H-Hepes, 10 glucose (pH 7.4). DMEM for cell culture contained 13.4 mg/mL DMEM, 44 mol/L $NaHCO_3$, 100 IU/mL penicillin G, 100 μg/mL streptomycin, 0.6% vitamin C and 10% FBS. The standard bath solution for SCG contained (mmol/L): 141 NaCl, 2.8 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 H-Hepes (pH 7.4).

DMEM and FBS were purchased from Gibco. All other chemicals were from Sigma, unless otherwise specified. All experiments were conducted at room temperature (22–24 ℃).

Line scan imaging SCG neurons were loaded with Fluo-4 AM (5 μmol/L, 15 min) (Molecular Probes). Fluo-4 was excited at 488 nm and the emitted fluorescence was collected at wavelengths >505 nm, with a Zeiss 510 inverted confocal microscope (40 oil immersion lens of numerical aperture 1.3). The horizontal and axial resolutions were set at 0.4 and 1.5 mm, respectively. Rectilineal scan, curve scan and 2D (*xy*) imaging modes were used to measure Ca^{2+} dynamics, while the transmission channel image of the cell were recorded simultaneously. Image processing and data analysis were performed using IDL 6.0 software (Research Systems, Boulder, CO) and Igor software 4.03 (WaveMetrix).

Local perfusion system Solutions were puffed locally onto the cell via an RCP-2B multichannel microperfusion system (INBIO, Wuhan, China), which allowed fast (<100 ms) electronic change of local solutions between seven solution channels. The tip (100 μm diameter) of the puffer pipette was located about 120 μm from the cell. As determined by the conductance tests, the solution around a cell under study was fully controlled by the application solution, provided the application flow speed was 100 μL/min or greater. All pharmacological experiments met this criterion[11].

Results

Subsurface local Ca^{2+} release events in the soma of superior cervical ganglion neurons Primary cultured SCG neurons were examined with confocal microscopy in conjunction with the Ca^{2+} indicator, Fluo-4. To monitor subsurface Ca^{2+} release events that participate in bidirectional Ca^{2+} signaling between the plasma membrane and the ER, high-resolution curve scan images were obtained by setting the scan trajectory along the periphery of the soma. Application of low concentration of caffeine (1.5 mmol/L), which sensitizes RyR opening, evoked a flurry of local Ca^{2+} transients at punctuated sites in quiescent cells (Figure 1A). Local release events apparently consisted of heterogeneous populations, two examples of which are shown in Figure 1A. At site 1, a

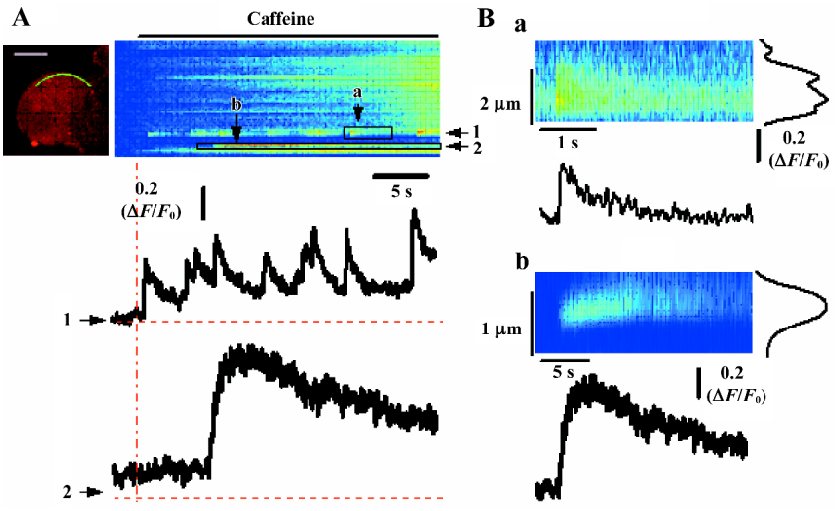

Figure 1. Subsurface local Ca^{2+} release events in the soma of SCG neurons. (A) Confocal image of SCG soma stained with Fluo-4 AM (inset). Curve line shows the scan trajectory, with the scan repetition rate of 326 Hz. Scale bar, 10 μm. Time courses at two representative local Ca^{2+} release sites are shown beneath the curve scan Ca^{2+} image, respectively. Repetitive Ca^{2+} sparks appeared at site 1, and a Ca^{2+} glow was observed at site 2. (B) Zoom in Ca^{2+} signals from (A). Box (a) marks a typical SCG Ca^{2+} spark at site 1. Its spatial profile (at peak Ca^{2+} level) and time course are shown to the right and the bottom of the image, respectively. Box (b) is for the Ca^{2+} glow at site 2.

train of repetitive release events similar to classic Ca^{2+} sparks were observed, and individual sparks were characterized by a rapid rise and a quasi-exponential decay (Figure 1B[a]). By contrast, release at site 2 displayed a prolonged rise time, a sustained plateau followed by a slow decay, with an overall release duration greater than 20 s (Figure 1B[b]). We named these long-lasting local release events (duration longer than 5 s) "Ca^{2+} glows". The majority subsurface release sites were of the spark rather than the glow type (66% spark sites from nine neurons).

On average, somatic Ca^{2+} sparks displayed 0.30±0.01 fold-increase of local Fluo-4 fluorescence ($\Delta F/F_0$, n=36 events from nine cells), whereas Ca^{2+} glows tended to have greater peak amplitude (0.61±0.13, n=5 events). The spatial width, indexed by the full width of half maximum (FWHM) at the peak Ca^{2+} level, was 1.79±0.19 and 0.66±0.21 mm for Ca^{2+} sparks and Ca^{2+} glows, respectively. The co-existence of Ca^{2+} sparks and Ca^{2+} glows in the soma of SCG neuron indicates distinctly different release kinetics at different release sites.

Neurite Ca^{2+} sparks and Ca^{2+} glows in SCG neurons Next, we used rectilineal scan imaging method to visualize local Ca^{2+} events in neurites, with the scan line placed in parallel with the neurite of interest (Figure 2A). Both Ca^{2+} sparks and Ca^{2+} glows were evoked by 1.5 mmol/L caffeine (Figure 2B, 2C), as was the case in the soma. However, the percent of Ca^{2+} glow sites was significantly greater in neurites (56/83 or 67%) than in the soma (χ^2 test, P<0.05). Furthermore, 42 out of 56 release sites displayed repetitive Ca^{2+} sparks on top of the Ca^{2+} glows (Figure 2B, site 1).

Thapsigargin blocked caffeine-evoked Ca^{2+} sparks To validate that caffeine-evoked Ca^{2+} release events arise from local intracellular Ca^{2+} release from the ER, 10 μmol/L thapsigargin (TG), an inhibitor of the ER Ca^{2+}-ATPase was applied 30 min prior to caffeine application. Under these experimental conditions, neither Ca^{2+} sparks nor Ca^{2+} glows were observed upon caffeine application (Figure 3). This result confirms the notion that both Ca^{2+} sparks and Ca^{2+} glows reflect local Ca^{2+} release from the ER.

Comparison between somatic and neurite Ca^{2+} sparks We analyzed the amplitude, FWHM, and the half maximum of full duration (FDHM) from 55 neurite Ca^{2+} sparks and 36 somatic Ca^{2+} sparks. Histogram distributions of these spark parameters are shown in Figure 4. The SCG spark amplitude in the neurites was greater than in the soma ($\Delta F/F_0$ 0.52 *vs* 0.30, P<0.01), and was much smaller than that in cardiac or skeletal muscles where Ca^{2+} sparks were first described. The lateral extensions of neurite and somatic Ca^{2+} sparks were similar (~1.8 mm). However, the FDHM of Ca^{2+} sparks was significantly smaller in the soma than in the neurites (381 *vs* 685 ms, P<0.05).

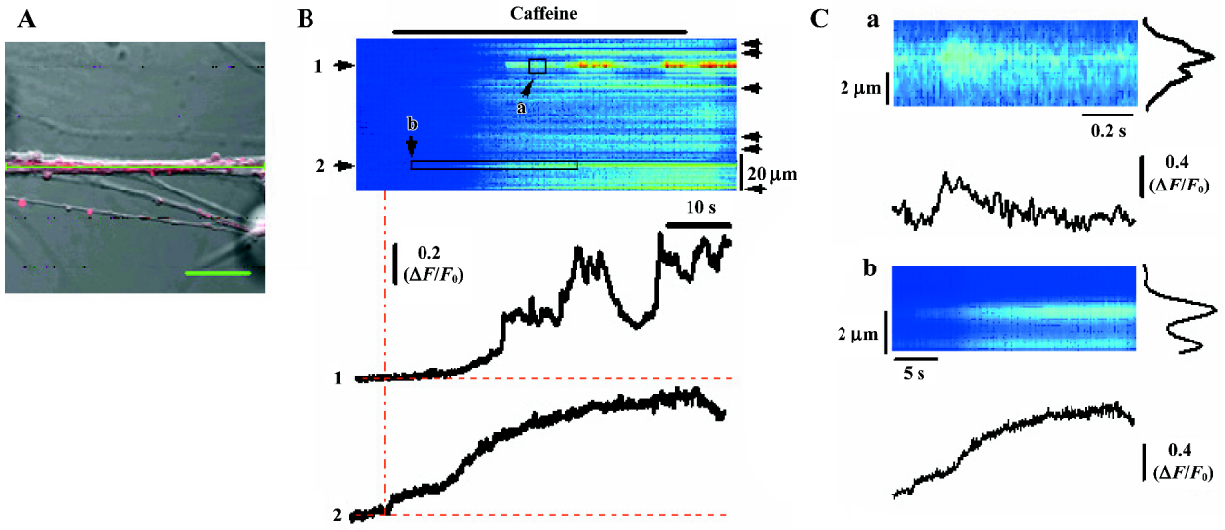

Figure 2. Neurite Ca^{2+} sparks and Ca^{2+} glows in SCG neurons. (A) Rectilineal scan of neurite. The green line denotes the scan position. Scale bar: 20 μm. (B) Representative line scan image. Solid arrows on the right margin of the image mark Ca^{2+} release sites. Sites 1: Ca^{2+} sparks appeared on top of a Ca^{2+} glow. Site 2: a Ca^{2+} glow. Note the difference of the latency of the Ca^{2+} response at different release sites. (C) Zoom-in view of events (a) and (b) boxed in B. The spatial profile and the time course of the local event are shown to the right and the bottom of the corresponding image, respectively.

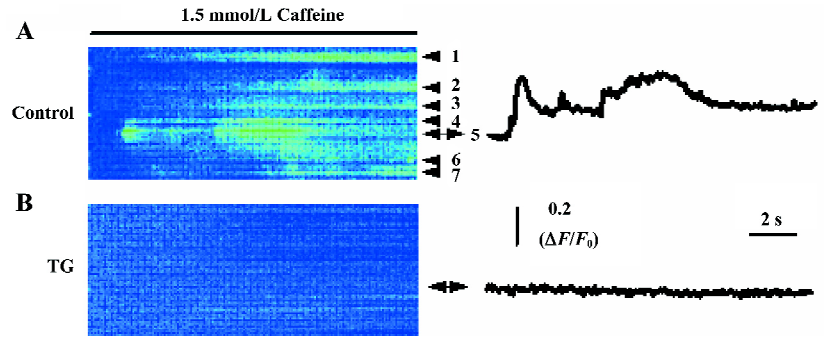

Figure 3. Thapsigargin blocked caffeine-evoked Ca^{2+} signals. (A) Control, 1.5 mmol/L caffeine evoked Ca^{2+} sparks and glows in the neurite of a SCG neuron. Arrows 1–7 mark multiple Ca^{2+} release sites. Right panel shows time course of local Ca^{2+} at site 5. (B) Treatment of the same neurite with thapsigargin (TG, 10 μmol/L, 30 min) completely abolished caffeine-evoked Ca^{2+} signals.

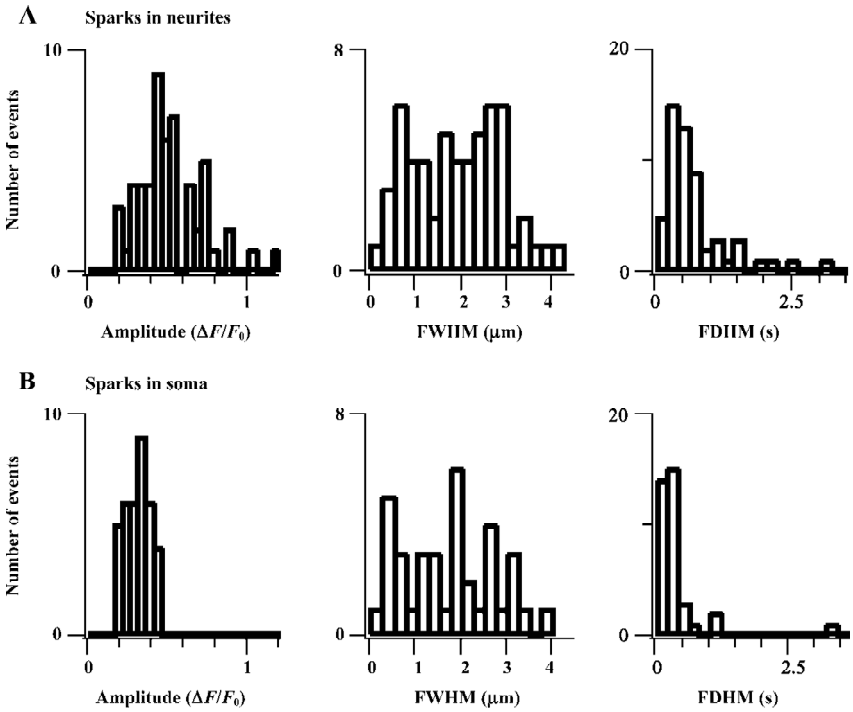

Figure 4. Unitary properties of SCG Ca^{2+} sparks detected in the neurites and the soma. (A) Histograms of spark amplitude (left), FWHM (full width at half maximum, middle) and FDHM (full duration at half maximum, right) in neurites of SCG neurons (n=55 sparks). (B) As in (A) except for somatic sparks (n=36).

Discussion

The ER Ca^{2+} signaling plays pluripotent roles in vital neuronal physiological and pathophysiological processes, such as neuronal excitability, neurotransmitter release, somatic secretion, synaptic plasticity, gene expression, neuronal growth and survival as well as circadian rhythms[12]. The specificity and versatility of Ca^{2+} signaling are in part determined by the spatiotemporal mode of local Ca^{2+} release. In the present study, we found that, in addition to Ca^{2+} sparks, there is a new class of local Ca^{2+} release events, namely Ca^{2+} glows, in SCG neurons. Once activated, local Ca^{2+} release in a glow lasts for many seconds and up to tens of seconds, as if there is no mechanism of release termination. Ca^{2+} sparks and Ca^{2+} glows can be observed both in the subsurface layer of the soma and the neurites of different thickness. However, the glow or spark type of response appeared to be a site-specific property, for preliminary data show that the response of a given site is rather stereotypic during repeated caffeine applications (interval ~20–30 s).

The similarities and differences among SCG, DRG, hippocampal and hypothalamic neurons reinforce the notion that the space-time architecture of intracellular Ca^{2+} signaling is highly neuronal cell type-specific. The presence of Ca^{2+} glows in neurons bears important ramifications. First, it indicates that inactivation of the release mechanism is non-existent or very weak. This is reminiscent of the situation in DRG neurons where type 3 RyR display little Ca^{2+}-dependent inactivation and rapidly repetitive Ca^{2+} sparks can be readily activated at given sites[7,13]. Second, there must be a rapid Ca^{2+} refilling and recycling mechanism to prevent ex-

haustion of local ER Ca^{2+} store, sustaining release in a Ca^{2+} glow. Functionally, the long-lasting Ca^{2+} release will retain a "memory" of the trigger signal well beyond the trigger duration. Indeed, we noticed that Ca^{2+} glows persisted for at least 10 s after washout of caffeine (data not shown). Hence, our finding may shed some new light on the encoding of cellular "memory" via Ca^{2+}-dependent mechanisms.

Conclusions

At present, the exact mechanism that confers a site the Ca^{2+} glow property remains elusive. Whether a release site can switch dynamically from a spark site to a glow site or vice versa is also an intriguing possibility that needs to be addressed over an extended timescale. The exact physiological role of the subsurface local Ca^{2+} glows, as well as Ca^{2+} sparks in the soma and in the neurites of SCG neurons, also warrants future investigation. Among others, subsurface Ca^{2+} is able to modulate the processes of membrane excitability[14,15], exocytosis[7] and synaptic transmission. In addition, sustained elevation of local Ca^{2+} will lead to the activation of Ca^{2+}-dependnet kinases, which are implicated in the long-term neuronal plasticity[16].

Acknowledgments

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