

Full-length article

Ca²⁺ sparks and Ca²⁺ glows in superior cervical ganglion neurons¹

Li-jun YAO^{2,3,4}, Gang WANG^{3,4}, Kun-fu OU-YANG³, Chao-liang WEI³, Xian-hua WANG³, Shi-rong WANG³, Wei YAO³, Hong-ping HUANG³, Jian-hong LUO², Cai-hong WU³, Jie LIU³, Zhuan ZHOU^{3,5}, He-ping CHENG^{3,5}

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

Key words

superior cervical ganglion neurons; Ca²⁺ sparks; ryanodine receptors; caffeine

¹ This work was supported by grants from the National Basic Research Program of China (ZZ and HC) and the National Natural Science Foundation of China (ZZ, HC, JL, XHW).

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

Received 2006-05-18

Accepted 2006-05-25

doi: 10.1111/j.1745-7254.2006.00402.x

Abstract

Aim: Ca²⁺ release from the endoplasmic reticulum (ER) is an integral component of neuronal Ca²⁺ signaling. The present study is to investigate properties of local Ca²⁺ 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 Ca²⁺ release from the ER Ca²⁺ store, and intracellular Ca²⁺ was recorded by high-resolution line scan confocal imaging and the Ca²⁺ indicator Fluo-4. **Results:** Two populations of local Ca²⁺ 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 Ca²⁺ 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 Ca²⁺ 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 Ca²⁺ sparks and Ca²⁺ glows in SCG neurons indicates distinctive local regulation of Ca²⁺ release kinetics. The local Ca²⁺ signals of variable, site-specific temporal length may bear important implications in encoding a “memory” of the trigger signal.

Introduction

Ca²⁺ is an ubiquitous intracellular messenger and regulates a great variety of physiological processes including excitability, secretion, development, learning and memory^[1]. In neurons, cytoplasmic Ca²⁺ elevation results primarily from two kinds of Ca²⁺ sources. One is the extracellular Ca²⁺ entry via voltage- and receptor-operated Ca²⁺ channels, the other is the Ca²⁺ release from intracellular Ca²⁺ stores via ryanodine receptors (RyR) or inositol 1,4,5-trisphosphate receptors (InsP₃R) in the endoplasmic reticulum (ER).

Ca²⁺ sparks as elementary Ca²⁺ release signals were first described in quiescent rat heart cells^[2]. Since then, Ca²⁺ 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 Ca²⁺ microdomains associated with Ca²⁺ sparks may stimulate high-threshold Ca²⁺-dependent signaling processes in the vicinity of the release channels. As well, spatially and temporally coordinated activation of Ca²⁺ sparks gives rise to propagating Ca²⁺ waves or near synchronous Ca²⁺ transients throughout the cell.

While Ca²⁺ 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 Ca²⁺ signaling. Since space-time organization of Ca²⁺ signals is critical to the efficacy, specificity and diversity of Ca²⁺ signaling, much remains to be learnt about organization of intracellular Ca²⁺ 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 °C 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 °C 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 μ g/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 °C).

Line scan imaging SCG neurons were loaded with Fluo-4 AM (5 μ mol/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 μ m, respectively. Rectilinear 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 μ m diameter) of the puffer pipette was located about 120 μ m 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 μ L/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

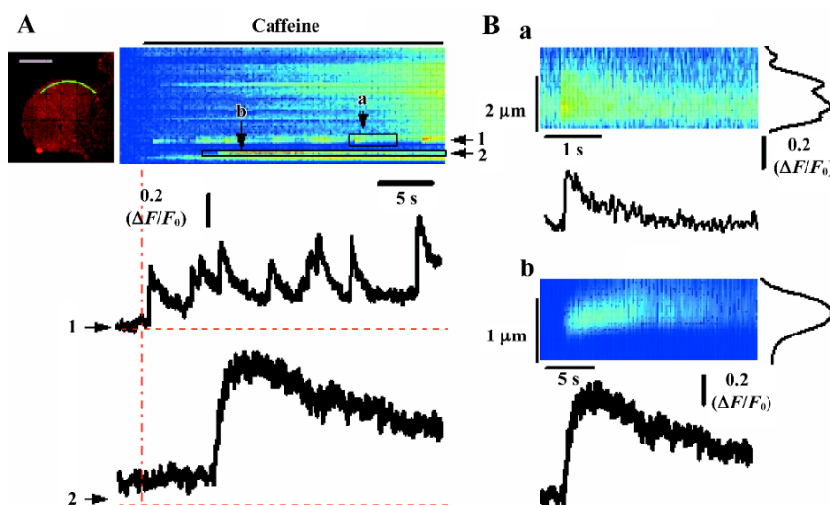


Figure 1. Subsurface local Ca^{2+} release events in the soma of SCG neurons. (A) Confocal image of SCG soma stained with Fluo-4 AM (inset). Curve line shows the scan trajectory, with the scan repetition rate of 326 Hz. Scale bar, 10 μ m. Time courses at two representative local Ca^{2+} release sites 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 μm 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 rectilinear 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 $\mu\text{mol/L}$ 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 μm). However, the FDHM of Ca^{2+} sparks was significantly smaller in the soma than in the neurites (381 vs 685 ms, $P < 0.05$).

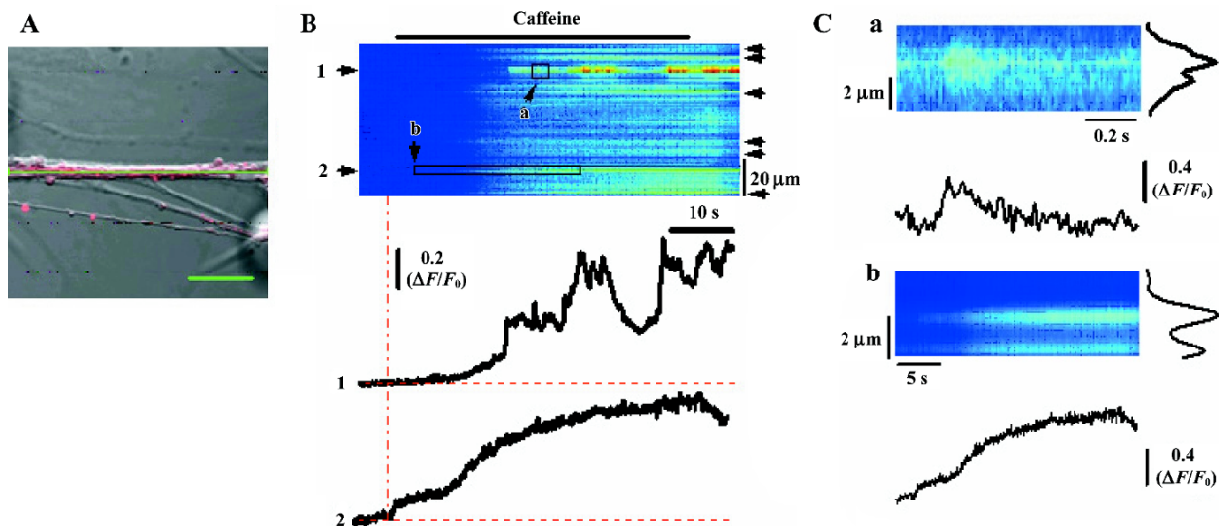


Figure 2. Neurite Ca^{2+} sparks and Ca^{2+} glows in SCG neurons. (A) Rectilinear scan of neurite. The green line denotes the scan position. Scale bar: 20 μm . (B) Representative line scan image. Solid arrows on the right margin of the image mark Ca^{2+} release sites. Site 1: Ca^{2+} sparks appeared 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.

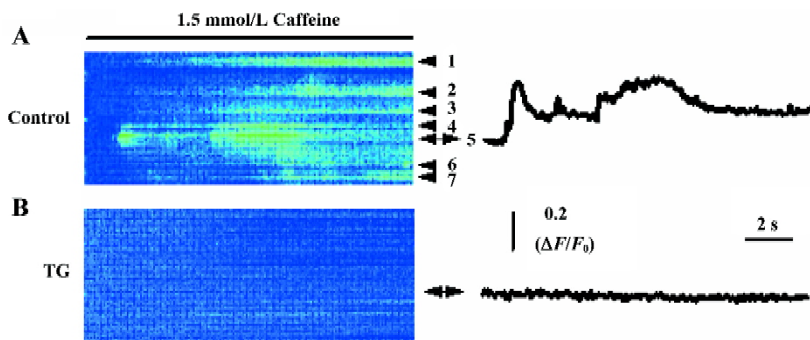


Figure 3. Thapsigargin blocked caffeine-evoked Ca^{2+} signals. (A) Control, 1.5 mmol/L caffeine evoked Ca^{2+} sparks and glows in the neurite of a SCG neuron. Arrows 1–7 mark multiple Ca^{2+} release sites. Right panel shows time course of local Ca^{2+} at site 5. (B) Treatment of the same neurite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

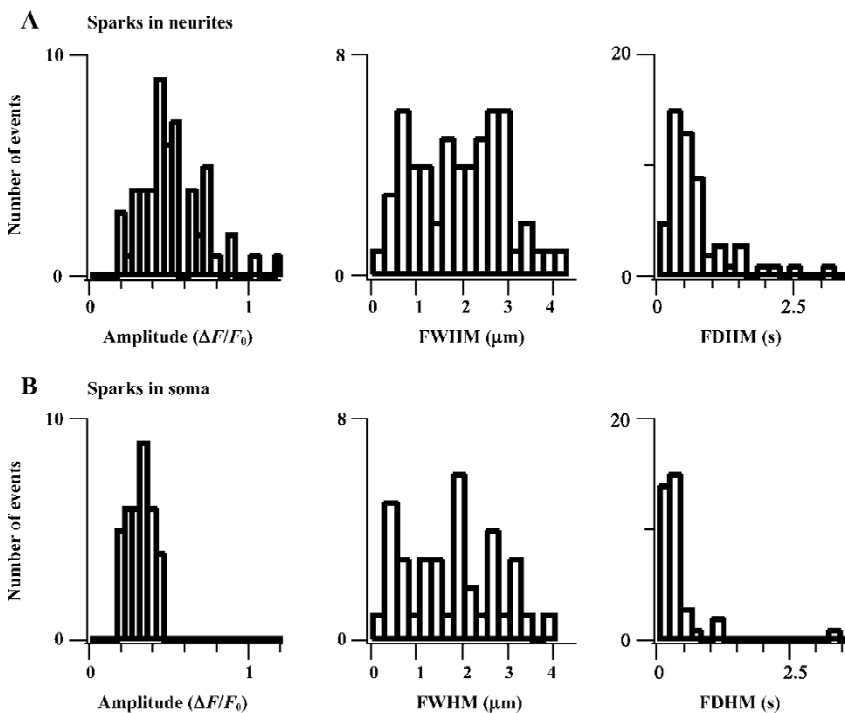


Figure 4. Unitary properties of SCG Ca^{2+} sparks detected in the neurites and the soma. (A) Histograms of spark amplitude (left), FWHM (full width at half maximum, middle) and FDHM (full duration at half maximum, right) in neurites of SCG neurons ($n=55$ sparks). (B) As in (A) except for somatic sparks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

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 $\sim 20\text{--}30$ 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 Ca²⁺ store, sustaining release in a Ca²⁺ glow. Functionally, the long-lasting Ca²⁺ release will retain a “memory” of the trigger signal well beyond the trigger duration. Indeed, we noticed that Ca²⁺ 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 Ca²⁺-dependent mechanisms.

Conclusions

At present, the exact mechanism that confers a site the Ca²⁺ 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 Ca²⁺ glows, as well as Ca²⁺ sparks in the soma and in the neurites of SCG neurons, also warrants future investigation. Among others, subsurface Ca²⁺ is able to modulate the processes of membrane excitability^[14,15], exocytosis^[7] and synaptic transmission. In addition, sustained elevation of local Ca²⁺ will lead to the activation of Ca²⁺-dependent kinases, which are implicated in the long-term neuronal plasticity^[16].

Acknowledgments

We thank Lin-ling HE and Lie-cheng WANG for their assistance in cell preparation.

References

- Berridge MJ. Neuronal calcium signaling. *Neuron* 1998; 21: 13–26.
- Cheng H, Lederer WJ, Cannell MB. Calcium sparks: elementary events underlying excitation-contraction coupling in heart muscle. *Science* 1993; 262: 740–4.
- Klein MG, Cheng H, Santana LF, Jiang YH, Lederer WJ, Schneider MF. Two mechanisms of quantized calcium release in skeletal muscle. *Nature* 1996; 379: 455–8.
- Nelson MT, Cheng H, Rubart M, Santana LF, Bonev AD, Knot HJ, *et al*. Relaxation of arterial smooth muscle by calcium sparks. *Science* 1995; 270: 633–7.
- Tsugorka A, Rios E, Blatter LA. Imaging elementary events of calcium release in skeletal muscle cells. *Science* 1995; 269: 1723–6.
- Koizumi S, Bootman MD, Bobanovic LK, Schell MJ, Berridge MJ, Lipp P. Characterization of elementary Ca²⁺ release signals in NGF-differentiated PC12 cells and hippocampal neurons. *Neuron* 1999; 22: 125–37.
- Ouyang K, Zheng H, Qin X, Zhang C, Yang D, Wang X, *et al*. Ca²⁺ sparks and secretion in dorsal root ganglion neurons. *Proc Natl Acad Sci USA* 2005; 102: 12259–64.
- De Crescenzo V, ZhuGe R, Velazquez-Marrero C, Lifshitz LM, Custer E, Carmichael J, *et al*. Ca²⁺ syntillas, miniature Ca²⁺ release events in terminals of hypothalamic neurons, are increased in frequency by depolarization in the absence of Ca²⁺ influx. *J Neurosci* 2004; 24: 1226–35.
- Alkadhi KA, Alzoubi KH, Aleisa AM. Plasticity of synaptic transmission in autonomic ganglia. *Prog Neurobiol* 2005; 75: 83–108.
- Zhou Z, Misler S. Amperometric detection of stimulus-induced quantal release of catecholamines from cultured superior cervical ganglion neurons. *Proc Natl Acad Sci USA* 1995; 92: 6938–42.
- Wu B, Wang YM, Xiong W, Zheng LH, Fu CL, Bruce IC, *et al*. Optimization of a multi-channel puffer system for rapid delivery of solutions during patch-clamp experiments. *Front Biosci* 2005; 10: 761–7.
- Verkhratsky A. Physiology and pathophysiology of the calcium store in the endoplasmic reticulum of neurons. *Physiol Rev* 2005; 85: 201–79.
- Ouyang K, Wu C, Cheng H. Ca²⁺-induced Ca²⁺ release in sensory neurons: low gain amplification confers intrinsic stability. *J Biol Chem* 2005; 280: 15898–902.
- Burdyga T, Wray S. Action potential refractory period in ureter smooth muscle is set by Ca sparks and BK channels. *Nature* 2005; 436: 559–62.
- Vinogradova TM, Lyashkov AE, Zhu W, Ruknudin AM, Sirenko S, Yang D, *et al*. High basal protein kinase A-dependent phosphorylation drives rhythmic internal Ca²⁺ store oscillations and spontaneous beating of cardiac pacemaker cells. *Circ Res* 2006; 98: 505–14.
- Deisseroth K, Heist EK, Tsien RW. Translocation of calmodulin to the nucleus supports CREB phosphorylation in hippocampal neurons. *Nature* 1998; 392: 198–202.