Biochemical and Biophysical Research Communications 431 (2013) 664–669

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Cell–cell communication induces random spikes of spontaneous calcium oscillations in multi-BV-2 microglial cells

Xian Wu, Leiting Pan *, Ying Liu, Pengchong Jiang, Imshik Lee, Irena Drevensek-Olenik, Xinzheng Zhang, Jingjun Xu

The Key Laboratory of Weak-Light Nonlinear Photonics, Ministry of Education, School of Physics and TEDA Applied Physics School, Nankai University, Tianjin 300457, PR China

ARTICLE INFO

Article history:
Received 10 January 2013
Available online 25 January 2013

Keywords:
Spontaneous calcium oscillations
Cell–cell communication
Random spikes
BV-2 microglial cells

ABSTRACT

As the first and main form of active immune defense in the central nervous system, microglial cells usually exhibit complicated intracellular calcium (Ca^{2+}) activity that can regulate the downstream components of signaling cascades. In the present work, spontaneous oscillations of the cytosolic calcium concentration ($[Ca^{2+}]_c$) in multi-BV-2 microglial cells were observed by video microscopy. These cells exhibited random spikes of Ca^{2+} oscillations. Cross-correlation analysis of the temporal dependence of the oscillations indicated the existence of cell–cell communication mediated by extracellular messengers. Numerical simulations based on a simple mathematical model suggested that these communications could induce random spikes of spontaneous Ca^{2+} oscillations in the multi-cell system. Short-time imaging analysis of random spikes in different regions of a single cell showed that spontaneous Ca^{2+} oscillations resulted from Ca^{2+} wave generated by other cells as well as from calcium elevation inside the cell. Taken together, our data demonstrate that cell–cell communication existed between the BV-2 microglial cells *in vitro* and further resulted in the random spikes of spontaneous Ca^{2+} oscillations.

1. Introduction

Microglial cells residing in the brain and the spinal cord act as the first line of immune defense in the central nervous system (CNS) by clearing damaged neurons, plaques, and infectious agents [1]. It has been demonstrated that microglial cells could rapidly congregate together and establish a potential barrier between the healthy and damaged tissue after the brain injury [2]. However, little is known about how microglial cells communicate with each other and exchange signals following the injury [3].

Calcium (Ca^{2+}) is a vital secondary messenger used to regulate a wide range of cellular processes including cell movement, exocytosis, contraction, metabolism, cell differentiation, transcription, and proliferation [4–7]. For instance, intracellular Ca^{2+} signals were found to promote the excitability of neuroglial cells. Propagating Ca^{2+} waves were found to serve as a form of long-range intercellular communication between astrocytes [8,9]. Intracellular Ca^{2+} oscillations, i.e., oscillatory changes of the cytosolic calcium concentration ($[Ca^{2+}]_c$), were reported to be advantageous for increasing the efficiency and specificity of gene expression [10]. These oscillations were controlled by inositol 1,4,5-trisphosphate ($InsP_3$) in response to external stimulation [4,6]. Furthermore, spontaneous Ca^{2+} oscillations are typically observed in the absence of any agonist stimulation [11]. It was demonstrated that such spontaneous Ca^{2+} oscillations could exist in microglial cells [12] and astrocytes [13,14].

BV-2 microglial cells have been widely used as a microglial model [15–17] because they can mimic various microglial responses [18,19]. A recent investigation reported that a single BV-2 microglial cell showed spontaneous Ca^{2+} oscillations, which occurred as random spikes [12]. The mechanism responsible for this behavior is still not clear. Previous studies focused mainly on the analysis of single cells. However, in a multi-cell system each BV-2 microglial cell can be affected by its interaction with the neighboring cells. Consequently, spontaneous Ca^{2+} oscillations could be influenced by cell–cell communication. In the present work, we used a multi-cell culture of BV-2 microglial cells to investigate the existence of cell–cell communication *in vitro* and to elucidate their relationship with the random spikes of spontaneous Ca^{2+} oscillations.

2. Materials and methods

2.1. BV-2 microglial cells culture

The cells were grown in Dulbecco's modified Eagle medium (Gibco, USA) supplemented with 10% (v/v) fetal bovine serum (Lanzhou National Hyclone Bio-engineering Co., China) and

* Corresponding author. Address: School of Physics and TEDA Applied Physics School, Nankai University, Tianjin 300457, PR China. Fax: +86 22 66229310.
E-mail address: plt@nankai.edu.cn (L. Pan).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.01.064

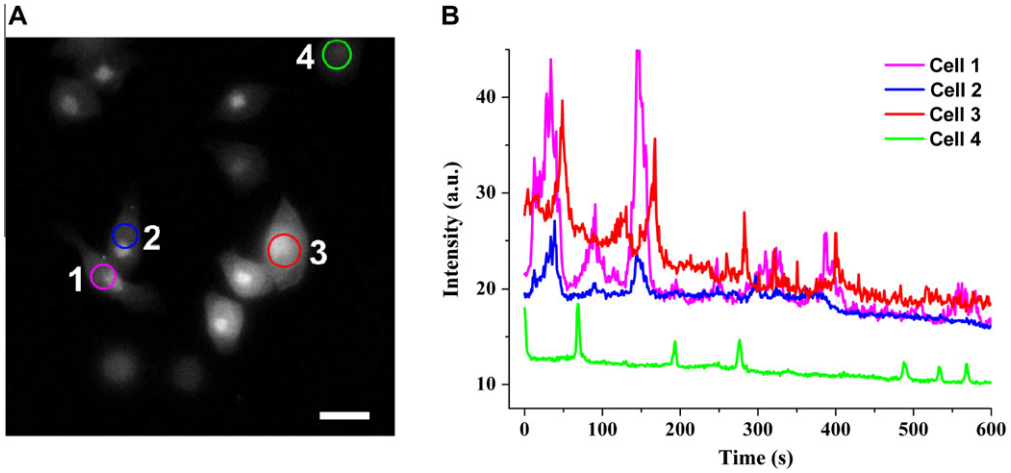

Fig. 1. Spontaneous Ca^{2+} oscillations in multi-BV-2 microglial cells. (A) A typical fluo-3 fluorescence image selected from the stack of images, which is shown in the Supplemental Movie 1. Scale bar, 20 μm. (B) Time-course of $[Ca^{2+}]_c$ changes detected from the four labeled cells exhibiting spontaneous Ca^{2+} oscillations.

100 U/mL penicillin + 100 μg/mL streptomycin (Gibco, USA) at 37 °C in a humidified incubator at 5% CO_2.

2.2. Microscopy

In all of the imaging experiments, the cells were placed in a temperature-controlled stage (providing a constant temperature of 37 °C) and were observed with an inverted fluorescent microscope (Axio observer D1, Carl Zeiss, Germany) with a Fluar 40×/1.30 oil objective. The fluorescence images were acquired by an electron multiplying charge coupled device (EMCCD) (DU-897D-CS0-BV, Andor, UK) connected to the left exit side of the microscope. The EMCCD was controlled by MetaMorph software version 7.1 (Universal Imaging Corp., USA).

2.3. Measurements of $[Ca^{2+}]_c$

The $[Ca^{2+}]_c$ was detected using the sensitive calcium indicator Fluo-3 AM (Invitrogen Co., USA). BV-2 microglial cells were loaded with 4 μM Fluo-3 AM in Hanks' Balanced Salt Solution for 30 min at 37 °C. The cells were then left for another 30 min to complete the de-esterification of the intracellular AM esters. The $[Ca^{2+}]_c$ was monitored by the micro-imaging system as described above. Fluo-3 AM-incubated cells were excited by a mercury lamp combined with a 485/20 nm excitation filter and the fluorescence was collected with a 510 nm long-pass dichroic mirror and a 540/50 nm emission filter. For long-time monitoring, sequences of images were acquired for 50 msec with 2 s intervals. For short-time monitoring, the fluorescence images were obtained for 100 msec without any interval (~10 frames/s). The changes in the fluorescence intensity within the selected regions of interest were quantitatively analyzed by using MetaMorph software 7.1. Representative results were selected and shown from three times repeatable independent experiments.

3. Results

3.1. Observation of the spontaneous Ca^{2+} oscillations in multi-BV-2 microglial cells

To monitor the spontaneous Ca^{2+} oscillations in a multi-cell system, the BV-2 microglial cells were randomly plated on glass for 24 h and then investigated in the absence of agonist stimulation. Long-time monitoring revealed that the probe-labeled cells exhibited spike-shaped Ca^{2+} oscillations as shown in Fig. 1A and Movie S1. The intervals between the spikes were irregular (Fig. 1B), indicating a stochastic nature of the Ca^{2+} oscillations.

3.2. Cell–cell communications between oscillatory cells

To test the presence of cell–cell communications during the Ca^{2+} oscillations, we performed a cross-correlation analysis of the fluorescence intensity data (shown in Fig. 1A and Movie S1). The Pearson correlation coefficient r given by Eq. (1) is a measure of the correlation between two variables X and Y. In our system, X and Y correspond to the temporal dependencies of $[Ca^{2+}]_c$ detected for two cells. If r is larger than 0.8, then the calcium signals of the two cells are strongly correlated, which suggests the presence of cell–cell communication between the two cells

$$r = \frac{\sum_{t=1}^{n}(X-\overline{X})(Y-\overline{Y})}{\sqrt{\sum_{t=1}^{n}(X-\overline{X})^2 \cdot \sum_{t=1}^{n}(Y-\overline{Y})^2}} \quad (1)$$

Because the cells are spread out over varying distances, a certain time interval is required for a selected cell to obtain signals from the other cells. Therefore, we calculated the value of r for a particular pair of cells by introducing a delay time (τ) as expressed in Eq. (2). The maximum value of r is associated with a specific value of τ, which corresponds to the most probable time interval necessary for the signal to travel from one cell to another cell

$$r_{xy}(\tau) = \frac{\sum_{t=1}^{n}(X(t)-\overline{X})(Y(t+\tau)-\overline{Y})}{\sqrt{\sum_{t=1}^{n}(X(t)-\overline{X})^2 \cdot \sum_{t=1}^{n}(Y(t+\tau)-\overline{Y})^2)}} \quad (2)$$

Fig. 2 shows the cross correlation function of the $[Ca^{2+}]_c$ for different pairs of cells calculated by Eq. (2). For cells 1 and 2, a maximum value of $r = 0.83$ is found for $\tau_{12} = 1$ s, meaning that these two cells were strongly correlated at $\tau = 1$ s. Furthermore, cells 1 and 2 were also strongly correlated with cell 3 at $\tau_{13} = 16$ s ($r = 0.81$) and $\tau_{23} = 15$ s ($r = 0.81$), respectively. In addition to cells 1–3 was correlated with cell 4 at $\tau_{34} = 21$ s ($r = 0.80$). These results imply that cell–cell communication among the cells exists *in vitro*.

3.3. Simulation of the random spikes of spontaneous Ca^{2+} oscillations

To test whether cell–cell communication could induce random spikes of spontaneous Ca^{2+} oscillations, we simulated the process of the $[Ca^{2+}]_c$ change using a simple mathematical model of calcium dynamics proposed by Goldbetter [20]. This model is based

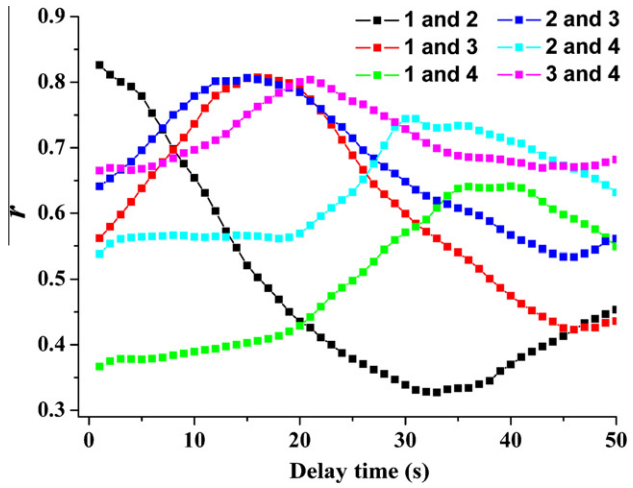

Fig. 2. Cross-correlation analysis of Ca^{2+} oscillations detected in different BV-2 microglial cells. Four cells (shown in Fig. 1) were selected to calculate the Pearson correlation coefficient *r*. Calcium signals from the two cells are strongly correlated when *r* is greater than 0.8.

on the phenomenon of Ca^{2+}-induced calcium release from the intracellular stores, as expressed in Eq. (3). The equation considers the changes in the $[Ca^{2+}]_c$ (Z) and the intercellular calcium stores (Y). The quantities v_0 and kZ determine the influx and efflux of Ca^{2+} into and out of the cell, respectively. Saturation of the $InsP_3$ receptors is denoted as β, and v_1 indicates the release rate of Ca^{2+} from an $InsP_3$- sensitive store into the cytosol. The v_2 corresponds to the transport rate of Ca^{2+} from the cytosol into the $InsP_3$-insensitive store, while v_3 denotes the rate of transport from this store into the cytosol. The k_fY represents the non-activated, leaky transport of Y into Z.

$$\begin{aligned}\frac{dZ}{dt} &= v_0 + v_1\beta - v_2 + v_3 + k_fY - kZ\\ \frac{dY}{dt} &= v_2 - v_3 - k_fY\\ v_2 &= V_{M2}\frac{Z^n}{K_2^n + Z^n}\\ v_3 &= V_{M3}\frac{Y^m}{K_R^m + Y^m}\cdot\frac{Z^p}{K_A^p + Z^p}\end{aligned} \tag{3}$$

The basic proposal of our theoretical consideration is that a random excitation signal produced by a specific cell can induce a rise in the saturation function (β) of other cells by triggering an increase in $InsP_3$, This increase in $InsP_3$ will subsequently result in a change of the $[Ca^{2+}]_c$ in the other cells. Accordingly, random changes of β (the magnitude and time interval) were used to simulate oscillations of $[Ca^{2+}]_c$ in a cell affected by neighboring cells. As shown in Fig. 3, we found that random changes of β could produce random spikes of Ca^{2+} oscillations, consistent with the experimental results (Fig. 1).

3.4. Ca^{2+} waves and global Ca^{2+} elevation

To further resolve the nature of spontaneous Ca^{2+} oscillations in a multi-cell system, we used short-time monitoring to detect $[Ca^{2+}]_c$ oscillations in different cell regions. Fig. 4 and Movie S2

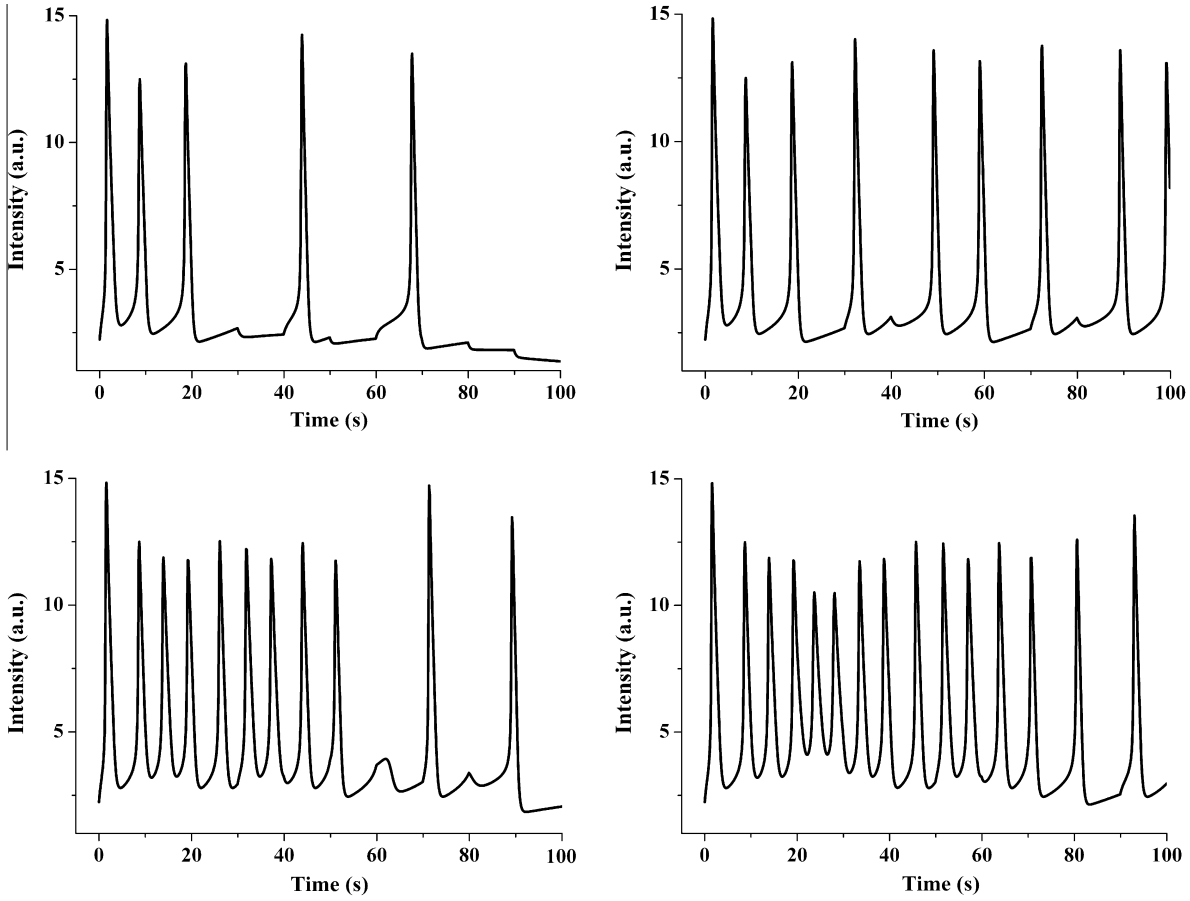

Fig. 3. Simulation of spontaneous Ca^{2+} oscillations in a cell. Random changes in the β value simulate the effect of neighboring cells on the selected cell. Four representative results are shown.

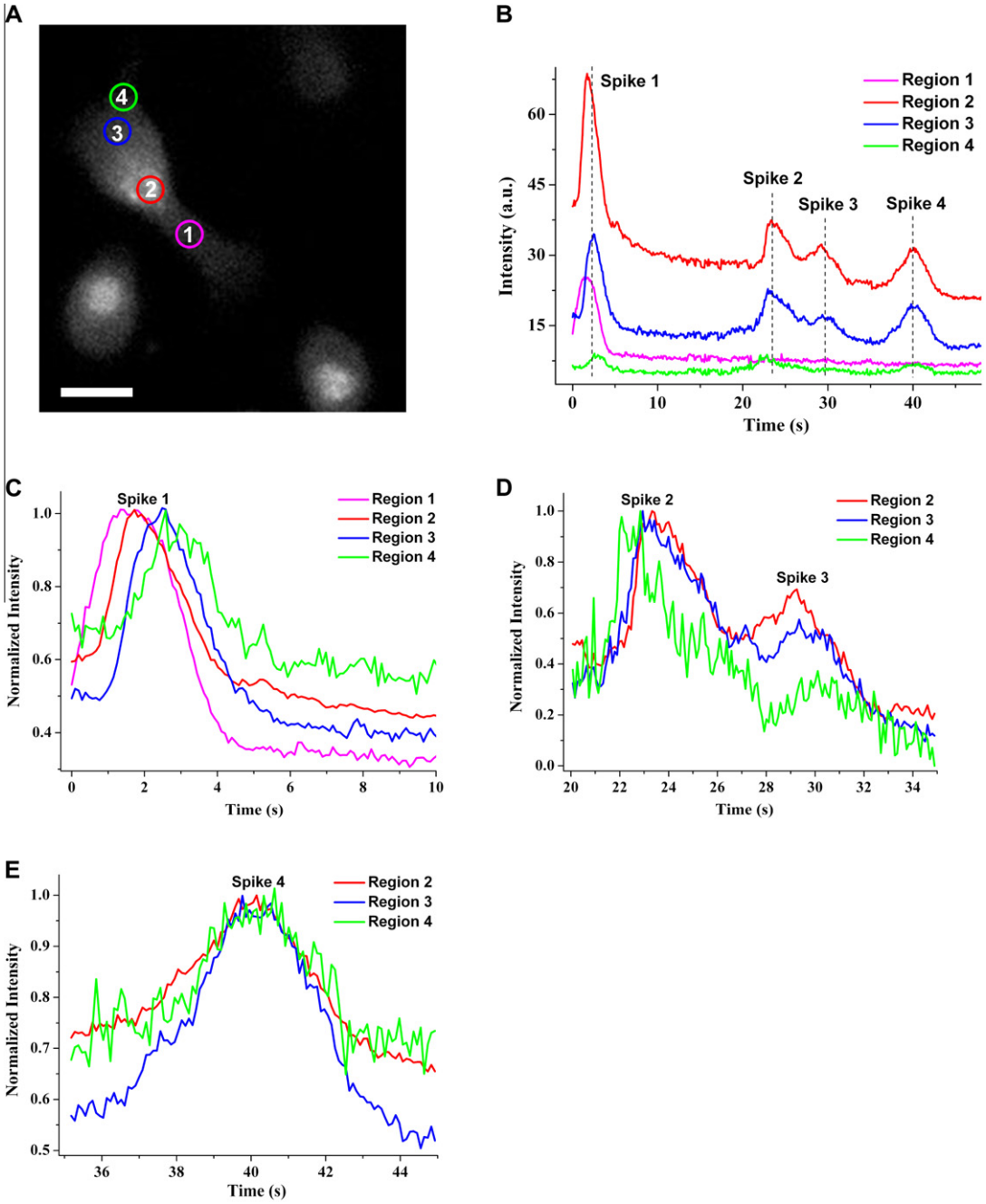

Fig. 4. Short-time imaging of Ca^{2+} oscillations in a single BV-2 microglial cell. (A) A representative fluorescence image of a cell that exhibited Ca^{2+} oscillations. Four regions of the cell were chosen to analyze $[Ca^{2+}]_c$ changes with respect to time. Scale bar, 10 μm. (B) Time-course of $[Ca^{2+}]_c$ changes in the four regions. Spontaneous Ca^{2+} oscillations consist of four profound spikes detected within 48 s of observation. (C–E) Normalized curves of four spikes are shown to resolve the Ca^{2+} waves more clearly. A Ca^{2+} wave was triggered in region 1 for the first spike (C), in region 4 for the second spike (D) and region 2 for the third spike (D), respectively. A synchronous global Ca^{2+} elevation was observed for the fourth spike (E).

show the calcium fluorescence images of a typical BV-2 microglial cell that exhibited $[Ca^{2+}]_c$ oscillations. We selected four different cell regions to analyze the calcium dynamics. As shown in Fig. 4B, four Ca^{2+} spikes were observed. For the first Ca^{2+} spike, the $[Ca^{2+}]_c$ change was delayed from region 1 to 4, indicating that a Ca^{2+} wave was triggered in region 1, which then propagated to other regions. The second and third Ca^{2+} spikes were initiated in regions 4 and 2, respectively. Interestingly, during the fourth spike the $[Ca^{2+}]_c$ detected in different regions of the cell was elevated simultaneously, meaning that this was a global Ca^{2+} elevation. These observations indicated that in multi-BV-2 microglial cells, spontaneous Ca^{2+} oscillations in a cell could be affected by other cells.

4. Discussion

It is difficult to analyze Ca^{2+} oscillations in a systematic way because experimental observations usually show patterns that are more complicated than simple, regular oscillations [10,21,22]. Skupin et al. found that $[Ca^{2+}]_c$ oscillated in a stochastic way and suggested that these oscillations resulted from a sequence of

randomly occurring global Ca^{2+} spikes [12]. Another work suggested that complex Ca^{2+} oscillations were a consequence of the interplay among the endoplasmic reticulum (ER), mitochondria and cytosolic proteins [23]. All of these explanations were focused on the evolution of the $[Ca^{2+}]_c$ on the level of a single cell. However, multi-cell interactions were not taken into consideration in their previous studies. However, our analysis of the multi-cell system showed that random spikes of spontaneous Ca^{2+} oscillations observed in different BV-2 microglial cells were strongly correlated. Therefore, we propose that cell–cell communication could play a key a role in the generation of random spikes of spontaneous Ca^{2+} oscillations.

It has been suggested that the main route of microglia Ca^{2+} signaling is associated with $InsP_3$ [8,24]. External signals activate G protein-coupled receptors and produce two important intracellular second messengers, $InsP_3$ and diacylglycerol. In turn, $InsP_3$ activates $InsP_3$-sensitive receptors in the ER, subsequently resulting in Ca^{2+} release and an elevation in the $[Ca^{2+}]_c$. Therefore, the elevation of $\nu_1\beta$ (see Eq. (3)), which denotes the $InsP_3$-modulated Ca^{2+} influx, can mimic the signal-induced Ca^{2+} elevation. Due to varying distances between the source and the target cells in a multi-cell system, it is evident that the different signals are sensed by the cells with varying effectiveness and time delays. Therefore, we set a small amount of random changes in β (the magnitude and time interval) to mimic the process of cell–cell communications. The random spikes of Ca^{2+} oscillations were clearly observed in our simulations (Fig. 3), which were similar to the previous finding that spikes of regular agonist-induced Ca^{2+} oscillations changed with the concentration of the incoming signal [7].

It was reported that exogenous histamine could stimulate Ca^{2+} oscillations in HeLa cells and that the spikes of the Ca^{2+} oscillations were spatially organized in the form of Ca^{2+} waves [25]. Meanwhile, exogenous agonist-induced Ca^{2+} waves were consistently initiated from the same cellular region in the HeLa cells [25] and hepatocytes [26]. Researchers suggested that the origin of the Ca^{2+} wave was in the most sensitive cellular region, although the whole cell can "sense" the agonist. In contrast to these findings, our results on spontaneous Ca^{2+} oscillations within a single BV-2 microglial cell showed that oscillations could appear not only in the form of Ca^{2+} waves but also as a global Ca^{2+} elevation. Meanwhile, Ca^{2+} waves evidently originated from different regions of the cell (Fig. 4 and Movie S2). Recent *in vivo* studies demonstrated that microglial cells were active sensor and versatile effector cells in the normal and pathologic brain [27]. In particular, these cells could sense subtle changes in the microenvironment and subsequently respond to these changes [3]. Thus, we suggest that BV-2 microglial cells could sensitively accept signals from different neighboring cells *in vitro*. Furthermore, these cells exhibited various spikes, which were organized in the form of a Ca^{2+} wave (Fig. 4).

It is well known that intercellular Ca^{2+} signals are triggered through two pathways [28], including messenger molecule transfer through gap junctions [29,30] and the release of paracrine messengers to the neighboring cells [31,32]. Because the cells were not in contact with each other, our results suggested that paracrine messengers played a significant role in the regulation of microglial Ca^{2+} waves. Several extracellular messengers have been reported to regulate Ca^{2+} wave communications. For instance, CCL6 was investigated as a mediator for cell–cell communication of microglial cells in CNC under physiological as well as pathological conditions [33]. ATP was suggested to modulate rapid microglial response to local brain injury [2]. Additionally, pancreatic β-cells are able to contract synchronously with each other via release of ATP [34]. Therefore, it seems that CCL6, ATP or other extracellular molecules play a special role in spontaneous Ca^{2+} oscillations of BV-2 microglial cells in the multi-cell systems, suggesting that further study is still necessary to identify these vital messengers.

In conclusion, we clearly observed that spontaneous Ca^{2+} oscillations in the form of random spikes took place in the multi-BV-2 microglial cell system *in vitro*. We showed that cell–cell communication existed between the cells, which then induced random spikes of spontaneous Ca^{2+} oscillations. Because Ca^{2+} mobilization plays an essential role in the immune defense mechanism of microglial cells, this work may contribute to understanding the role that microglial cells play in the CNS immediate immune response.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 11204142), the National Basic Research Program of China (No. 2010CB934101), International S&T Cooperation Program of China (2011DFA52870), the Specialized Research Fund for the Doctoral Program of Higher Education (No. 20110031120004), the National Natural Science Foundation of China (No. 11074133), the Fundamental Research Funds for the Central Universities (No. 65010861) and the 111 Project (No. B07013).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.01.064.

References

[1] F. Aloisi, Immune function of microglia, Glia 36 (2001) 165–179.
[2] D. Davalos, J. Grutzendler, G. Yang, et al., ATP mediates rapid microglial response to local brain injury in vivo, Nat. Neurosci. 8 (2005) 752–758.
[3] A. Nimmerjahn, F. Kirchhoff, F. Helmchen, Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo, Science 308 (2005) 1314–1318.
[4] M.J. Berridge, P. Lipp, M.D. Bootman, The versatility and universality of calcium signalling, Nat. Rev. Mol. Cell Biol. 1 (2000) 11–21.
[5] M.J. Berridge, M.D. Bootman, P. Lipp, Calcium-a life and death signal, Nature 395 (1998) 645–648.
[6] M.J. Berridge, M.D. Bootman, H.L. Roderick, Calcium signalling: dynamics, homeostasis and remodelling, Nat. Rev. Mol. Cell Biol. 4 (2003) 517–529.
[7] M.J. Berridge, The AM and FM of calcium signalling, Nature 386 (1997) 759–760.
[8] H. Kettenmann, U.K. Hanisch, M. Noda, et al., Physiology of microglia, Physiol. Rev. 91 (2011) 461–553.
[9] G. Perea, A. Araque, Glial calcium signaling and neuron-glia communication, Cell Calcium 38 (2005) 375–382.
[10] R.E. Dolmetsch, K.L. Xu, R.S. Lewis, Calcium oscillations increase the efficiency and specificity of gene expression, Nature 392 (1998) 933–936.
[11] S. Kawano, K. Otsu, A. Kuruma, et al., ATP autocrine/paracrine signaling induces calcium oscillations and NFAT activation in human mesenchymal stem cells, Cell Calcium 39 (2006) 313–324.
[12] A. Skupin, H. Kettenmann, U. Winkler, et al., How does intracellular Ca^{2+} oscillate: by chance or by the clock?, Biophys J. 94 (2008) 2404–2411.
[13] T.F. Wang, C. Zhou, A.H. Tang, et al., Cellular mechanism for spontaneous calcium oscillations in astrocytes, Acta Pharmacol. Sin. 27 (2006) 861–868.
[14] H.R. Parri, T.M. Gould, V. Crunelli, Spontaneous astrocytic Ca^{2+} oscillations in situ drive NMDAR-mediated neuronal excitation, Nat. Neurosci. 4 (2001) 803–812.
[15] M.A. Burguillos, T. Deierborg, E. Kavanagh, et al., Caspase signalling controls microglia activation and neurotoxicity, Nature 472 (2011) 319–324.
[16] S.X. Jiang, C.L. Benson, L.I. Zaharia, et al., Abscisic acid does not evoke calcium influx in murine primary microglia and immortalised murine microglial BV-2 and N9 cells, Biochem. Biophys. Res. Commun. 401 (2010) 435–439.
[17] L.C.H. Park, H. Zhang, K.F.R. Sheu, et al., Metabolic impairment induces oxidative stress, compromises inflammatory responses, and inactivates a key mitochondrial enzyme in microglia, J. Neurochem. 72 (1999) 1948–1958.
[18] F.C. Lau, D.F. Bielinski, J.A. Joseph, Inhibitory effects of blueberry extract on the production of inflammatory mediators in lipopolysaccharide-activated BV-2 microglia, J. Neurosci. Res. 85 (2007) 1010–1017.
[19] A. Henn, S. Lund, M. Hedtjarn, et al., The suitability of BV-2 cells as alternative model system for primary microglia cultures or for animal experiments examining brain inflammation, Altex Altern. Tierexp. 26 (2009) 83–94.
[20] A. Goldbeter, G. Dupont, M.J. Berridge, Minimal model for signal-induced Ca^{2+} oscillations and for their frequency encoding through protein phosphorylation, Proc. Natl. Acad. Sci. USA 87 (1990) 1461–1465.

[21] S. Schuster, M. Marhl, T. Hofer, Modelling of simple and complex calcium oscillations- from single-cell responses to intercellular signalling, Eur. J. Biochem. 269 (2002) 1333–1355.
[22] U. Kummer, L.F. Olsen, C.J. Dixon, et al., Switching from simple to complex oscillations in calcium signaling, Biophys. J. 79 (2000) 1188–1195.
[23] M. Marhl, T. Haberichter, M. Brumen, et al., Complex calcium oscillations and the role of mitochondria and cytosolic proteins, Biosystems 57 (2000) 75–86.
[24] T. Moller, Calcium signaling in microglial cells, Glia 40 (2002) 184–194.
[25] M.D. Bootman, M.J. Berridge, Subcellular Ca^{2+} signals underlying waves and graded responses in HeLa cells, Curr. Biol. 6 (1996) 855–865.
[26] T.A. Rooney, E.J. Sass, A.P. Thomas, Agonist-induced cytosolic calcium oscillations originate from a specific locus in single hepatocytes, J. Biol. Chem. 265 (1990) 10792–10796.
[27] U.K. Hanisch, H. Kettenmann, Microglia: active sensor and versatile effector cells in the normal and pathologic brain, Nat. Neurosci. 10 (2007) 1387–1394.
[28] E. Scemes, Components of astrocytic intercellular calcium signaling, Mol. Neurobiol. 22 (2000) 167–179.
[29] G.C. Lin, J.K. Rurangirwa, M. Koval, et al., Gap junctional communication modulates agonist-induced calcium oscillations in transfected HeLa cells, J. Cell Sci. 117 (2004) 881–887.
[30] C. Giaume, L. Venance, Intercellular calcium signaling and gap junctional communication in astrocytes, Glia 24 (1998) 50–64.
[31] P.B. Guthrie, J. Knappenberger, M. Segal, et al., ATP released from astrocytes mediates glial calcium waves, J. Neurosci. 19 (1999) 520–528.
[32] A. Charles, Intercellular calcium waves in glia, Glia 24 (1998) 39–49.
[33] M. Kanno, S. Suzuki, T. Fujiwara, et al., Functional expression of CCL6 by rat microglia: a possible role of CCL6 in cell–cell communication, J. Neuroimmunol. 167 (2005) 72–80.
[34] B. Hellman, H. Dansk, E. Grapengiesser, Pancreatic beta-cells communicate via intermittent release of ATP, Am. J. Physiol. Endocrinol Metab. 286 (2004) E759–E765.