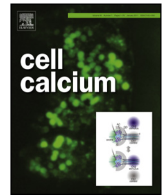
Cell Calcium 64 (2017) 102–108

Contents lists available at ScienceDirect

Cell Calcium

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



Monitoring single-synapse glutamate release and presynaptic calcium concentration in organised brain tissue

Thomas P. Jensen [a,*], Kaiyu Zheng [a], Olga Tyurikova [a,b], James P. Reynolds [a], Dmitri A. Rusakov [a,*]

[a] UCL Institute of Neurology, University College London, Queen Square, London WC1N 3BG, UK
[b] Institute of Neuroscience, University of Nizhny Novgorod, 603950 Nizhny Novgorod, Russia

ARTICLE INFO

Article history:
Available online 30 March 2017

ABSTRACT

Brain function relies in large part on Ca^{2+}-dependent release of the excitatory neurotransmitter glutamate from neuronal axons. Establishing the causal relationship between presynaptic Ca^{2+} dynamics and probabilistic glutamate release is therefore a fundamental quest across neurosciences. Its progress, however, has hitherto depended primarily on the exploration of either cultured nerve cells or giant central synapses accessible to direct experimental probing *in situ*. Here we show that combining patch-clamp with time-resolved imaging of Ca^{2+} –sensitive fluorescence lifetime of Oregon Green BAPTA-1 (Tornado-FLIM) enables readout of single spike-evoked presynaptic Ca^{2+} concentration dynamics, with nanomolar sensitivity, in individual neuronal axons in acute brain slices. In parallel, intensity Tornado imaging of a locally expressed extracellular optical glutamate sensor iGluSnFr provides direct monitoring of single-quantum, single-synapse glutamate releases *in situ*. These two methods pave the way for simultaneous registration of presynaptic Ca^{2+} dynamics and transmitter release in an intact brain at the level of individual synapses.

© 2017 Published by Elsevier Ltd.

1. Introduction

Ca^{2+}-dependent, stochastic release of neurotransmitter quanta is a long-recognised, fundamental function of neurons [1]. Monitoring synaptic release events and Ca^{2+} concentration ($[Ca^{2+}]$) dynamics at individual axonal terminals has since provided critical advances in our understanding of brain function [2,3]. Historically, accurate measurements of axonal $[Ca^{2+}]$ *in situ* have relied on the experimental probing of giant calyceal synapses in acute brain slices. These preparations allow direct experimental access to individual, superficially occurring presynaptic terminals thus enabling whole-cell patch recording combined with ratiometric Ca^{2+} imaging [4–8]. Whilst ratiometric indicators can provide $[Ca^{2+}]$ readout, their use in organised brain tissue is limited by light absorption and scattering, which are strongly wavelength-dependent [9] and thus could affect the ratio of chromatically separated signals. Furthermore, fluorescence signals from small presynaptic boutons of common excitatory synapses typically have the signal-to-noise ratio which is too low to obtain reliable ratiometric measurements. Instead, monitoring presynaptic $[Ca^{2+}]$ dynamics *in situ* has been routinely carried out using fluorescence intensity measures [10–12]. This approach however has low sensitivity to low basal $[Ca^{2+}]$ (<100 nM) and normally requires non-stationary kinetic modelling and various controls to translate recorded fluorescence into $[Ca^{2+}]$ dynamics [13–15].

These complications in large part can be dealt with by using fluorescence lifetime imaging (FLIM) of Ca^{2+} indicators whose fluorescence lifetime is specifically sensitive to free $[Ca^{2+}]$, such as Oregon Green BAPTA-1 (OGB-1) [16–18]. We have recently advanced a two-photon excitation (2PE) FLIM technique which, by optimising photon counting, boosts image acquisition rate hence spatiotemporal resolution of recorded $[Ca^{2+}]$ in neurons and astroglia, *in situ* and *in vivo* by [19]. Here we take this technique one step further, implementing the FLIM scanning mode (Tornado) that enables readout of single action potential-evoked presynaptic $[Ca^{2+}]$ dynamics, with nanomolar sensitivity, in individual small presynaptic boutons in acute brain slices.

As for the monitoring of neurotransmitter release events, for decades it relied on the registration of postsynaptic receptor currents using a patch-clamp technique. An important advance came in the shape of an optical quantal analysis in which

* Corresponding authors.
E-mail addresses: t.jensen@ucl.ac.uk (T.P. Jensen), d.rusakov@ucl.ac.uk (D.A. Rusakov).

http://dx.doi.org/10.1016/j.ceca.2017.03.007
0143-4160/© 2017 Published by Elsevier Ltd.

successful single-synapse glutamate releases *in situ* are registered by detecting (NMDA receptor-dependent) Ca^{2+} entry in postsynaptic dendritic spines [20–22]. The signal-to-noise ratio in such recordings is however relatively low, the slow NMDA receptor kinetics complicates registration of repetitive releases, and these observations are usually uncoupled from the monitoring of presynaptic Ca^{2+}. Another powerful method to track synaptic release has been the presynaptic uptake of fluorescent FM dyes in the course of vesicular exocytosis [23,24] which could be combined with presynaptic $[Ca^{2+}]$ monitoring [14]. This was further advanced with single-vesicle imaging employing pH-sensitive indicators (pHluorins) targeted to the synaptic vesicle lumen [25,26]. The use of this potent method *in situ* could be however complicated, mainly because of its stringent requirements to the optical environment which has to favour reliable signal detection from individual release sites.

Recently, a genetically encoded high-affinity glutamate sensor iGluSnFr has been developed providing robust registration of synaptically evoked glutamate transients in the extracellular space *in situ* [27]. Here we show that combining whole-cell single axon tracing with local iGluSnFr expression enables reliable registration of spike-evoked quantal glutamate release events from individual axonal boutons of principal neurons *in situ*. The approach opens a new window into our quest to understand the principles of synaptic signal integration in the brain.

2. Material and methods

2.1. iGluSnFr transduction of hippocampal neurons

All animal procedures were conducted in accordance with the European Commission Directive (86/609/EEC) and the United Kingdom Home Office (Scientific Procedures) Act (1986). Young C57BL/6 mice (3–4 weeks of age) male and female, were anaesthetised using isoflurane (5% induction, 1.5–2.5% v/v). Upon loss of pedal withdrawal reflexes, the animal was secured in a stereotaxic frame (David Kopf Instruments, CA, USA). Perioperative analgesics were administered (subcutaneous buprenorphine, 60 $\mu g\,kg^{-1}$) and the scalp was shaved and disinfected using three washes of topical chlorhexidine. A small midline incision was made and the skull was exposed. A craniotomy of approximately 1–2 mm diameter was performed over the right hemisphere using a high-speed hand drill (Proxxon, Föhren, Germany), at a site overlying the medial hippocampus. Stereotactic coordinates were 60% of the anterio-posterior distance from bregma to lambda and 2.5 mm lateral to midline. Once exposed, a warmed aCSF variant (cortex buffer; in mM, 125 NaCl, 5 KCl, 10 HEPES, 10 glucose, 2 $CaCl_2$, 2 $MgSO_4$) was applied to the skull and cortical surface throughout the procedure.

Pressure injections of AAV9 *hSyn iGluSnFr* (totalling $0.1–1 \times 10^{10}$ genomic copies in a volume not exceeding 200 nL, supplied by Penn Vector Core, PA, USA) were carried out using a pulled glass micropipette stereotactically guided to a depth of 1.3 mm beneath the cortical surface, at a rate of approximately 1 $nL\,s^{-1}$. The total injection volume was delivered in three steps, reducing depth by 100 μm at each step. Once delivery was completed, pipettes were left in place for 5 min before being retracted. The surgical wound was closed with absorbable 7-0 sutures (Ethicon Endo-Surgery GmbH, Norderstedt, Germany) and the animal was left to recover in a heated chamber. Meloxicam (subcutaneous, 1 $mg\,kg^{-1}$) was subsequently administered once daily for up to two days following surgery. Mice were killed by transcardial perfusion with ice-cold sucrose-enriched slicing medium (in mM, 105 sucrose, 60 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 15 glucose, 1.3 ascorbic acid, 3 Na pyruvate, 0.5 $CaCl_2$ and 7 $MgCl_2$, saturated with 95% O_2 and 5% CO_2) after a 2–4 week AAV incubation period and acute cortical slices prepared for imaging and electrophysiological recordings as below.

2.2. Brain slice preparation

Acute 350 μm thick hippocampal slices were obtained from either 3–4 week old Sprague-Dawley rats or 5–7 week old C57BL/6 mice, as specified, in full compliance with national guidelines on animal experimentation. Slices were prepared in an ice-cold slicing solution described above, stored in the slicing solution at 34 °C for 15 min before being transferred to a submersion chamber for storage in an extracellular solution containing (in mM) NaCl 119, KCl 2.5, $MgSO_4$ 1.3, NaH_2PO_4 1, $NaHCO_3$ 26, $CaCl_2$ 2, glucose 10 (osmolarity adjusted to 295–305 mOsM with glucose). All solutions were continuously bubbled with 95% O_2/5% CO_2. Slices were allowed to rest for at least 60 min before recordings started.

2.3. Axon tracing and Tornado scanning in pre-synaptic boutons

We used a Femtonics Femto3D-RC or Femto2D-FLIM imaging system, integrated with patch-clamp electrophysiology (Femtonics, Budapest) and both linked on the same light path to two femtosecond pulse lasers MaiTai (SpectraPhysics-Newport) with independent shutter and intensity control as previously described [28]. Patch pipettes were prepared with thin walled borosilicate glass capillaries (GC150-TF, Harvard apparatus) with open tip resistances 2.5–3.5 or 3.5–4.5 MOhms for CA3 pyamidal cells or CA1 interneurones respectively. Internal solution contained (in mM) 135 potassium methanesulfonate, 10 HEPES, 10 di-Tris-Phosphocreatine, 4 $MgCl_2$, 4 Na_2-ATP, 0.4 Na-GTP (pH adjusted to 7.2 using KOH, osmolarity 290–295), and supplemented with the morphological tracer dye Alexa 594 (50 μM) with addition of Oregon Green BAPTA-1 (300 μM) for FLIM imaging.

Pre-synaptic imaging was carried out using an adaptation of pre-synaptic Ca^{2+} imaging methods previously described [22]. Following break-in 45–60 min were allowed for Alexa 594 to equilibrate across the axonal arbour. Axons, identified by their smooth morphology and often torturous trajectory were followed in frame scan mode to their respective targets in CA1. Discrete boutons were identified by criteria previously demonstrated to reliably match synaptophysin labelled punctae [29,30]. Once identified, spiral shaped (Tornado) line scans of diameter equivalent to the bouton were produced from single points using the MES software (Femtonics). Depending on the bouton size, one spiral scan typically takes 1–1.5 ms, thus providing comprehensive, high-resolution readout of axonal fluorescence.

2.4. 2PE Tornado-FLIM readout of Ca^{2+} concentration in small axonal boutons

Here we identified and patched CA1 *stratum radiatum* interneurons located close to the apical border of the pyramidal cell layer. Axons were followed as above to boutons present in, around or within the pyramidal cell layer and, initially, line scans were carried out with a single action potential initiated by brief positive voltage steps in voltage clamp mode (V_m holding −70 mV). Tornado scan data were recorded by both standard analogue integration in Femtonics MES and in TCSPC in Becker and Hickl SPCM using dual HPM-100 hybrid detectors. Next we used the fast-FLIM analysis procedure described previously [19] to handle individual Tornado scans (also see in [19] the general diagram illustrating the TCSPC data conversion into dynamic $[Ca^{2+}]$ changes). Thus, the fast-FLIM linescan data were collected and stored as 5D-tensors (*t,x–z,T*) to be analysed with a custom written data analysis software available online (https://github.com/zhengkaiyu/FIMAS).

Download English Version:

https://daneshyari.com/en/article/5530522

Download Persian Version:

https://daneshyari.com/article/5530522

Daneshyari.com