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Long-lasting spontaneous calcium transients in the striatal cells

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Abstract

The striatum plays an important role in linking cortical activity to basal ganglia output. We conducted the calcium (Ca^{2+}) imaging to investigate the spontaneous activities of the striatum using acute slice preparations. Corticostriatal slices of rat brain were stained with Fura-PE3-AM. Long-lasting spontaneous intracellular Ca^{2+} ($[Ca^{2+}]_i$) transients, which lasted up to about 250 s, were observed. The amplitudes of the transients were variable even in a single cell. Most cells exhibited irregular frequencies, but some exhibited oscillatory features. These $[Ca^{2+}]_i$ transients were not induced by action potentials because they were not inhibited by tetrodotoxin. Antagonists of the ionotropic glutamate receptors, 6-cyano-7-nitroquinoxaline-2,3-dione and D,L-2-amino-5-phosphonovaleric acid, did not block these transients. These results suggested that the action potentials and the excitatory synaptic inputs in these striatal network were not involved in the induction of the $[Ca^{2+}]_i$ transients. In contrast, the number of the active cells, which exhibited the $[Ca^{2+}]_i$ transients, was greatly reduced by the intracellular Ca^{2+} store depletor, thapsigargin. Therefore, the intracellular Ca^{2+} store is likely to contribute to the $[Ca^{2+}]_i$ transients.

Keywords: Basal ganglia; Striatum; Calcium oscillation; Calcium imaging; Spontaneous activity

Calcium ion (Ca^{2+}) is an important messenger for signal transduction and it has been shown that the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) changes in response to the excitation of the cell [4,7,15]. In neuronal cells, depolarization of the membrane potential opens the voltage-dependent Ca^{2+} channels and $[Ca^{2+}]_i$ increases. Endoplasmic reticular Ca^{2+} stores (ER) are another source of $[Ca^{2+}]_i$ elevation and are crucial for second messenger-induced intracellular Ca^{2+} signaling [3,5].

In the basal ganglia, the striatum receives inputs from the cortex and is thought to play a crucial role in controlling somatic motor movements, behavioral pattern, cognition, learning and memory [6,8]. The physiological properties of the striatum remain unclear. Therefore, we conducted the Ca^{2+} imaging of the rat corticostriatal slices to investigate the spontaneous activity of the striatal cells and observed the long-lasting $[Ca^{2+}]_i$ transients in the striatal cells. The preliminary results of this work have been reported in an abstract form [12].

Postnatal Day 12 (P12) to P18 Sprague–Dawley rats were anesthetized with halothane and were decapitated. The cerebrum was rapidly isolated and placed in ice-cold normal artificial-cerebrospinal fluid (ACSF) bubbled with 95% O_2–5% CO_2. Sagittal slices of striatum with cortex (corticostriatal slice), 300 μm thick, were prepared with a vibratome tissue slicer (Leica, VT-1000S). Slices were incubated at room temperature in a submerged chamber containing gassed ACSF for at least 60 min. The composition of ACSF was (in mM): 137 NaCl, 2.5 KCl, 0.58 NaH_2PO_4, 1.2 $MgCl_2$, 2.5 $CaCl_2$, 21 $NaHCO_3$, and 10 glucose.

$[Ca^{2+}]_i$ in slices was measured with the membrane-permeant acetoxy-methyl ester of Fura-PE3-AM (CalbioChem) [19] dissolved in DMSO. The corticostriatal slices were incubated in 100 μl ACSF containing 20 μM Fura-PE3-AM and 0.02% Chremophore EL (Sigma) gassed with 95% O_2–5% CO_2 at 35 °C for 45 min. The slices were then washed with 100 μl ACSF at 35 °C for 15 min. Fura-PE3-loaded slices were transferred to a continuously superfused (2–2.5 ml/min) recording chamber on a stage of an upright microscope equipped with epifluorescence illumination (BX51WI, Olympus). $[Ca^{2+}]_i$ changes were imaged with a 20×, NA 0.95 water-immersion objective (Olympus) at 29–31 °C. The Fura-PE3-loaded slices were alternately excited at the wavelengths of 340 and 380 nm using a filter changer (DG-4; Sutter, exposure time was 100 ms on individual wavelengths)

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and fluorescence signals were captured (F340 and F380) every 2 s with a cooled-CCD camera (Cool SNAP HQ, Photometrics). All equipment was controlled by the MetaFluor software (Roper).

In the measurement of Ca^{2+} signals from imaged cells, we identified Fura-PE3-loaded cells in images of the slices, and measured the average fluorescences (F340 and F380) of these cells as a function of time. The $[Ca^{2+}]_i$ in the striatal cell was estimated by the fluorescence ratio (R = F340/F380) from each imaged cell [9].

To detect the individual $[Ca^{2+}]_i$ transients, a baseline of each trace was obtained by fitting the local minimum values of the trace using a higher order Hanning filter (window length, 22–42 s) with a polynomial function. The changes of the R from the baseline (ΔR) were obtained by subtracting the baseline from the raw trace (cf. [7]). To reduce the amount of noise, we applied the Hanning filter (window length, 6 s) as a low-pass filter for the ΔR trace. $[Ca^{2+}]_i$ transients were discriminated from fluctuations of the baseline using a threshold ($\Delta R = 0.01$). The transient rate was defined as the number of onsets per unit time during the recording period (>1000 s). We selected the cells which showed a transient rate higher than 0.001 s^{-1}. In some cells, the activity of $[Ca^{2+}]_i$ transients diminished during a long period of recording. In the pharmacological experiments, we discarded the cells which did not show any $[Ca^{2+}]_i$ transient within 600 s just before the application of pharmacological agents to confirm their effects. All drugs were applied by perfusion.

A fluorescence image of the Fura-PE3-loaded cells in a striatal slice is shown in Fig. 1A. In captured images, 9–37 cells were found to exhibit the spontaneous $[Ca^{2+}]_i$ transients in a field of view (~450 μm × 330 μm). We analyzed the $[Ca^{2+}]_i$ transients obtained from 97 cells in five slices from four animals. The frequency and patterns of activity were variable including highly variable frequency, rhythmic oscillations, and bursting activity. Four typical patterns of observed $[Ca^{2+}]_i$ transients are shown in Fig. 1A (lower panel). Pattern # 1 showed the $[Ca^{2+}]_i$ transients with relatively short duration and sometimes bursting activity. Pattern # 2 showed a relatively regular $[Ca^{2+}]_i$ oscillations, but had variable durations and peak amplitudes. Pattern # 3 showed the $[Ca^{2+}]_i$ transients with slow time courses and small amplitudes. In pattern # 4, the $[Ca^{2+}]_i$ transients appeared with a low frequency but had large amplitudes. An individual cell exhibited a mixed profile of these patterns and we were not able to classify the cells in terms of these patterns.

Most of these spontaneous $[Ca^{2+}]_i$ transients were long in duration (typically 2–20 s; maximum = 266 s). The distribution of the duration and amplitude of the transients are shown in Fig. 1B and C, respectively. The average values of the duration and the peak amplitude were 17.5 ± 28.7 s in 1504 events and 0.0213 ± 0.0155 in 1508 events, respectively (mean ± S.D.). The average duration is much longer than that observed in $[Ca^{2+}]_i$ transients during the up-state of striatal neurons [10]. These long-lasting spontaneous $[Ca^{2+}]_i$ transients were not observed in the area of the cortex attached with the striatal slice (data not shown). These spontaneous $[Ca^{2+}]_i$ transients occurred

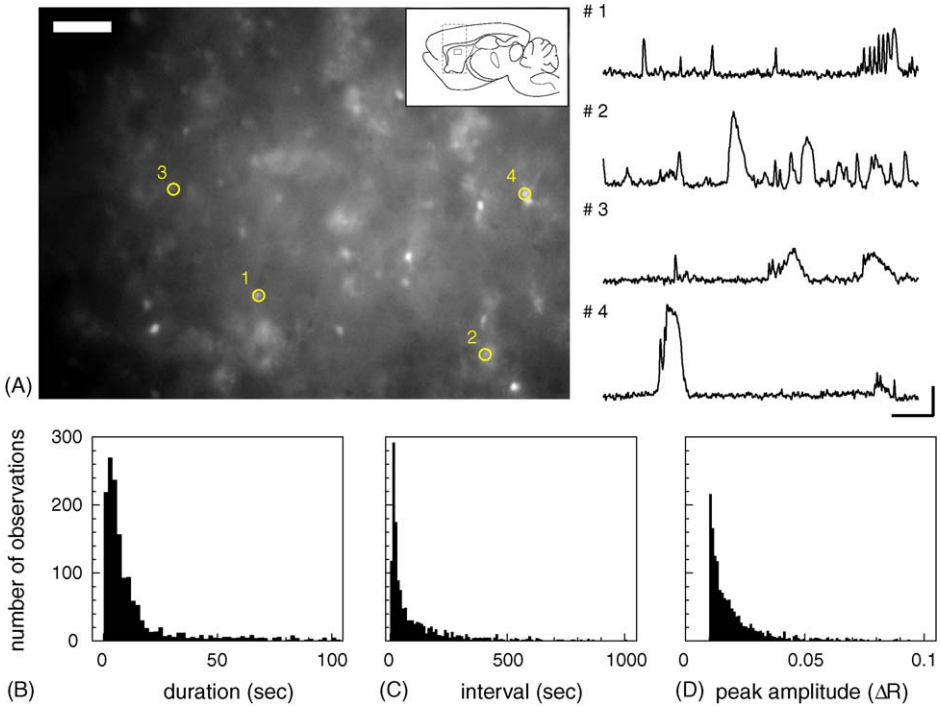

Fig. 1. (A) Left, fluorescence image of a rat striatal slice loaded with the acetoxy-methyl ester of the Ca^{2+} indicator dye, Fura-PE3-AM. The $[Ca^{2+}]_i$ transients were observed from 37 cells in this slice. Scale bar, 50 μm; inset, schematic diagram illustrating the sagittal cross-section of the brain and the imaged region (box). The box illustrated by dashed lines indicates the region of the slice which we used; right, the time courses of the $[Ca^{2+}]_i$ transients. The number of each panel corresponds to the cell number in the left fluorescence image. Scale bar, 200 s, $\Delta R = 0.03$; and (B–D) the frequency histogram of the duration (B), interval (C) and peak amplitude (D) of the $[Ca^{2+}]_i$ transient. The median value of each distribution was 8 s, 44 s and 0.0157, respectively. The bin size of each histogram is 2 s, 10 s and 0.001, respectively.