Calcium Imaging in mDA neurons

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ABSTRACT

Calcium imaging using Fura-2, Fluo-4 and X-Rhod-1.

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1 To measure cytosolic calcium, 5 uM Fura-2 or Fluo-4 was added to cells at room temperature for 40 minutes made up in HBSS (Invitrogen).

To measure mitochondrial calcium, cells were incubated with 2µM X-Rhod-1 in HBSS for 40 minutes.

1.1 All probes are from Molecular Probes (Thermo Fisher Scientific). Fura-2-AM is

a ratiometric dye with a high affinity for Ca2+. It is used to measure [Ca2+]c.

Fluo-4-AM is a calcium indicator.

X-Rhod-1-AM is a calcium indicator which localises to mitochondria.

2 Cells are then washed 2x in HBSS at room temperature. They are now ready for imaging.

3 [Ca2+]c is monitored in cells by obtaining the ratio between the excitation at 340 nm (high Ca2+) and 380 nm (low Ca2+) for which fluorescence light was reflected through a 515 nm long pass filter.

4 Microscopy for Fluo-4:

4.1 Live-cell imaging was performed excited by a 488 nm laser and measured at 520 nm. A time-series with 5 second intervals was performed to establish basal fluorescence before 50mM KCl was added to depolarise the membrane and measure fluorescence intensity increase, and recovery.

5 Microscopy for Fura-2 [Ca2+]c:

5.1 The cells were excited sequentially at 340 and 380nm using light from a Xenon arc lamp. A time-series with 1 or 5 second intervals was performed to establish basal fluorescence before 50mM KCl was added to depolarise the membrane.The emitted fluorescence was measured at 515 nm on a cooled camera device (CCD).

5.2 $[Ca^{2+}]_c$ using Fura-2 AM was also imaged on a Nikon Ti2 inverted microscope with Perfect Focus System, an ASI motorised XY stage with piezo Z and an Okolab environmental chamber with a CO^2 mixer. Imaged were acquired using an Andor iXon Ultra897 EMCCD camera. Cells were excited with a Cairn FuraLED light engine optimised for 340 and 380 nm with a dichroic mirror T400lp (Chroma) and an emission filter ET510/80m (Chroma), using a 40x 1.3 NA S Fluor objective. The microscope was controlled with Micro-Manager v2.0

6 Microscopy for X-Rhod-1:

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6.1 Live-cell imaging was performed excited by a 561 nm laser. A time-series with 5 second intervals was performed to establish basal fluorescence before 50mM KCl was added to depolarise the membrane and measure fluorescence intensity increase, and recovery giving readouts for mitochondrial calcium.

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