Exposing cells to a single pulse of light of user-defined wavelength and duration using the FDSS6000

Abstract

In the described model system the GPCR receptor optimally transduces signal from light at 480 nm (light as a wave) by absorbing photon energy (light as a particle) and undergoing isomerization.
As a consequence the excitation wavelength 480 nm used for Fluo-4 AM based calcium dyes also agonizes the receptor. In this model the assay design 1) Precludes collecting a pre-agonist (background) signal and 2) Continuously bombards the receptor with stimulus.
In this study we used 1) The UV ratiometric dye Fura-2 AM to separate reporter dye excitation/emission from receptor agonism and 2) A novel "pulse" feature in the FDSS6000 software to expose cells for specific times to 480 nm light. The result is an assay that can collect background signal and determine the minimal light exposure time at 480 nm (or any other wavelength) for maximal agonism.

Materials and Methods

Adherent cells were washed and dye loaded using 2 µM Fura-2 for 1 h @37 °C, 5 % CO_2. Following extensive washing the cells were placed at 37 °C no CO_2 for 1 h then read using the FDSS6000.
For each experiment cells were exposed to 340 nm and 380 nm light prior to the light pulse at 480 nm. Following the light pulse further data was collected at 340 nm and 380 nm.

Results

The data in Figure 1 shows the effect of light exposure duration on calcium mobilization. The results indicate maximal calcium mobilization is 1.5-3 sec exposure while 0.2 sec exposure is insufficient for a significant response.

The results in Figure 2 show the effect of LOPAC compounds in columns 5-24 on light mediated calcium mobilization (2 sec @ 480 nm) against Fura-2 AM loaded cells. Columns 1-3 are the control compounds of various concentrations, column 4 is buffer only. The Z' score was over 0.5 using columns 3 and 4. The blue wells are compounds eliciting putative antagonism or are autofluorescent (F9, M9, P18). Putative antagonists may be nonspecific agonists desensitizing cells; such compounds are identified by adding compounds online and looking for agonism. Since wavelengths 340 nm and 380 nm do not activate the receptor this protocol is possible only using Fura-2 AM and not Fluo-4 AM.
Further, light as an agonist spontaneously agonizes all wells along with eliminating ligand diffusion issues, solubility issues, quencher binding issues, etc.

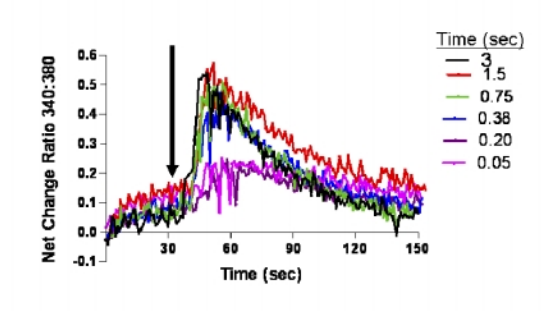

Fig. 1: Effect of exposure time of 480 nm light on calcium mobilization. For each group n=16, the %CV range is 8-16. Arrow indicates start of 480 nm exposure. The conditions listed are in seconds. Note maximal response is a 1.5-3 sec light pulse. Exposure time 0.2 sec and lower elicited no/minimal response.

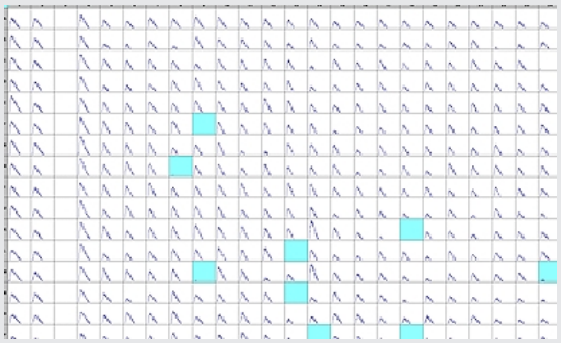

Fig. 2: Effect of LOPAC on antagonizing light mediated calcium mobilization. Columns 1-2 are control antagonist each at a different concentration (n=16/group), column 3, buffer only, and column 4-24 LOPAC. The blue wells are 9 wells with the most inhibition. (Data presented using CeuticalSoft).

Summary

FDSS6000 hardware and software versatility enables new applications, in this case a single light pulse of user-defined length and wavelength.

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