Intracellular Ca^{2+} monitoring

Cerebrocortical neuron cultures were used for $[Ca^{2+}]i$ measurements at day 12 *in vitro* (DIV12) as previously described (Cao et al., 2010). Briefly, the growth medium was removed and replaced with dye loading medium (100 µl per well) containing 4 µM fluo-3 AM and 0.04% Pluronic acid in Locke's buffer (8.6 mM HEPES, 5.6 mM KCl, 154 mM NaCl, 5.6 mM glucose, 1.0 mM MgCl2, 2.3 mM CaCl2, 0.0001 mM glycine, pH 7.4). After 1 hour incubation in dye loading medium, the neurons were washed four times in fresh Locke's buffer (180 µl per well) using an automated microplate washer (Bio-Tek Instruments Inc, VT, USA) and transferred to a FLIPR II (Molecular Devices, Sunnyvale, USA). Cells were excited at 488 nm and Ca^{2+}-bound fluo-3 emission was recorded at 515-575 nm at 1.2 second intervals for calcium influx and 0.5 second intervals for calcium oscillations. For calcium influx, baseline fluorescence was recorded for 1 minute, 20 µl of 10X concentrations of compounds were added to wells at a rate of 20 µl/s, the fluorescence was monitored for an additional 5 minutes. For calcium oscillations, baseline fluorescence was recorded for 2 minutes, 20 µl of 10X concentrations of compounds were added to wells at a rate of 20 µl/s, the fluorescence was monitored for an additional 2 minutes.

Data analysis

Graphpad Prism 7 software (San Diego, CA) was used to analyze time and concentration response relationships. Calcium oscillation data is a plot of the raw Fluo3 fluorescence reads over time. With calcium influx, Fluo3 fluorescence was expressed as (*F*max - *F0*), where Fmax is the maximum and Fo is the baseline line fluorescence measured in each well.

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