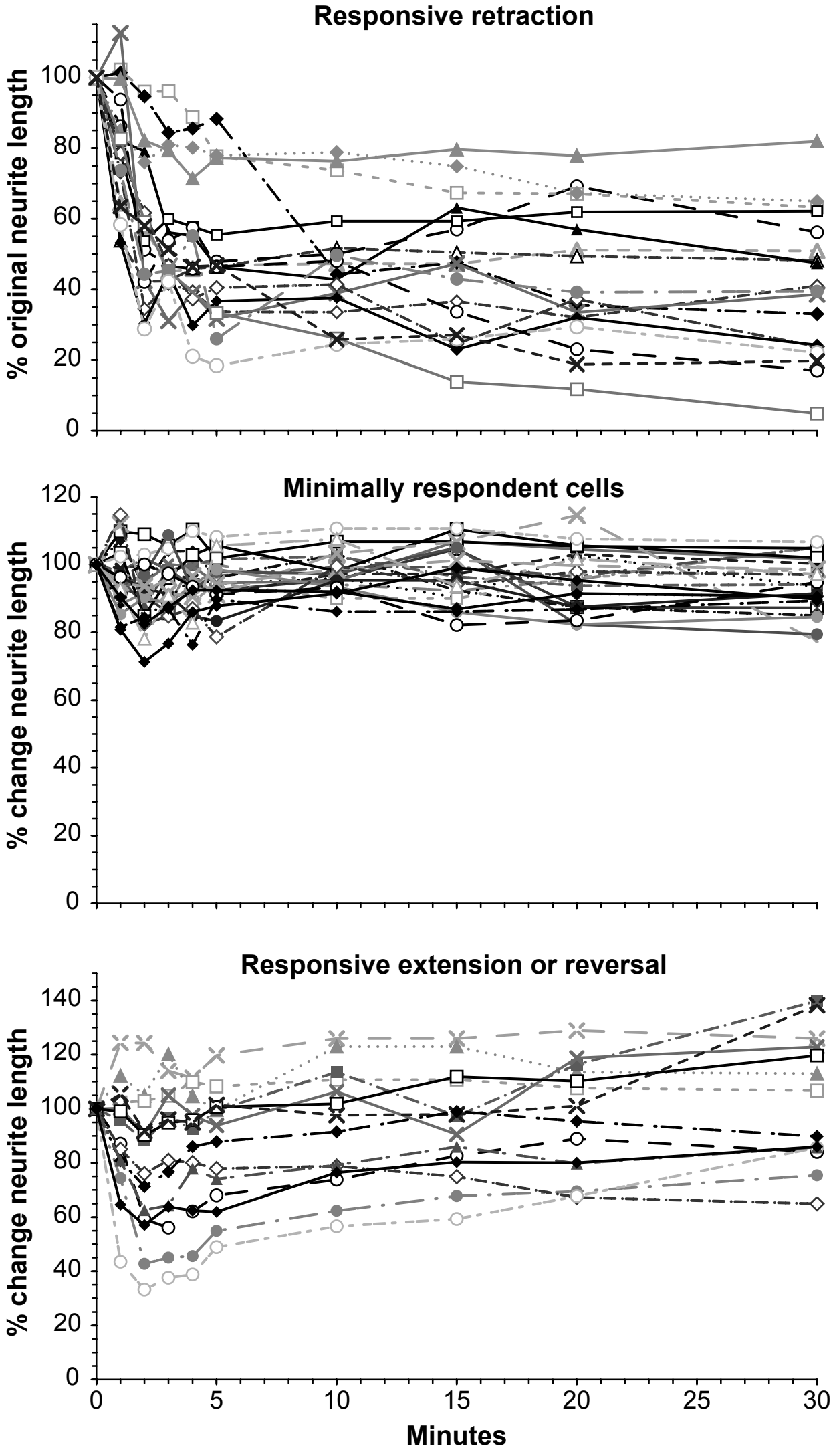
Supplementary Material

Figure S1 Complexity of responses of Neuro-2A cells pre-treated with antisense oligonucleotides targeting myosin IIC sequence subsequent to the addition of LPA.

The broad distribution of data seen in Figure 11 (Panel Myosin IIC, Antisense) was re-examined to display the underlying time course of each individual treatment. In this set of experiments, fifty-five Neuro-2A cells had been separately treated with a pulse of LPA (1μM) following 48 hours incubation with antisense oligonucleotides targeting myosin IIC sequence. Over each subsequent 30-minute period, DIC images were taken of the same cell at various intervals (1, 2, 3, 4, 5, 10, 15, 20 and 30 minutes), prior to neurite length measurement.

As evidenced by the line plots displaying all individual time courses, the dataset can be broken down into three broad groupings. There are cells (n = 16) that display responsive retraction (>20% decrease in neurite length as compared with the starting value) to a pulse of LPA (Top panel); cells (n = 25) that are minimally respondent (<20% change in neurite length as compared with the starting value) (Middle panel); and cells (n = 14) that respond to the LPA pulse either by slow extension (>10% increase in neurite length as compared with the starting value) or by immediate retraction that reverses within a few minutes to a slow extension. Together, these results make up the dataset seen in the myosin IIC, antisense panel in Figure 11. This makes clear the complexity of responses that underlie Neuro-2A neurite length change with respect to myosin IIC antisense treatment – opposite extremes to a single treatment being encapsulated through visualisation of the responses of two adjacent cells (Figure 12).


Responsive retraction
% original neurite length
0
20
40
60
80
100
Minimally respondent cells
% change neurite length
0
20
40
60
80
100
120
Responsive extension or reversal
% change neurite length
0
20
40
60
80
100
120
140
0
5
10
15
20
25
30
Minutes

Confocal Stack 1
http://www.rvc.ac.uk/AboutUs/Staff/pchantler/Stacks/ConfocalStack1.cfm

Animated confocal stack showing the localisation of Myosin IIA and F-actin in Neuro-2A cells that have been treated for 96 hours with sense oligonucleotides corresponding to myosin IIC sequence.

Cells are stained for both myosin IIA (green; indirect immunofluorescence; isoform-specific primary antibody; secondary antibody conjugated to Alexa-633, visualised as green) and F-actin (red; visualised using rhodamine phalloidin).
Interval between slices: 0.4μm
Cell height: 10 μm

The stack is viewed from bottom to top. F-actin first becomes apparent as broad, non-uniform puncta. Although the location of myosin IIA coincides with many of these structures, it is also broadly distributed throughout the cell when viewed in horizontal planes within the first 2 microns. At higher levels (3-5 microns) the full extent of actin puncta can be seen, together with actin-rich filopodia that lack myosin staining. Myosin IIA remains dispersed throughout the cytoplasm. At higher levels still (6-10 microns) actin staining becomes more cortical, concentrating around the perimeter of the cell, and is also seen in the growth cone. Myosin IIA distribution, in turn, becomes more cortical and overlaps considerably with the F-actin distribution but displays a broader distribution subjacent to the plasma membrane.

Confocal Stack 2
http://www.rvc.ac.uk/AboutUs/Staff/pchantler/Stacks/ConfocalStack2.cfm

Animated confocal stack showing the localisation of Myosin IIB and F-actin in Neuro-2A cells that have been treated for 96 hours with sense oligonucleotides corresponding to myosin IIC sequence.

Cells are stained for both myosin IIB (green; indirect immunofluorescence; isoform-specific primary antibody; secondary antibody conjugated to Alexa-633, visualised as green) and F-actin (red; visualised using rhodamine phalloidin).
Interval between slices: 0.4μm
Cell height: 8.6 μm

The stack is viewed from bottom to top. Within 2 microns of the substratum, F-actin is visible as a series of discrete puncta, especially within the cell body and processes, and is also prominent within growth cones (see those visualised at base of image). The distribution of myosin IIB does not appear to coincide with these structures, although there is some overlap; mostly, it is broadly distributed throughout the cell at this level, exhibiting increased concentration in cell body and parts of the growth cone as compared with the process shafts. Above this level (3 microns upwards) F-actin exhibits a thin rim of cortical staining, concentrated immediately below the plasma membrane. Myosin IIB distribution, in turn, is also cortical though is relatively uniform in intensity as compared with the F-actin distribution.

Confocal Stack 3
http://www.rvc.ac.uk/AboutUs/Staff/pchantler/Stacks/ConfocalStack3.cfm

Animated confocal stack showing the localisation of Myosin IIC and F-actin in Neuro-2A cells that have been treated for 96 hours with sense oligonucleotides corresponding to myosin IIC sequence.

Cells are stained for both myosin IIC (green; indirect immunofluorescence; isoform-specific primary antibody; secondary antibody conjugated to Alexa-633, visualised as green) and F-actin (red; visualised using rhodamine phalloidin).
Interval between slices: 0.4μm
Cell height: 8.6 - 10 μm

The stack is viewed from bottom to top. Within 2 microns of the substratum, F-actin is observed as a series of discrete puncta, especially within the cell body and growth cones; there is a particularly prominent group of larger, densely stained puncta in the central cell towards the base of the image. While the localisation of myosin IIC overlaps with these puncta the distribution appears broader and extends throughout the cytoplasm, showing no particular concentration in regions such as the growth cone, for example. Above this level (3 microns upwards), where there is an abrupt change in the distribution of F-actin to one showing cortical staining and intense staining within growth cones, myosin IIC is seen to display an intense cortical ring and some, albeit less prominent, staining within the growth cones.

Confocal Stack 4
http://www.rvc.ac.uk/AboutUs/Staff/pchantler/Stacks/ConfocalStack4.cfm

Animated confocal stack showing the localisation of Myosin IIC and F-actin seen at high power (100x objective) in the neurite processes of Neuro-2A cells following 96 hours incubation with sense oligonucleotides corresponding to myosin IIC sequence.

Cells are stained for both myosin IIC (green; indirect immunofluorescence; isoform-specific primary antibody; secondary antibody conjugated to Alexa-633, visualised as green) and F-actin (red; visualised using rhodamine phalloidin).
Interval between slices: 0.1μm
Process thickness: <2 μm

Similar to **Confocal Stack 3** but uses a 100x objective lens so as to view detail within the neuritic processes. Actin staining within the processes includes discrete puncta as well as a fine rim of cortical staining; F-actin within filopodia is also visible. At this resolution, we can also see significant correspondence between the localisation of F-actin and myosin IIC; the latter is found in the growth cones, in regions of the cortex where the processes emerge from the cell body, and even coincident with some of the puncta that we consider to be structures engaged in adhesion. However, the myosin IIC localisation is also distinctive: note, for example the continuous "ribbon" of staining found in the uppermost process emerging from the central cell – which appears to be independent of actin and may be related to myosin IIC filament formation.

Confocal Stack 5
http://www.rvc.ac.uk/AboutUs/Staff/pchantler/Stacks/ConfocalStack5.cfm

Animated confocal stack showing the localisation of Myosin IIA, Myosin IIB and F-actin in Neuro-2A cells that have been treated for 96 hours with antisense oligonucleotides targeting myosin IIC.

Triple-stained stack: myosin IIA (green; indirect immunofluorescence; isoform-specific primary antibody; secondary antibody conjugated to fluorescein), myosin IIB (magenta; indirect immunofluorescence; isoform-specific primary antibody; secondary antibody conjugated to Alexa-633, visualised as magenta); F-actin (red; visualised using rhodamine phalloidin).
Interval between slices: 0.4μm
Cell height: ~7μm

The stack is viewed from bottom to top. Below ~1.6 microns above the substratum the distribution of F-actin can be seen to be localised within microspikes and within growth cones. Puncta, while present, are diminished in number. Above this height, F-actin exhibits peripheral cortical staining. Below ~1.6 microns the distributions of Myosin IIA and Myosin IIB, while different from each other in detail, are diffuse and show considerable overlap. Above this height, both myosins adopt more cortical staining patterns, overlapping with F-actin yet appearing to extend further into the cytoplasm. Although the stack terminates before the upper boundaries of the cells are reached, it is clear that 96 hours exposure to myosin IIC antisense regime, while not disrupting myosin IIA or myosin IIB distributions to any noticeable extent, has brought about considerable flattening of the cells.

Confocal Stack 6
http://www.rvc.ac.uk/AboutUs/Staff/pchantler/Stacks/ConfocalStack6.cfm

Animated confocal stack showing the localisation of Myosin IIB, Myosin IIC and F-actin in Neuro-2A cells that have been treated for 96 hours with antisense oligonucleotides targeting myosin IIC.

Triple-stained stack: myosin IIC (green; indirect immunofluorescence; isoform-specific primary antibody; secondary antibody conjugated to fluorescein), myosin IIB (magenta; indirect immunofluorescence; isoform-specific primary antibody; secondary antibody conjugated to Alexa-633, visualised as magenta); F-actin (red; visualised using rhodamine phalloidin).
Interval between slices: 0.4μm
Cell height: ~8μm

The stack is viewed from bottom to top. Below ~1.6 microns F-actin staining within microspikes, growth cones and the cortical rim are apparent; some puncta can be seen. Above this height, most F-actin staining is confined to the cortical rim. Antisense treatment targeting Myosin IIC has led to a severe attenuation of Myosin IIC immunofluorescence. Below ~1.6 microns the distribution of Myosin IIB is diffuse and found throughout the cytoplasm. Some fibrous myosin staining in the main process emanating from the central cell does not coincide with F-actin immunofluorescence. Above 1.6 microns, Myosin IIB distribution appears more cortical. While overlapping with F-actin, myosin IIB extends further into the cytoplasm than F-actin, which is concentrated around the rim. Myosin IIC antisense treatment does not appear to disrupt the original distribution of myosin IIB in these cells yet leads to significant flattening of the cell bodies.

Confocal Stack 7
http://www.rvc.ac.uk/AboutUs/Staff/pchantler/Stacks/ConfocalStack7.cfm

Animated confocal stack showing the localisation of Myosin IIA, Myosin IIC and F-actin in Neuro-2A cells that have been treated for 96 hours with antisense oligonucleotides targeting myosin IIC.

Triple-stained stack: myosin IIC (green; indirect immunofluorescence; isoform-specific primary antibody; secondary antibody conjugated to fluorescein), myosin IIA (magenta; indirect immunofluorescence; isoform-specific primary antibody; secondary antibody conjugated to Alexa-633, visualised as magenta); F-actin (red; visualised using rhodamine phalloidin).
Interval between slices: 0.4μm
Cell height: ~7μm

The stack is viewed from bottom to top. Below ~1.6 microns F-actin staining is observed within microspikes, growth cones and the cortical rim; puncta, staining for F-actin, are relatively few in number. Above this height, most F-actin staining is confined to the cortical rim and the front of advancing processes. Antisense treatment targeting Myosin IIC has led to a severe attenuation of Myosin IIC immunofluorescence. Below ~1.6 microns the distribution of Myosin IIA is diffuse and found throughout the cytoplasm. Apparent fibrous myosin staining in the process of the cell on the right overlaps, but does not coincide exactly, with F-actin immunofluorescence. Above 1.6 microns, Myosin IIA overlaps with cortical F-actin staining but is more extensive than the rim-like localisation seen for F-actin. Myosin IIC antisense treatment does not appear to disrupt the original distribution of myosin IIA observed in these cells yet does lead to a significant flattening of the cell bodies.