Dear editor,

Please find below the revisions made to the manuscript.

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

This has been done.

2. JoVE cannot publish manuscripts containing commercial language. This includes company names of an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents. Examples of commercial language in your manuscript include Durcupan, etc.

All commercial langage has been removed.

3. Please ensure that the references appear as the following:  
Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. Source. **Volume** (Issue), FirstPage – LastPage, doi: DOI (YEAR).  
For more than 6 authors, list only the first author then et al.

The reference list has been updated.

4. The highlighted protocol steps are over 2.75 page limit. Please adjust the highlighting.

The highlighting has been adjusted. Highlighted protocol can be found below:

**1. Determination of calcium binding protein or protein content of interneurons and biocytin visualization following electrophysiological recordings and biocytin filling.**

1.2 In a fume cupboard, carefully pick up the brain slice with a paintbrush and roll it onto a small piece of fine quality filter paper. Place another piece of moistened filter paper onto the slice and place the two pieces of wet filter paper in a small plastic pot containing 5-10 mL of fixative solution and store in the fridge overnight at 4 °C.

1.4 Replace the fixative solution with 2 mL of 0.1 M PB. Place the tissue in a petri-dish (9cm D- 1.4 cm H) ensuring that it lies flat with no folds or creases and remove the excess buffer using a dry paintbrush.

1.5 Cover the tissue with the warm gelatin solution and place the petri-dish onto a frozen block to quickly cool the solution.

1.7 Move the dish of setting gelatin to the fridge and leave at 4 °C for 30-60 min.

1.8 In a fume cupboard, cut out a small block (~ 1x1 cm) containing the gelatin-embedded tissue of the dish using a scalpel blade, lift the block using a small spatula and carefully place it in the same but fresh fixative solution used to fix the slices for at least 30 min at 4 °C.

1.9 Wash the gelatin block in 5 mL of 0.1 M PB three times, dry it using a piece of paper tissue and stick the block side up (*i.e.* with the tissue at the top) onto a vibratome chuck using superglue.

1.10 Remove excess glue with a piece of filter paper and use a scalpel blade to cut the corners of the block off, leaving a diamond shape.

1.11 Section the slice at 50 µm thickness using a vibratome and place each section carefully in a glass vial containing 10 % sucrose.

1.12 Carefully pick up a section from the vial, place it flat into a petri-dish lid. Using a dissecting microscope and a fresh scalpel blade, remove the gelatin from around the section and return the section to a vial containing 2ml of fresh 10 % sucrose

1.13 Cryo-protect the sections in 0.1 M PB-based sucrose-glycerol solution at room temperature by incubating them for 10 min in 10 % sucrose solution, 20 min in 20 % sucrose-6 % glycerol solution twice and finally 30 min in 30 % sucrose-12 % glycerol solution twice under constant agitation.

1.14 Place the sections flat onto a small rectangle of tin foil using a paintbrush. Remove any excess liquid from the sections and carefully fold the tin foil into a parcel.

1.15 Hold the tin foil close to the surface of liquid nitrogen without touching the surface for 30 s and then allow the sections to thaw completely for approximately 30 s. Repeat the freeze-thaw another two times.

1.16 Remove all sections with a paintbrush and place them in a glass vial containing 2 mL of 0.1M PB under constant agitation to wash off excess sucrose.

1.17 Remove the PB with a Pasteur pipette and incubate the sections in 2 mL of 1 % aqueous H2O2 for 30 min. Wash sections in 2 mL of 0.1M PB 3 x 5 min.

1.18 Remove the 0.1 M PB with a Pasteur pipette and add 1 % sodium borohydride (NaBH4) in 0.1 M PB Do not cap the vial, as NaBH4 solution gives off hydrogen gas.

1.19 Remove the sodium borohydride with a Pasteur pipette and wash the sections thoroughly in 2 mL of 0.1 M PB 5x 5 min.

1.20 Replace the 0.1M PB with 10% normal goat serum (NGS) in 0.1M PB for 30 min.

1.21 Remove the goat serum and incubate the sections overnight at 4 °C in a mixture of mouse monoclonal and rabbit polyclonal antibodies made up in ABC solution.

1.22 Incubate the sections for 2 h in the dark in a mixture of fluorescently-labelled secondary antibodies (Table of reagents).

1.23 Mount the sections onto slides in mounting medium and cover with a coverslip.

1.24 Take images of fluorescence labelling at 40X magnification. (Representative images in Fig. 1Bb).

1.27 Perform the avidin-HRP reaction by firstly incubating the sections in ABC for at least 2 h to amplify the HRP reaction product.

1.29 Wash the sections with PBS three times for 10 min and then with Tris buffer twice for 10 min. Remove the Tris buffer after the last wash.

1.30 Quickly add one drop of 8 % NiCl2 solution to the DAB solution, pipette the solution in and out to mix and quickly add 1 mL of this solution over the sections. Incubate the sections in the DAB/NiCl2 solution for 15 min.

1.31 Add 10 µL of 1 % H2O2 to the DAB solution. Allow the reaction to proceed in the dark under constant agitation for about 1 to 2 min and monitor the labelling of the filled cells with a dissecting microscope.

1.32 Stop the reaction by removing the DAB/NiCl2/H2O2 solution and wash the sections with Tris buffer twice for 5 min.

1.33 In a fume cupboard, place a small circle of filter paper into a Petri-dish and dampen it with a little 0.1 M PB. Lift the sections one at a time from the glass vial using a paintbrush, place them carefully flat upon the paper.

1.34 Cover the sections with another moistened circle of filter paper and remove excess buffer by gently touching tissue paper to the surface.

1.35 Apply 8-9 drops of 1% osmium tetroxide in 0.1 M PB to the top paper, cover the dish and retain in the fume hood for at least 30 min, but no more than 1 h.

1.36 Open the petri-dish and lift the top filter paper. Lift the sections carefully one at a time with a paintbrush, place them in a glass vial and rinse them in distilled water twice.

1.38 Place each section flat onto a glass slide and coverslip the sections. Transfer the slide into a Petri dish, place an empty glass vial over the coverslip to retain it in place and cover with 50% absolute alcohol. After 15 min, remove the slide from the solution and remove the sections from the slide. Place the sections back on the slide and then place the slide in a solution of 70% alcohol for 15 min. Repeat the same process with 95% and finally 100% alcohol solution.

1.39 Transfer the sections to a glass vial containing a solution of 100 % alcohol on a shaker in a fume cupboard. Replace the alcohol solution with a solution of propylene oxide (C3H6O) and wash three times for 5 min. Following the last wash, keep ~2mL of propylene oxide in the vial and add resin (1:1 ratio). Ensure that the resin is dissolved and keep the sections under constant agitation for 30 min.

1.40 Place each section in an aluminium planchette containing epoxy resin using a wooden stick and incubate overnight.

1.41 Place the planchette over a hot plate for approximately 10 min. Pick up each section with a wooden stick and place them on a clean slide. Keep the orientation of each section consistent using a dissecting microscope. Place a coverslip over the sections. Place the slide in the oven for 48 h at 56 °C for curing.

**2. 3D neuronal reconstructions using a Neurolucida software**

2.1 Place a slide on the stage and secure with stage clip and open the neuron reconstruction software. Click on ‘Acquire’ tab and select ‘live image’.

2.3 Use a low-magnification objective to focus on the ‘home’ section containing the cell body. When the cell body is in focus, click in the center to mark the reference point.

2.5 To trace the soma in 3D using a 100X objective, select ‘Contour’ from the ‘Trace’ tab and select the ‘Cell Body’ contour. Use the joystick to move the focus to the very top of the cell body. Place points by clicking around the perimeter of the part of the cell body that is currently in focus. Right-click and select ‘Close Contour’ to finish this first outline. Focus down a little, and repeat. Repeat this process at different z positions until the bottom of the cell body is reached (Figure 2).

2.7 To trace the dendritic arbor, select ‘Dendrite’ or ‘Apical Dendrite’ in the ‘Neuron’ menu. First trace a short, initial segment for each dendrite. Trace along each dendrite using the joystick to move across the section and the mouse scroll wheel to adjust the diameter of the cursor to match the diameter of the dendrite.

2.9 When a node in the tree is reached, right-click and select ‘Bifurcating Node’ or ‘Trifurcating Node’ from the drop-down menu.

2.10 When the end of a branch has been reached, select an ending from the drop-down menu in the ‘Neuron’ menu. Select the correct ending type, i.e. ‘High Ending’ or ‘Low Ending’ to facilitate matching across sections.

2.12 To identify matching points between dendrites in a section that matches the completed section, Click on ‘Move’ tab and select ‘Match points’. Select the number of points needed to be matched (three or more points is preferred) and then press OK. Click on the ending of a completed branch and then click on the branch it will match to on the live microscope image. Do this for each match point. Repeat this process at higher magnifications at 1000X to ensure accurate matching.

2.14 Once all the dendrites of each section have been traced, trace the axon using the same process (steps 2.2.6-2.2.13) by selecting ‘Axon’ from the ‘Neuron’ menu.

5. Step 1.7: What’s the size of the small block? Done  
6. 1.31: Issecting microscope? Typo has been rectified.   
7. 2.14: Please write this step in imperative tense. Done