FEMTO-SECOND LASER DENDROTOMY

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1. INTRODUCTION

Focused laser light has found use in cellular surgery as an ultra-sharp scalpel. The nonlinear nature of two-photon (2P) absorption localizes interaction to a narrow focus with minimal damage to surrounding tissue. We use a custom-built 2P laser microscope to prune the dendritic tree of layer V pyramidal neurons and assess how this affects firing.

2. EXPERIMENT

We imaged neurons filled with 100µM of Alexa-488 in 300µm slices of somatosensory cortex from 15-19 day-old rats using 12-22mW of 800nm laser light. The site of a potential cut was chosen from this image and cuts were made with 100ms pulses of 30-150mW at 720nm. The holding current of the neuron was monitored in whole-cell voltage-clamp and firing patterns in current-clamp were compared before and after the cut. Cuts were verified by generating EPSPs via 2P uncaging of MNI-glutamate at identified spines proximal and distal to the cut and via biocytin staining post hoc. Dendritic cuts were followed by a sudden and large increase in holding current (>200 pA) and were evidenced by retained proximal uncaging responses but lost distal ones. They were verified in biocytin stainings proximal to the point of the cut. Cuts of small 3rd or 4th order dendritic segments did not change neuronal firing rate significantly. However, cuts of 1st and 2nd order segments resulted in an increase in firing rate for the same current injected. Figure 1a shows the 2P fluorescence image of a layer V pyramidal neuron (magnified in 1b and 1c made before and after the cut, respectively). The neuron’s firing patterns before and after the cut are compared in Fig. 1d.

3. SUMMARY

We have demonstrated the use of fs Ti:S laser surgery to dynamically prune the neuron’s dendritic arbor. This approach can be used for arbitrarily pruning dendritic morphology and investigating the relationship between dendritic structure and neuronal function.

Figure 1. Femtosecond-laser surgery. (a) Fluorescence image of a layer V pyramidal neuron. Red line indicates cut site. Scale bar 50 µm. (b-c) Magnified view of boxed area in a before (b) and after (c) the cut. Scale bar 10 µm. (d) Neuronal firing frequency versus injected current before (pre) and after (post) the cut.