

**Fast Rhythmic Bursting Cells:
The Horizontal Fiber System in the Cat's Primary Visual Cortex**

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Abstract

One of the cellular mechanisms underlying the generation of gamma oscillations is a type of cortical pyramidal neuron named fast rhythmic bursting (FRB) cells. After 58 cells from 21 cats' primary visual cortices were filled with Neurobiotin, the brains were cut, and the cells were photographed. From all cells, 1 non-pyramidal and 4 pyramidal cell (3 regular spiking (RS) cells & 1 FRB cell) were confocaled, reconstructed with NeuroLucida, and analyzed with NeuroExplorer. All 5 cells showed a linear correlation (>0.94) between length and number of intersections. Their polar histograms indicated that the FRB cell has triple dendritic length, twice the number of dendritic tree orders and mean length compared to the 4 other cells. We propose that FRB cells are key elements of the horizontal fiber system that links cell populations with similar feature selections throughout the primary visual cortex.

Introduction

As the mammalian visual system has an infinite ability for object recognition and localization, the ultimate "binding or combinatorial problem" remains unsolved. In order for the brain to perceive and locate an object, the brain has to locate the object's viewing position, distance, and identify its illumination, shape, color, size, etc. [1]. The temporal coding mechanism or the temporal correlation hypothesis [2], suggests that precise synchronization of feature-related neurons codes for the perceptual coherence. Over the years, synchronous gamma (γ , 30-60 Hz) frequency bands were found in visual cortical areas [3] olfactory systems [4], somato-sensory and motor cortices [5], and the hippocampi [6] of cats and monkeys. By increasing the activity summation through the synchronizing inputs, gamma oscillations enhance response saliency [7] and synaptic information transmission [8] because the individual cells in a cell assembly need only contribute few spikes [9].

Most cortical neurons have longer vertical processes than lateral processes, and they are classified morphologically into two classes. One class is the non-pyramidal or stellate cells that have no or few spines. Their axons spread out locally, and they release a neurotransmitter, GABA, to generate inhibitory postsynaptic potentials. These cells include fast spiking (FS) cells and low threshold spikes (LTS) [10] cells. Whereas FS cells fire very thin spikes with short duration (usually 0.4-0.6ms) at very high frequencies, LTS cells generate small bursts of 2-3 spikes that do not have spike inactivation.

The second class is the pyramidal cells that make up 60-70% of the total cortical neuron population [11], with a high density of spines. As output cortical neurons, the pyramidal cells release glutamate to generate excitatory postsynaptic potentials that synapse in the spines. Examples of pyramidal cells include regular spiking (RS) cells, intrinsically bursting (IB) cells, and fast rhythmic bursting (FRB) or also known as chattering cells. While RS cells fire long (usually over 1ms), sustained, repetitive spikes, IB cells fire clustered sequential (3-5) spikes with slow depolarizing phases. FRB cells respond to depolarizing current injections with intrinsic gamma (γ , 20-50 Hz) frequency bands [12, 13, 14]. The resulting repetitive, high frequency (350-700Hz) burst of action potentials are very short (<0.5 ms) and with almost no spike inactivation.

FRB cells can be both simple and complex and are distributed throughout cortical layers II to VI [15]. Because the evoked γ oscillations are mostly due to rhythmic synaptic inputs [15], we believe that FRB cells mainly amplify and distribute γ oscillations instead of generating the γ activity as their intrinsic properties. Since short duration firing pattern made FRB cells to reflect little information on the input's time, they may be essential in providing internal temporal structure to the cortical network [12]. By linking cell populations with similar feature selection, distance cells [16] that make up a horizontal fiber system [17] reinforce the interconnections by amplifying their inputs. FRB cells have many horizontal axon collaterals that expand horizontally and ramify through layers I-III and V [12, 18]. Since the high frequency bursts of FRB cells enable them to generate powerful excitatory synaptic potentials, FRB may be the optimal horizontal cells that we have been searching for. The essential role of the FRB cells is undeniable when blocking of FRB firing [19] or FRB gap junctions [20] eliminate the γ activity.

Materials and Methods

Surgical Protocol. The experiments were conducted according to the ethical guidelines of the National Institutes of Health and with the approval of the Institutional Animal Care and Use Committee of the University of Pennsylvania. Since our knowledge of the primary visual cortex in cats exceeds that of any other mammals, we used

male and female barbiturate-anesthetized adult cats (2.5-3.5 kg). The cats were inspected by a veterinarian, and continuously infused with anesthetics throughout the experiments. To insure that the animals were not in pain, we had them in light sleep and monitored them through EEG. At the end of the experiment, the animals were euthanized with the guidelines of the Panel on Euthanasia of the American Veterinary Association.

Completed Intracellular recording procedures. To obtain the FRB cells, V1 cortical neurons in vivo were recorded through a direct current injection. Their electrophysiological cell types of FS, RS, IB, and FRB was then classified based on their firing patterns.

Histology. After filling the FRB cells with Neurobiotin, an intracellular label for neurons, their morphologies and exact locations were identified *a posteriori* based on the depth of the neurons through the micromanipulator reading, and their position relative to recognizable landmarks such as the sulcus. The cats was euthanized with pentobarbital and perfused with chilled saline followed by paraformaldehyde (4%) in phosphate buffered saline (PBS). The brain was removed and the visual cortex was cut into small blocks. The tissue blocks was then post fixed (4°C) overnight and stored in 30% sucrose-0.02 % thimerosal solution for cryoprotection until the tissue blocks sunk. Next, 80 um thick sagittal sections was obtained by using a freezing microtome and stored in 0.1% PBS-0.02% thimerosal at pH 7.4 and 4°C. To identify the Neurobiotin-filled cells, we washed the sections three times in PBS and then submerged the sections in a blocking solution of 10% normal goat serum (NGS, ImmunoResearch, West Grove, PA), 0.4% triton X-100 (Sigma), 1% bovine serum albumin (BSA, Jackson ImmunoResearch) for an hour, and then in Cy3-conjugated*Strptavidin, NGS, Triton X-100, Boccine Serum Albumin in PBS overnight. After the cells are rinsed in PBS, the tissue was mounted on gelatinized glass slides and cover-slipped with Vectashield (Vector Laboratories).

Morphological analyses. Neurons was seen under Olympus (Meville, NY) BX51 microscope with a filter cube set for tetramethylrhodamine isothiocyanate/Dil/Cy3 (excitation, 540nm; dichoric, 565nm; emission, 605nm; Chroma Technology, Rockingham, VT). Images were captured by a 12-bit CCD, Olympus MagnaFire digital camera. The identified cells were also taken under confocal microscopy through the Leica TCSNT software (Leica Mikroskopie und systeme Bmbml, 1995). Finally, the neurons were reconstructed three-dimensionally, using Neurolucida, a computer software for brain mapping, (MicroBrightField, Williston, VT) to trace out each dendrite through the confocal stacks. The final neurons were then analyzed with NeuroExplorer (MicroBrightField).

Results

From 2001 till 2007, we obtained a total of 58 cells only from 21 cats out of 119 cats that we have experiments with, a mere 18%. Out of these 21 cats, we have total 37 or 63.8% pyramidal cells: 2 FRB cells and majority are RS cells, although we found cells throughout layers I to VI, majority of the pyramidal cells lies within layer III and VI while most of the non-pyramidal cells are located in layers VI. We obtained only 6 or 10.34% non-pyramidal cells, and 15 or 25.86% indistinguishable ghost cells. The chance of acquiring cells with good morphology is extremely limited since the lab focus mainly on obtaining electrophysiology data. After 13 hours of cell recording, injected neurobitin usually has faded or cells have already atrophied resulting faint or damaged morphologies. Distant arborizations and fine axons are especially hard to recover. As they have greater resistance to dye passage, axons are also less complete than dendrites. Some amounts of dentritic tree were also lost through sectioning and pipetteing.

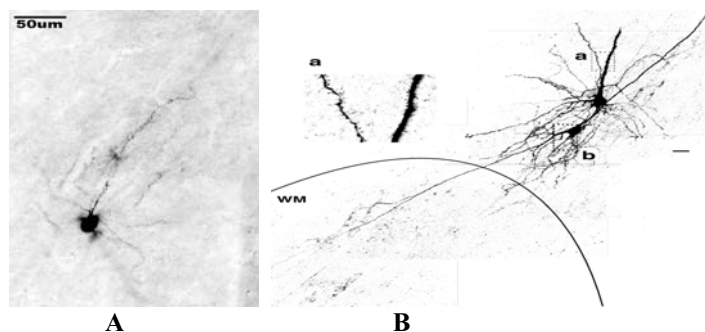
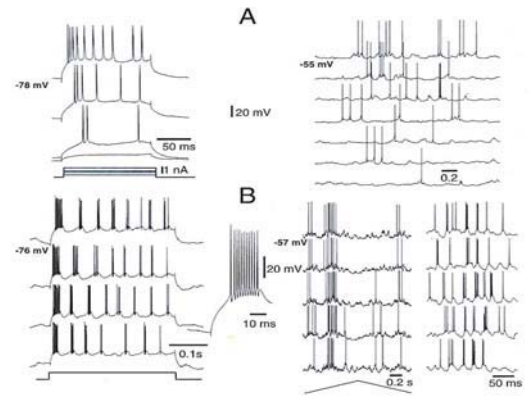


Figure 1: **A:** Fluorescence micrograph of a pyramidal-FRB cell in layer 6 with magnification of 20X. **B:** Confocal micrograph montage of 2 pyramidal shaped cells in layer 6. The cell closer to the white matter (WM, below the line, layer 6 is above the line) is a complex RS1 cell with basal dendrites extending into the white matter, The more superficial cell was a simple FRB cell with a very spiny dendritic tree (inset a). All calibration bars are 20 μm.

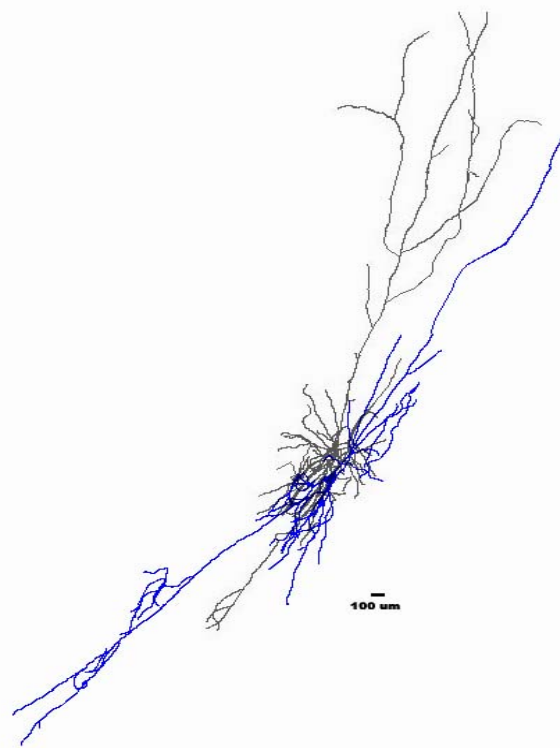
Electrophysiology.

Figure 2. **A** is the RS1 cell and **B** is the FRB cell. Both were activated by direct current injection through micropipette recording (left) and receptive field stimulation (right). The FRB cell's intraburst frequency was 600 Hz. The firing pattern induced by the current pulses was very close to that of the visual stimulation. While the RS cell was stimulated with gratings of logarithmical increasing contrasts from 0 to 64%, the FRB cell was stimulated with a bar drifting TO and FRO over the RF (its response to TO motion is expanded to the right). The Calibration bar for voltage is the same for all traces except the detail from the FRB cell.

Confocal microscopy & NeuroLucida tracings.

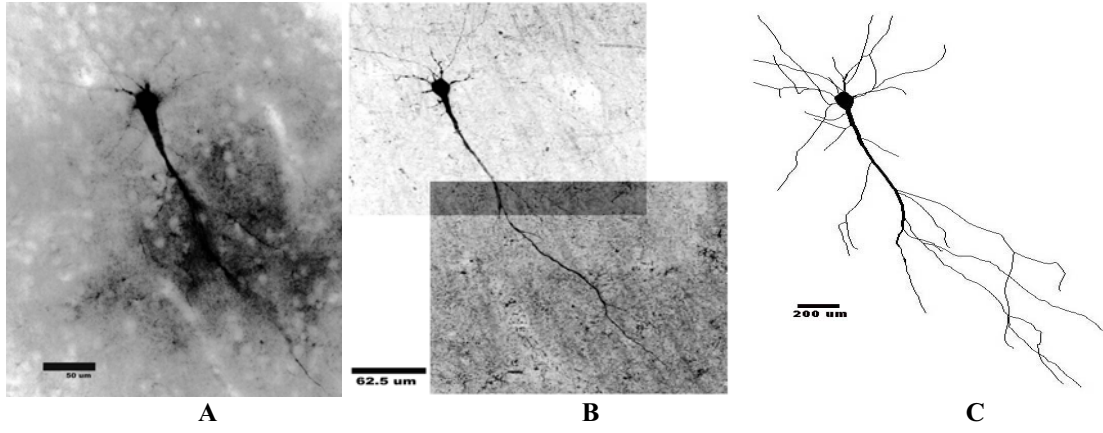
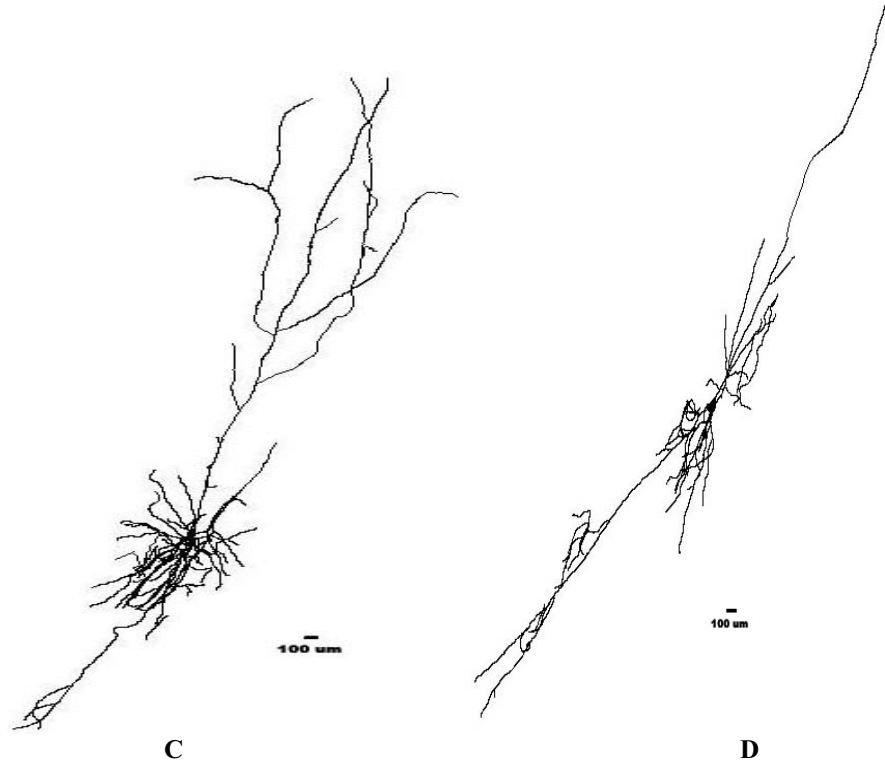


A



B

Figure 3. **A** Confocal micrograph montage of 2 pyramidal shaped cells in layer 6: the same FRB cell (superficial) and a more downfield RS cell as figure 7. **B:** NeuroLucida tracing of the two cells. **C:** NeuroLucida tracing of the FRB cell. **D:** NeuroLucida tracing of the RS cell.



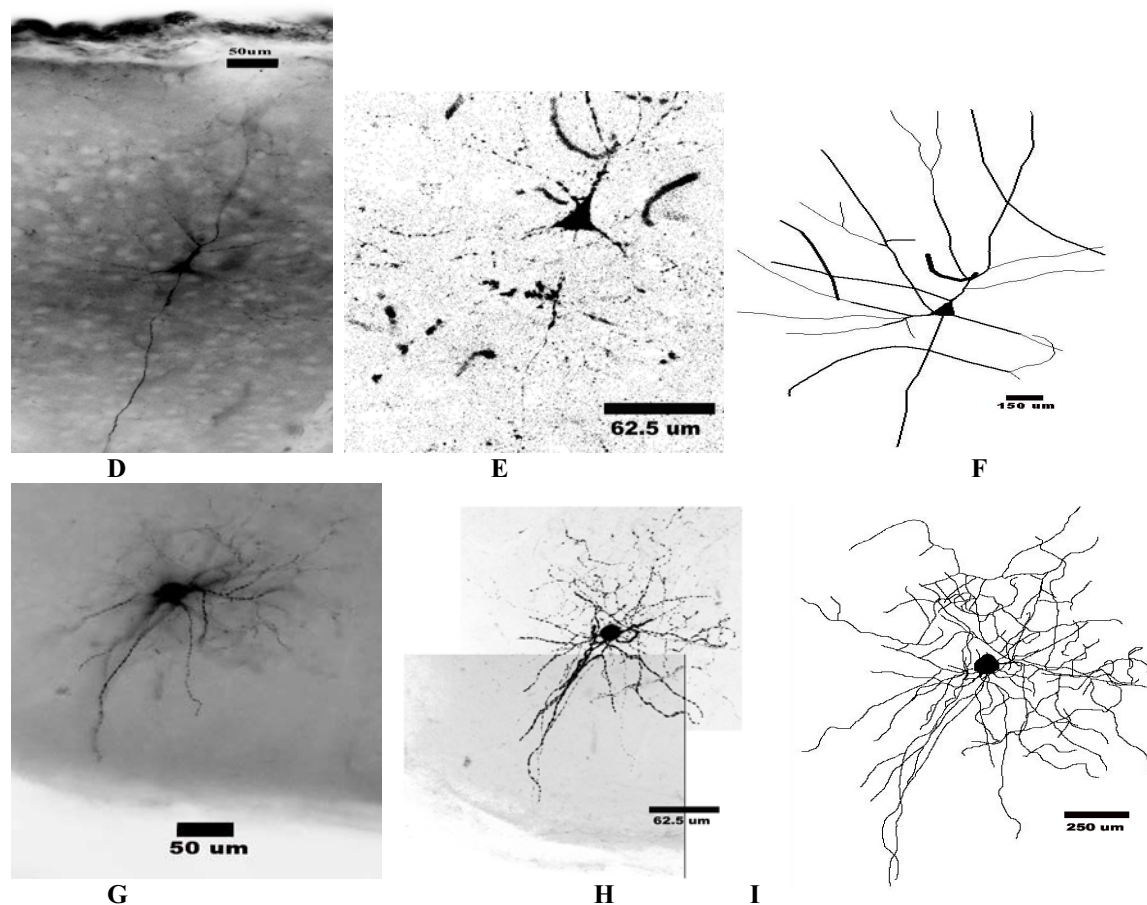


Figure 4: RS2- **A:** Fluorescence micrograph from layer 5 with magnification 40X. **B:** Confocal micrograph montage **C:** Nuerolucida tracing. RS3- **D:** Fluorescence micrograph from layer 3 with magnification 20X. **E:** Confocal micrograph montage **F:** Nuerolucida tracing. Non pyramidal, smooth basket cell with an FS phenotype - **G** Fluorescence micrograph from superficial layer 2 with magnification 20X. **H:** Confocal micrograph montage. **I:** Nuerolucida tracing.

From all cells, 1 non-pyramidal and 4 pyramidal cell (3 regular spiking (RS) cells & 1 FRB cell) were captured in a fluorescence microscope, confocaled, reconstructed with Nuerolucida, and analyzed with NeuroExplorer. Cell types were concluded based on their electrophysiology. (Fig. 2). The branching pattern for all 3 cell types were similar in that most dendritic branching happens near the soma with terminal segments being much longer and smaller in diameter (tapering off) than the intermediate segments (Fig. 1, 3, 4). Bifurcation dendrites have bigger diameters while thin dendrites tend to synapse on thick dendrites. Some of the dendrites overlap each other to maximize signal transduction (Fig. 1B, 3). This implies that each point in the visual field is detected by more than one type of pyramidal cell at any given time.

Depending on the plane where the neurons were cut, some dendrite seem to be different size at different layer. As the cell atrophies after 13 hours of experiment, most dendrites have bulge out segments because the dying cell contracts its membrane during the suffering and cause the dye to concentrate in certain part of dendrite. This artifact was eliminated by tracing the neuron according to their mean diameters (Fig 3, 4). Even though circular circuits seem to be common among the cells, circular pathways are not possible because currents will be running in circles rather than propagating to the axon.

The 4 pyramidal cells (1 FRB & 3 RS) have 6-8 main dendrites arising from one or two poles of their soma, giving them octopus-like appearance (Fig. 1, 3, 4). Because of the close proximity of the FRB and RS cells (Fig 3A, B), the two tracings were manually separated (Fig 3C, D). The two cells seem to synapse morphologically, mostly with dendritic spines (Fig 3A, B). Like a typical pyramidal cell, all FRB and 3 RS cells have their somas in

the lower neocortical layer with their apical dendrites extending at right angles to layer I or II and sent collaterals to layers VI ((Fig. 1, 3, 4). Other the other hand, the interneuron seems to have larger soma and dendrites branching in all directions, like a web, making the tracing much more difficult to follow (Fig. 4G, H, I). The dendrites of the pyramidal cell seem to be more curved compare to the interneuron (Fig. 4).

Spines. The FRB cells appeared to have much more spines than other 4 cells while the non-pyramidal interneuron showed typical aspiny dendritic arbors (Fig 1B). The dentritic spines were observed in thin, stubby, and mushroom shapes. No cup-shaped dentrites was seen. All spines were connected to the neuron by a thin spine neck, and aroused from the soma, dendrites, and the axon hillock of a neuron. The spine density of a neuron differs within its dentritic trees and cortical layer. Because the spines have high dynamics, the observed spines fixtures are mere snapshots of them during their morphological transition in response to the particular visual stimuli at that time.

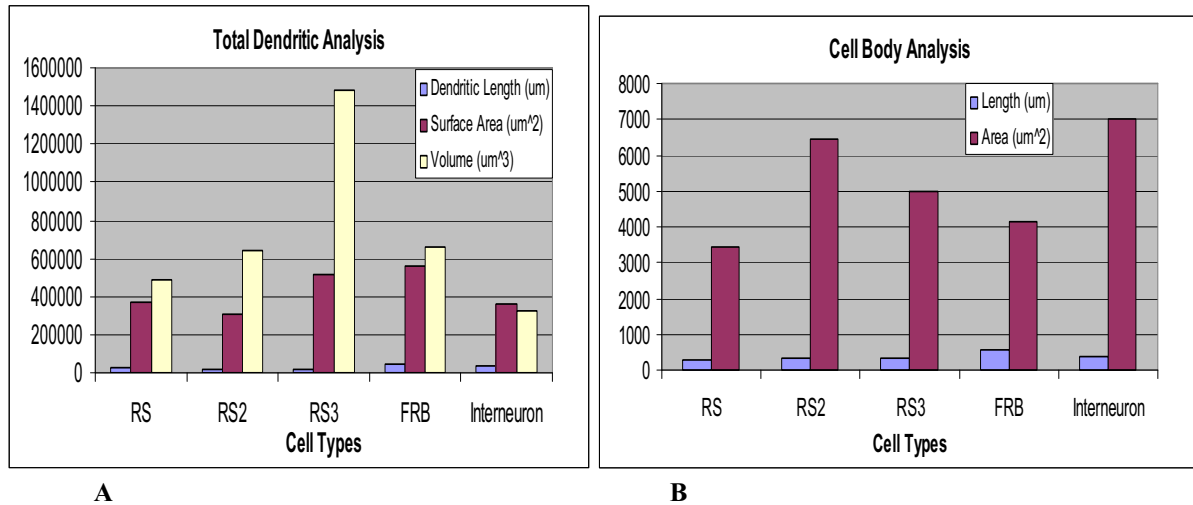
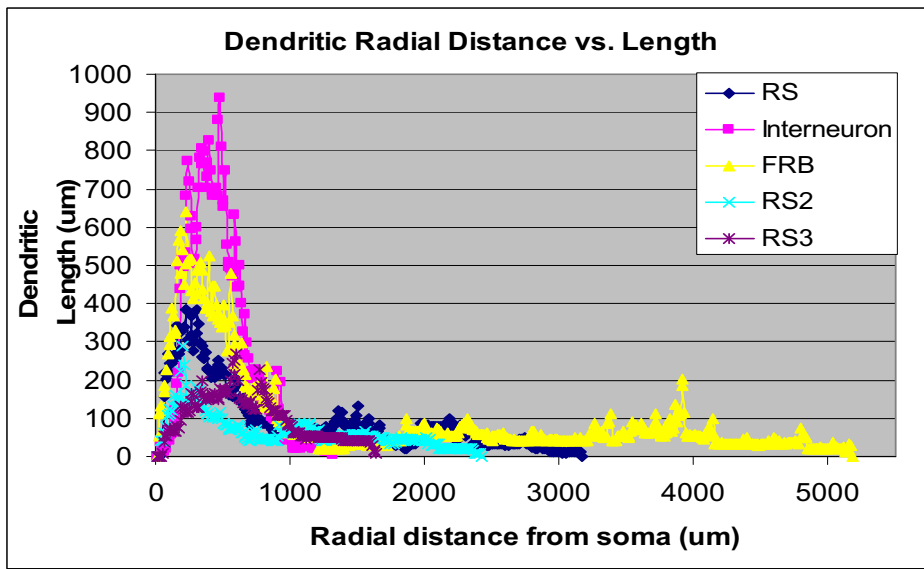


Figure 5: A: Total Dendritic Analysis. B: Cell body analysis.



A

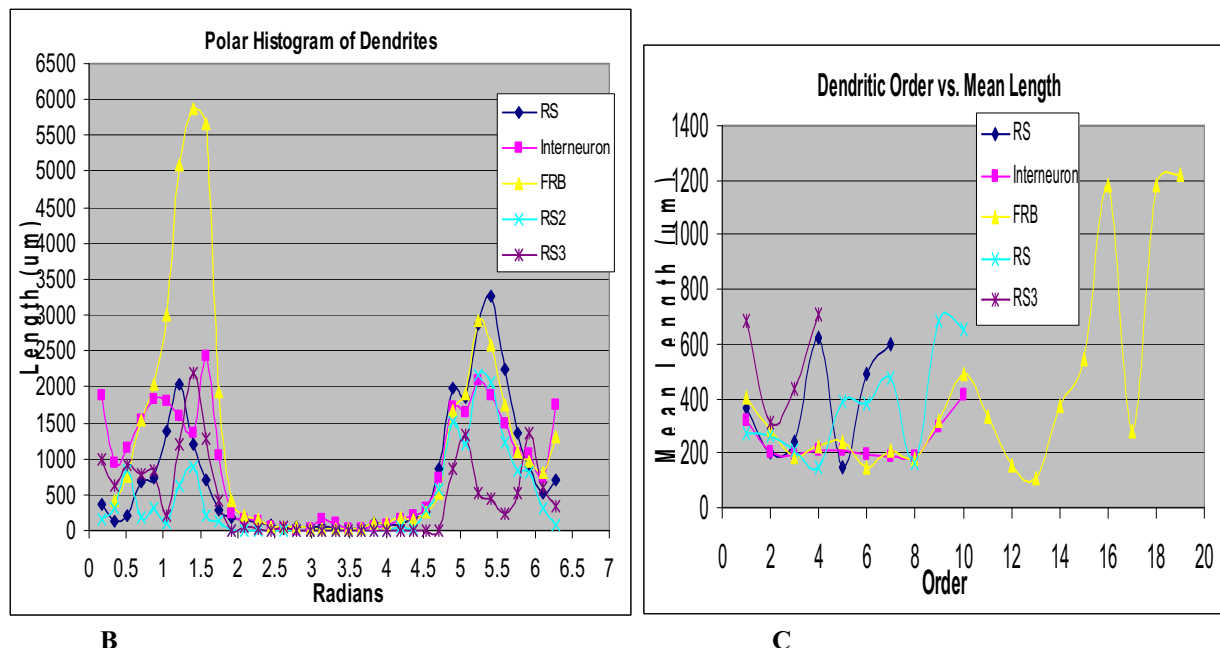
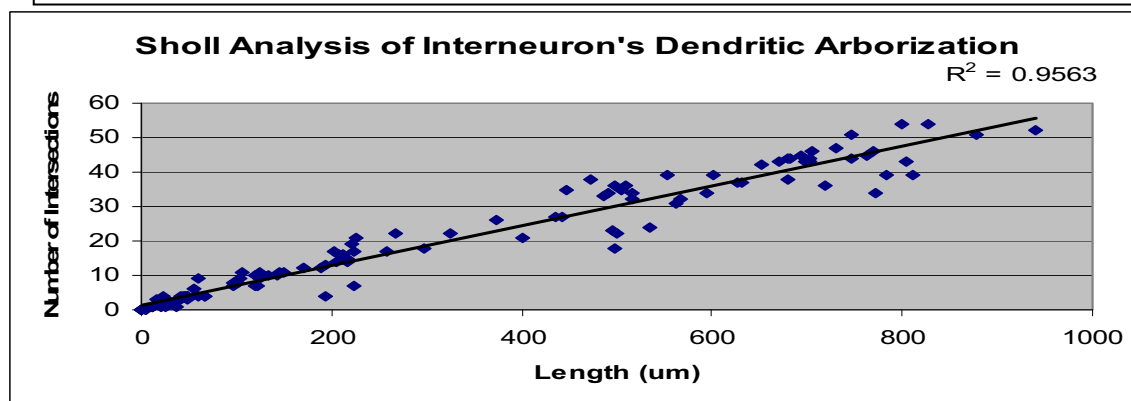


Figure 6: A: Dendritic radial distances vs. length. B: polar histogram comparison of 3 cell type dendrites. C: Dendritic Order vs. Mean Length.

Figure 7: An Example of sholl analysis of dendritic arborization of Interneuron.



Quantifications from the NeuroExplorer software indicated that RS3 has the largest total volume while the FRB cell has the largest total surface area and dendritic length (Fig 5A). All 5 cells displayed similar cell body length with the FRB cell having the longest. the interneuron has the biggest cell body area compared to the rest 4 cells (Fig 5B). All the cells showed a peak dendritic length at 250-500um radial distance from their somas, with the FRB cell having double radial distance than the 3 RS cells, and 5 times the distance of the non-pyramidal cell (Fig 6A). The FRB cell has 3 times longer dendrites compared to other cell types at their peak of 1.5 radians. All 5 cells shows zero length between radian 2 to 4.5 (Fig 6B). The FRB cell also has twice the number of dendritic tree orders (2ary, 3ary and so on branching) and mean length compared to the 4 other cells (Fig 6C). The 3 RS cells seems to have the longest mean length at the dendritic order of 4 and 7 while the interneuron has the longest length at order 10 and the FRB cell at order 16 and 19. All the 4 pyramidal cells has their maximum mean length at one order follow by a drastic drop in the next order and then rise again in the following order. This shows that all 3 cells types have shorter mean dendritic length near their somas to allow for more branching. Sholl analyses of the 5 cells were done where different size spheres spaced at 10- μ m increments were used. The total number of sphere-dendrite intersections was also analyzed at various dendritic lengths from the soma. All 5 cells showed a linear correlation (>0.94) between length and number of intersections, indicating a strikingly regular pattern of ramification as a

function of distance from soma across cell types (Fig 7). Cells with more complete morphology such as RS2, RS3, and the FRB cell showed that the neurons have reached the maximum arborization.

Discussion/Conclusion

In order to validate that most fast rhythmic bursting cells have extensive horizontal axon collaterals that amplify and distribute long distance response synchronization throughout the primary visual cortex in cats, we used various histo-chemical methods and fluorescence microscopy. Even though previous studies have found FRB cells only in superficial cortical layers [12, 14], the simple FRB cell with almost a complete morphology used in this study was found in layer 6. In agreement with previous studies, all cells display typical cell morphology.

By having longer dendritic radial distances from the soma, longer mean dendritic length, and more dendritic tree branching orders, FRB cells could form a horizontal fiber system to amplify and distribute long distance response synchronization throughout the primary visual cortex. The rapid firing within a burst of a FRB cells can lead to greater temporal summation post-synaptically [21, 22] and will significantly increase neurotransmitter release from the presynaptic neurons [21, 23]. More dendritic branches also mean greater opportunities for synapses. This higher dendritic order of the FRB cell enables it to have a greater degree of signal propagation and convergent processing. As a FRB cell projects to other FRB cells [12, 20], the interconnections it forms can further amplify and distribute the rich γ oscillation input throughout the cortex. The horizontal system may link similar functional columns, increase feature binding function and lateral input.

Possible research in the future will be to trace more cells to confirm the current conclusion. Since most synapses occur on the spines, we hope to count the spines to see whether FRB cells have denser spines than other cell types. If better confocal stacks are obtained, we hope to investigate the morphology of axons and the difference between the base and apical oblique dendritic arborizations. We also hope to use the Neuron program to generate computerized data by inserting ion channels into the current model to compare with the electrophysiological data *in vivo*. The computer model will allow to modify parameters that cannot be modified *in vivo* such as cell morphology (dendrites can be made shorter) in order to test more hypothesis.

Even though our knowledge of the primary visual cortex has greatly increased since the last decade, much is still needed to be done. By clarifying the linkage between FRB's morphology and electrophysiology in the cat's visual cortex, this research deepens our understanding of visual processing in mammals. As the FRB cells are the key in generating the synchronous cortical activity, further research is needed in determining FRB's functional consequences in the mammalian primary visual cortex.

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