Overlap among Dendrites in Neuronal Networks Is a Designed Entity onto Which Functional Topology Is Coded

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Abstract. Information processing in the brain is performed by propagating data through an array of neuronal networks, each having unique structural and topological architectures. However, the mechanisms that specify these architectures are not well understood. We found that neuronal networks *in vitro* determine the pattern and strength of their connectivity by designing the way dendrites overlap. The branches of neighboring dendrites converge in a collective and ordered fashion, leading to a network configuration that enables axons to innervate multiple and remote dendrites using short wiring lengths. In addition, the convergence sites are associated with synaptic clusters of higher density and strength than found elsewhere, leading to patchy distribution of synaptic strength in the network. Thus, controlled design of the overlap among dendrites patterns and strengthens neuronal connectivity in neuronal networks.

Keywords: neuronal networks, dendro-dendritic contact, synaptic strength.

1 Introduction

Neurons integrate information through tree-like protrusions extending from their cell body termed dendrites. Dendrite arborization patterns are critical determinants of neural circuit formation and function as they can influence the type and location of inputs a neuron is able to receive, and how these inputs are integrated [1, 2]. The mechanisms that underlie these influences are not clear, but are likely to be found within the context of dendritic morphogenesis.

Dendritic arbor development is a highly dynamic process, characterized by extension, branching and retraction of branches, followed by their stabilization [3-5]. This process is influenced largely by the combined actions of intrinsic signals, guidance cues, and neuronal activity [3, 6, 7]. But, the action of these diffusible cues is too broad to resolve specific tree architectures.

A finer tuning of dendritic morphogenesis *in vivo* occurs through stabilization of dendritic branches through dendrite-dendrite physical interactions [8]. This mechanism has a profound influence on determining the size and shape of the dendritic tree by specifying growth directions and by allowing individual cells to refine dendritic targeting to their appropriate area and ensure appropriate synaptic contacts [9]. Also, the increase in the dendrite-dendrite proximity at the contact area has physiological consequence. When such distances are shorter than a few microns, the current produced by one active branch can spread through the extracellular matrix space to alter the membrane potential of an adjacent branch, potentially causing activity synchronization.

Hence, physical interactions among dendrites play a role in both structure and function of dendrites and may serve as a link between them. Therefore, considering the geometrical map of dendro-dendritic contacts is essential for understanding development and function of neuronal networks.

Based on this conclusion, we raised the following **working hypothesis:**

a. Dendrite-dendrite contacts are allocated in an ordered and controlled fashion. This structure wiring principle leads to development of distinct distribution maps of dendrite-dendrite contacts.
b. Contact maps serve as the template onto which specific topological and synaptic maps are coded.

We found that dendritic branches form stable contacts preferably at bifurcations and at pre-existing contacts on branches of neighboring dendrites in a non-random and activity-promoted fashion [10-12]. This directed growth led to clustering and strengthening of synaptic connections at the contact sites and formation of an Economical Small World network configuration [13], which broadens network connectivity. Hence, this new dendritic behavior shapes and links structure and topology in neuronal networks.

2 Methods

Imaging the Structural Dynamics: The main working system here was cultured neuronal networks, prepared from rat brain hippocampus (an organ related to learning and memory), since in culture dendrites and axons are relatively sparse and their wiring is readily monitored. Neural cells were extracted, plated on a glass dish and allowed to grow and reconnect while being imaged through a phase contrast light microscope.

Imaging Wiring and Synaptic Connectivity: In addition, cultured cells were tagged by fluorescent antibody markers specific for dendrites (anti-MAP2), axons (anti-NFM) and synaptic connections (anti-synaptophysin), and imaged through a fluorescence microscope. The strength of synaptic connections was imaged using the synaptic vesicle recycling fluorescence probe FM1-43. In one set of experiments, cells were labeled by transfection of the green-fluorescent protein cDNA for visualizing inter-dendritic contacts. Several experiments were performed on rat brain tissue sections.

Definition of Dendrite-dendrite Contacts: Contacts were identified using MAP2 images. Contacts of more than two dendritic branches were considered only if the branches were not associated through fasciculation.

Analysis of Network Configuration: Checking for ESWN was performed by manually converting MAP2 images into a graph, using MATLAB.

3 Results

3.1 Ordered Dendrite-Dendrite Interactions That Shape Network Structure

We first defined three basic structural components (Fig. 1):

Dendritic segments – sections of dendritic branches, spanning between two branch points or between a branch point and dendrite endings
Dendritic bifurcations – sites where a dendritic segment splits into two daughter segments
Dendrite-dendrite intersection (or contact) – a single point of overlap between two dendritic segments or between a segment and a bifurcation

These three components interacted in various manners forming three contact motifs (see Fig. 1):

Structural motif 1 [10]: ***directed construction of multi-dendritic intersections*** **(MDIs):** Dendritic branches grow directly toward pre-existing intersections between other branches and cross them, forming multi-dendrite intersections (Fig. 1, see also Fig. 2A1-A4). Such directed growth could begin dozens of microns away from the intersection.

Structural motif 2 [11]: ***directed crossing of dendritic bifurcations:*** dendro-dendritic contacts occur frequently between dendritic branches at sites of bifurcations. We termed the new structure **bifurcation dendrite intersection (BDI).**

Structural motif 3 [12]. ***collective branch convergence:*** Time lapse recordings of cultures at different ages revealed massive convergence of dendritic branches, either by the growth of processes towards preexisting contact sites between other processes or by the lateral movement of several processes towards a single area (see Fig. 2b1-b3). Such behavior resulted in the formation of clusters, several microns in width, comprising contact sites of multiple processes. We termed these structures **dendrite-dendrite contact clusters (DCCs)**.

Motif stability: How stable are the structural motifs? We performed time lapse experiments over 7 days, which revealed that many of the motifs were stable throughout the experiment duration (Figs. 2C, 2D). We also found that rates of formation and dissolution of the motifs were approximately equal and constant, keeping the overall motifs density per cell constant during the entire experiment. Thus, dendrites seem to form stable and long lasting contacts at the above structural motifs.

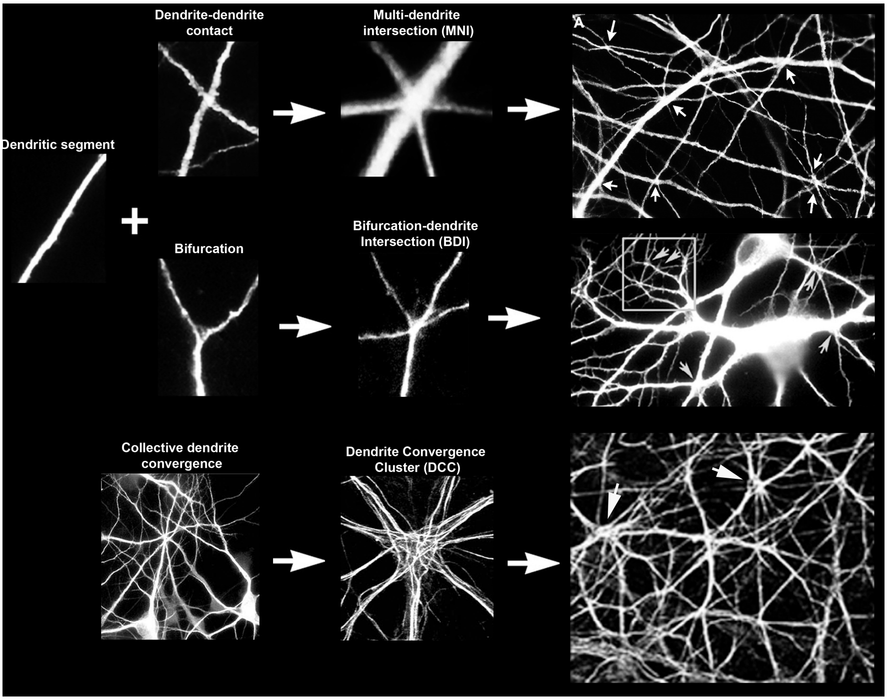

Fig. 1. MDIs, BDIs and DCCs — novel structural motifs of dendro-dendritic contact: All images are of MAP2 labeled 12 days old cultures of hippocampal neurons. (Upper two rows) Contact between three elementary dendritic structural units, segments, dendrite-dendrite contact (or intersection) and bifurcations produces MDIs and BDIs at high frequencies (arrows, upper right and middle right). To be considered part of a contact structure, each dendrite must be distinct and not arrive at the contact structure by fasciculation. (Bottom row) dendritic branches converge, producing DCCs. DCCs are frequent and when linked to each other produce ordered network (bottom right).

Evidence for non-randomness in the formation of the above motifs

High frequency of occurrence: The directed growth of dendritic branches toward the site of the motif construction (Fig. 2A1-A4) led us to assume that the motifs are formed non-randomly. A support for a directed formation came from the finding that the frequency of the motifs in the real network surpassed that found in simulations of random neuron distribution (Fig. 2E).

Motifs construction involves non-self recognition: We found that the occurrence of MDIs and BDIs between dendritic branches of different neurons was significantly higher than within single dendritic trees (Fig. 2F). Thus, neurons employ a mechanism of non-self recognition to construct hetero-cellular structural motifs among their dendrites.

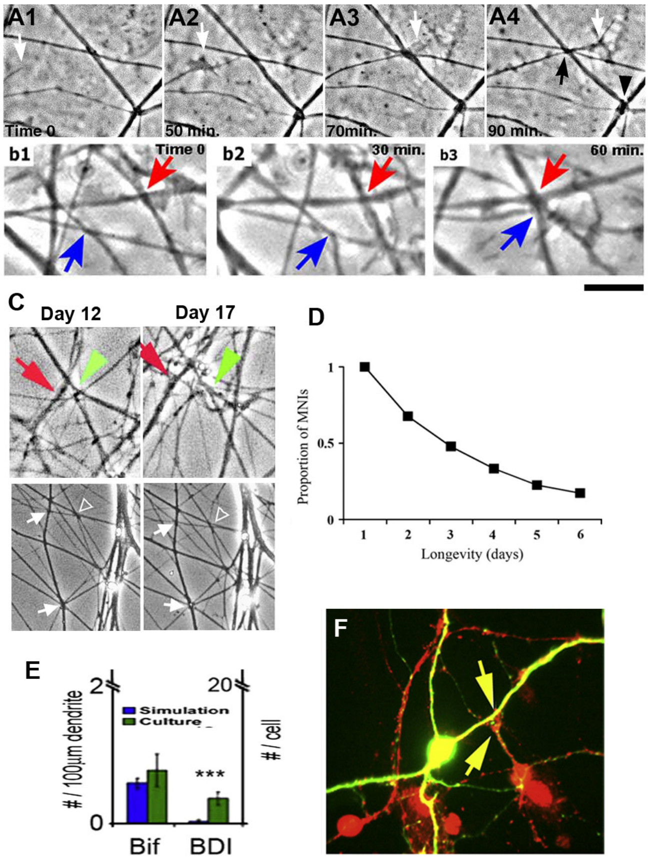

Fig. 2. MDIs, BDIs and DCCs are stable, hetero-cellular non-random entities: (A1-A4) growth of neuronal processes toward pre-existing intersections is directed (white arrows). (B1--3) Lateral movements of intersections produce sites of convergence. (C) Example of the dynamic character of the network structure. Upper two panels show contacts configuration disabled after 5 days. Bottom panels show a stable configuration (white arrows – stable, arrow-head –dismantled). (D) Longevity distribution of contacts made by three processes, as an example. Note that 20% lasted more than 6 days. (E) Neuronal cultures exhibit significantly higher level of BDIs per dendritic length compared to that found in simulations of random dendritic distribution. (F) BDI preferably form by the interaction of dendritic branches of two different cells (arrows). (Yellow – a combined MAP2 and GFP staining, red – MAP2). Scale bar: A-C – 15μm; F – 25μm.

The role of the motifs in the design of dendritic and network structures

The three motifs are expected to affect the morphology of single dendritic trees and the network as follows:

a. The growth of dendritic branches toward the motif sites shapes dendritic trees by affecting the growth direction and branch length (Figs. 1, 2).

b. From the second week in culture on, most dendritic branches were involved in at least one motif and many were involved in more than one (Figs. 1, 2), suggesting that the motifs are frequent enough to affect the structure of entire dendritic network.
c. The 'non-self' manner by which dendritic branches contract the motifs indicates that sister branches undergo 'self avoidance', and that by preferentially associating with non-sister branches they highly increase the overlap among different dendritic trees.

Studies describing dendritic morphology based on analysis of single dendritic trees often have led to the conclusion that dendritic ramification is random and that the growth directionality is unbiased toward specific targets. We present here a different explanation for dendritic tree morphogenesis, where the interaction of a tree with other trees is a major player in the design of the final dendritic morphology.

According to our model, the growth of dendritic branches is preferentially directed toward areas of high dendritic proximity and to sites of bifurcation and intersection to form MDIs, BDIs and DCCs. Thus, the development of particular dendritic tree architectures can be predicted by considering the distribution and density of DCs around the growing trees. By the same token, the morphology of entire networks of dendritic trees can be described by considering the number, location and size of their DCs, bundles and DCCs. Thus, studying dendritic proximity maps may enable us to proceed beyond the structure of individual dendritic arbors to that of full dendritic networks.

3.2 Evidence for a Role of the Motifs in Network Functional Connectivty

Dendrite-dendrite contacts and their structural motifs influence the growth pattern of axons, their choice of targets and the efficiency of connectivity in the entire network in the following ways:

Motifs are preferable crossing sites for axons: The tendency to prefer dendritic intersections as a contacting target appeared also in axons. Many axonal edges directed their growth toward the center of intersections and crossed them (Fig. 3a), developing their structure according to the distribution of the surrounding intersections (Fig. 3b). This type of growth also leads axons to select specific dendritic targets, namely those located at the crossed intersections.

Motifs facilitate target switching by axons: Many of the axons fasciculate with dendrites and follow their path, but frequently when reaching an intersection they turn and switch dendrites (Fig. 3C1-C3). At DCCs, due to the high proximity among targets, only a few microns of growth suffice for axons to switch between many targets (up to several dozen, depending on the DCC size) (Figs. 3D-3F). This high targets/axon ratio means that single neurons would connect to a higher number of neurons in the network than would be the case in non-aggregated networks. The outcome of this wiring mechanism may be an all-to-all connectivity.

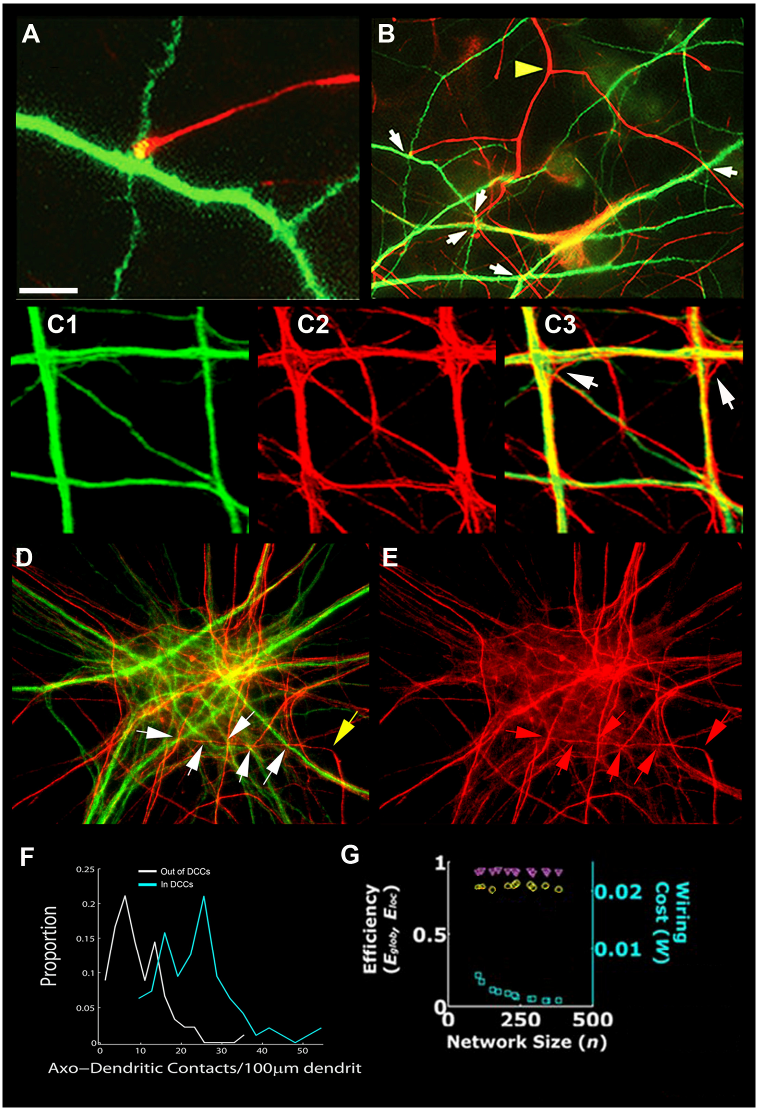

Fig. 3. MDIs, BDIs, and DCCs shape axonal wiring and increase network connectivity: In all images, red=axons, green=dendrites. (A) A portion of the axons grow directly toward dendrite-dendrite contacts. Shown is an axonal growth cone approaching such a contact. (B) An axon shaping its structure by crossing five dendrite-dendrite contacts. (C1-C3) An ordered growth of dendrites (C1) leads to organized axonal growth (C2, same area as C1), as many of axons fasciculate with the dendrites and follow their paths. Several axons turn at intersections and switch targets (arrows in C3, a merger of C1 and C2). (D, E) A large DCC in which axons turn (an example pointed at by a yellow arrow), and form a complex mesh (see only axons in (E)). The turning axons make contact with several different dendritic branches at relatively short lengths (white arrows in (D)). (F) Quantification showing a shift to the right in the number of axo-dendritic contacts per axonal length at in vs. outside DCCs. Scale bar: (A-C) - 10μm; (D, E) – 20μm.

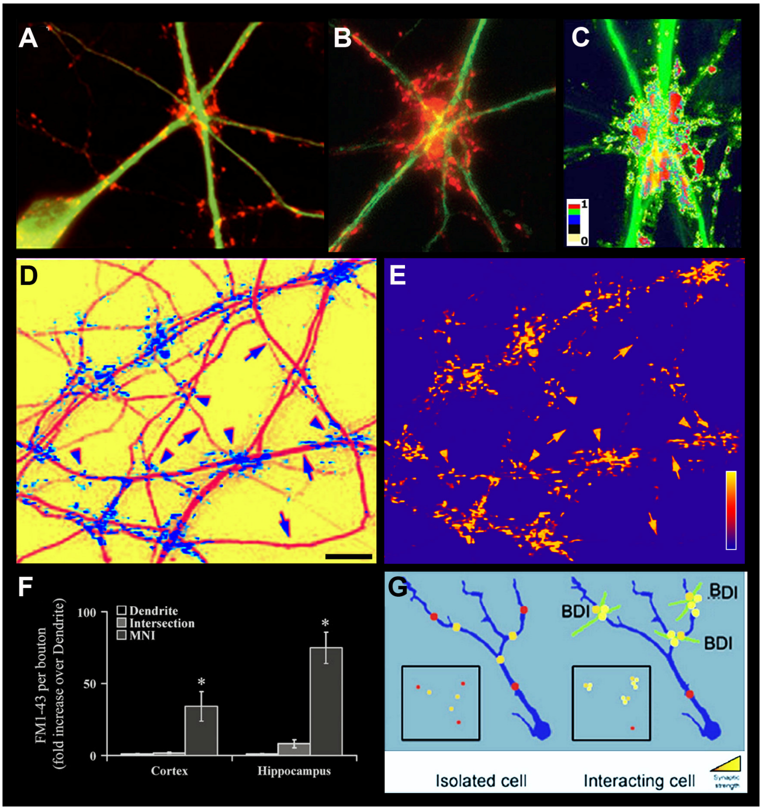

Fig. 4. MDIs, BDIs and DCCs lead to clustering and strengthening of synaptic connectivity: (A, B) At the contact site among dendritic branches (green), the density of synaptic connections (red, anti-synaptophysin) is higher than along non-crossing regions, and the size of the connections increases (B). (C) A DCC in which the strength of synaptic connections (secretion level by FM1-43) is higher than elsewhere. (D) Due to the synaptic enrichment at contacts, the map of denditic contacts and motifs (red) dictates a patchy distribution of synaptic connections in the network. (E) A look up table of the synaptic image in (D) showing that synaptic connections of the highest strength are located in hubs of DCCs. (F) Synaptic strength per connection increases with increased number of dendritic branches participating in the studied motif. (G) Dendro-dendritic intersections cause a patchy distribution of synaptic connections and synaptic strength along the dendritic arbor. Scale bar: (A-C) 10µm; (D, E) 15µm.

Ordered dendrite overlap increases efficiency of connectivity [12]: In relating to dendritic proximity by describing dendritic networks as graph of connections among dendrite-dendrite contacts we were able to show that in culture, such networks assemble into ESWN configurations (Fig. 3G). The main anatomical consideration of such a configuration is that a dendritic network exhibits 'shortcuts' that connect distant dendrites. Such an arrangement would have significant implications for axonal directionality and patterning, as many of the axons fasciculate with dendrites and follow their tracks (Fig. 3C1-C3). This means that if axons have access to 'shortcuts', their chances of innervating distant dendrites are increased, enhancing the connectivity of the entire network.

3.3 A Role for the Structural Motifs in Network Activity

Causing synaptic clustering: It was found that synaptic connections assembled into clusters at dendrite-dendrite contacts, and the synaptic density of such clusters was further enhanced in MDIs, BDIs (Figs. 4A, 4B) and DCCs (Figs. 4C-4E). Hence, synaptic density is elevated in the presence of dendritic contacts and structural motifs.

Leading to synaptic strengthening: Using a fluorescent probe for the strength of synaptic secretion (see methods), we found that synaptic connections accumulating at dendrite-dendrite contacts were of higher strength than found elsewhere (Figs. 4C-4E). Moreover, the increase in synaptic strength was proportional to the number of intersecting dendrites in the motifs (Fig. 4F). Eventually, the presence of the structural motifs led to increase in synaptic strength in the network and produced patchiness in it distribution (Figs. 4D, 4G).

Motifs formation and synaptic clustering are regulated by the network activity: The density of motifs and clustering of synaptic connections were reduced in the presence of inhibitors of synaptic activity. Thus, ordered contacts, synaptic clustering and strengthening are all activity-dependent.

4 Discussion

Our work demonstrates that interactions of a dendritic tree with its dendritic neighbors are non-random and therefore should be included when attempting to model or explain dendritic trees morphogenesis. Our results imply that the pattern of branching in a dendritic tree is related to the pattern of contacts that this tree makes with adjacent trees of other neurons. A broader consequence of such a relation is that structural modification of a particular tree, due to growth or retraction, may be propagated to other trees and alter their structure via generation and disassembly of MDIs, BDIs and DCCs. Hence, the dynamic ramification of single and network of dendrites can be better understood by considering the pattern of their branching and hetero-neuronal contacts.

We conclude that the proximity among dendritic branches of neighboring neurons is a functional structural entity. Being upregulated by synaptic activity and associated with enrichment in synaptic density and strength, dendritic proximity affects the conversion of synaptic information into a map of synaptic connections and synaptic strength distributions (see fig. 5). Accordingly, when neuronal network activity increases, the network architecture becomes more aggregated through DCC and bundle formation, leading to an increase in synaptic clustering and strength. This structure-mediated, activity-dependent synaptic strengthening may serve as a novel structural-based mechanism of plasticity.

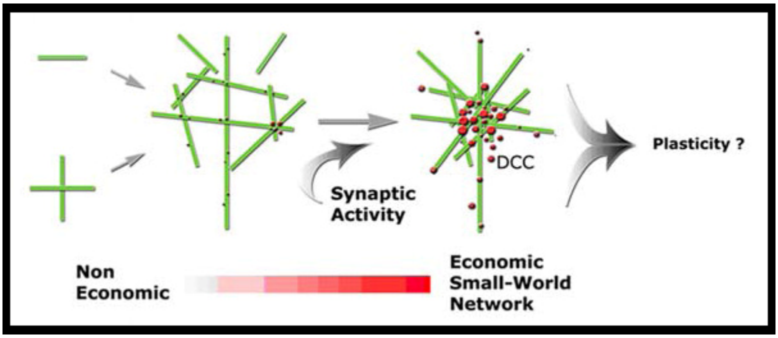

Fig. 5. Conceptual model of network structure-based data consolidation. Green rods - dendrites, Red circles - synaptic clusters (darker colors refer to higher density and synaptic strength). For simplicity, axons are omitted from the model. When single or intersecting dendritic branches converge, DCC are formed and the network becomes more aggregated. This process is promoted by synaptic activity. Synaptic clustering and strengthening becomes prominent at the DCCs. The geometric architecture of the dendritic network results in an Economic Small-World organization, a scenario that increases network connectivity and it produces local enhancement in synaptic strength. It therefore may serve as a new mechanism of synaptic plasticity.

Acknowledgment. I thank Drs. Pablo Blinder, Joshua Cove, Elia Abi-Jaoude and Miriam Lafreniere-Roula and Professor Luc Devroye for participating in this research.

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