Carmichael’s Concise Review

Localizing Channels for Learning

Stephen W. Carmichael
Mayo Clinic, Rochester, MN 55905
carmichael.stephen@mayo.edu

The most widely accepted model of learning at the level of the cell involves associative synaptic plasticity in brain neurons that include cortical pyramidal cells (PC). To oversimplify, for a neuron to “learn” from information coming into it, electrical events need to occur in a fairly precise pattern throughout the cell membrane.

An essential prerequisite of this model of learning at the cellular level is that input synapses that are distributed over a very large dendritic tree must be capable of sensing the precise timing of the output signal. In order to coordinate these events, it is thought that fast sodium action potentials (APs) are initiated at the axon initial segment (AIS) and propagated back through the body (soma) into the dendrites of the neuron. Physiological studies have detected voltage-gated Na⁺ (Nav) currents in PCs and other neurons. Nav currents differ in their activation and inactivation properties, which suggests that the possibility that different Nav subunits may be involved. Andrea Lorincz and Zoltan Nusser have combined immunofluorescent techniques with a very sensitive electron microscopic immunocytochemical technique to demonstrate the unequal distribution of one Nav subunit among different parts of the PC [1].

To increase the sensitivity of the immunofluorescent techniques, Lorincz and Nusser chemically fixed rat brain samples in an acidic medium, resulting in better preservation of the immunogenicity of certain receptors and ion channels. Whereas immunolabeling for Navs was stronger in the AIS and nodes of Ranvier, some signal could be detected in the dendritic tree. However, it was not possible to determine the subunit composition of the Navs or their precise localization.

To examine the subunit composition of the Navs, they used specific antibodies against different Nav subunits, including a subunit referred to as Nav 1.6. After confirming the specificity of the immunoreactions, they demonstrated the distribution of the Nav subunits in various parts of the neuron. Of particular interest was the strong signal for Nav 1.6 at the AIS and nodes of Ranvier, and also the weak signal from apical dendrites, which had no signal for the other Nav subunits.

To more precisely localize Nav 1.6 subunits on dendrites, Lorincz and Nusser used a very sensitive, quantitative electron microscopic immunogold method known as SDS-digested freeze-fracture replica-labeling (SDS-FRL). Under optimal circumstances, this method is thought to have a labeling efficiency close to 100%, which means every protein, on average, is represented by a gold particle. The spatial resolution is about 25 nm. As expected, the AISs and nodes of Ranvier were intensely labeled. Relatively low densities of gold particles could be found along dendritic shaft membranes, but not on dendritic spines (see Figure 1). After accounting for non-specific labeling, gold particles were displayed at a significantly higher density on the neuronal cell bodies, and proximal apical, proximal oblique, and distal apical dendrites. Thus, for the first time, the exceptionally high sensitivity of the SDS-FRL method revealed low, but significant, densities of the Nav 1.6 subunit in these dendritic compartments. The much higher density (about 40-fold) of labeling on AISs is consistent with earlier physiologic studies.

Lorincz and Nusser have convincingly demonstrated that Nav 1.6 is the main Nav subunit in the somatodendritic compartments of PCs in the rat hippocampus. Whereas there may be other factors involved, these data support the view that the distribution of Navs, particularly the Nav 1.6 subunit, plays an important role in learning at the neuronal level [2].

References
[2] The author gratefully acknowledges Dr. Zoltan Nusser for reviewing this article.
More than 160 years of experience in optics has laid the foundation for pioneering electron and ion beam microscopes from Carl Zeiss. Superior integration of imaging and analytical capabilities provides information beyond resolution, unlocking the best kept secrets of your sample. Our mission at all times: Maximum Information – Maximum Insight.

**SEM**  
EVO® MA and LS Series

**FE-SEM**  
MERLIN®  
SUPRA® Series  
ULTRA Series  
ΣIGMA™ Series

**TEM**  
LIBRA® 200 Series  
LIBRA® 120

**CrossBeam® FIB-SEM**  
AURIGA®  
NVision 40

**HIM**  
ORION® PLUS

---

Carl Zeiss NTS  
One Corporation Way  
Peabody, MA 01960  
USA  
Tel. +1 978 / 826 1500  
info-usa@smt.zeiss.com  
www.zeiss.com/nts