ω-Agatoxin IV A (AgaIVA), a peptidyl toxin from Agelenopsis sperta venom, specifically binds to P/Q-type calcium channels. Pharmacological and electrophysiological studies showed that AgaIVA-sensitive channels are widely distributed in both the central nervous system and in neuromuscular junctions. Using biotin-conjugated AgaIVA, it was possible to determine which cells in freshly prepared mouse cerebellar and hippocampal slices possess binding sites for this toxin (Nakanishi, S. et al., 1995). Biotinylated AgaIVA was also applied to transcardially fixed brain slices prepared with various fixatives (4 % paraformaldehyde with 0.1 % glutaraldehyde, Zamboni's fixative, and acrolein). AgaIVA did not bind to fixed tissues from P/Q-type calcium channel knockout mice, confirming that the binding to normal, fixed tissues was not an antifact. With transmission electron microscopy, it was shown that the toxin also binds to fixed tissue. Using immunoelectron microscopy, the locations of biotinylated AgaIVA binding sites were compared to those of binding sites identified with an antibody specific for the α-1A subunit of P/Q-type voltage-gated calcium channels. Biotinylated AgaIVA binding sites visualized with FluoroNanogold-streptavidin showed a similar pattern to those visualized with antibody. This ability of biotinylated AgaIVA to bind to fixed tissue provides a new tool to study the molecular architecture of excitatory synapses. Reference: Nakanishi, S. et al., (1995) J Neurosci Res 41: 532-539

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Fig. 1: Biotinylated AgaIVA bound to chemically fixed P/Q-type calcium channels in the medial nucleus of the trapezoid body (left), but without toxin no binding was seen (middle). Immunohistochemical examination using an antibody to recombinant protein (aa856-888) showed similar results (right).

Fig. 2: P/Q-type calcium channels were visualized on the presynaptic membrane (black arrow heads) with peptide-binding cytochemistry (left) and with immunoelectron microscopy (right). Tissues shown in Fig.1 were reprocessed for TEM. Bars, 50 nm