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DYNAMIC, EXPERIENCE-DEPENDENT MODULATION OF SYNAPTIC ZINC WITHIN THE EXCITATORY SYNAPSES OF THE MOUSE BARREL CORTEX

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Abstract—Increasing evidence suggests that synaptic zinc, found within the axon terminals of a subset of glutamatergic neurons in the cerebral cortex, is intricately involved in cortical plasticity. Using the vibrissae/barrel cortex model of cortical plasticity, we have previously shown manipulations of sensory input leads to rapid changes in synaptic zinc levels within the corresponding regions of the somatotopic map in the cortex. Here, using electron microscopy, we show how some of these changes are mediated at the synaptic level. We found that the density of zincergic synapses increased significantly in layers II/III, IV, and V. In layers IV and V, this change occurred in the absence of a significant increase in excitatory synapse density, which seems to indicate that excitatory synapses, which previously did not contain synaptic zinc, begin to newly house zinc within its synaptic vesicles. Our results show that excitatory neurons can dynamically change the phenotype of the vesicular content of their synapses in response to changes in sensory input. Given the range of modulatory effects zinc can have on neurotransmission, such a change in the complement of vesicular contents presumably allow these neurons to utilize synaptic zinc to facilitate plasticity. Thus, our results further support the role of zinc as an active participant in the processes contributing to experience-dependent cortical plasticity.

Key words: barrel cortex, cortical plasticity, ultrastructure, whisker, zinc.

Cortical plasticity is mediated by a milieu of factors working in concert with one another. The divalent cation zinc, found within the synaptic vesicles in the axon terminals of a subset of glutamatergic neurons (Frederickson, 1989; Beaulieu et al., 1992), is one such factor. Evidence indicative of a role for synaptic zinc in cortical plasticity is robust (Nakashima and Dyck, 2009). Synaptic zinc is released in an activity- and calcium-dependent manner (Assaf and Chung, 1984; Howell et al., 1984). At the postsynaptic neuron, zinc can exert effects on a number of neurotransmitter receptors and modulate signaling cascades within the neuron itself (Westbrook and Mayer, 1987; Paoletti et al., 1997; Huang et al., 2008). Long-term potentiation and depression (long-term potentiation (LTP) and long-term depression (LTD), respectively), believed to be the cellular substrates of plasticity, can be powerfully modulated by zinc (Izumi et al., 2006; Huang et al., 2008).

The role of synaptic zinc in cortical plasticity is also supported by *in vivo* studies. Modifications of sensory input, which induces plasticity in cortical sensory maps, are accompanied by alterations in synaptic zinc levels. In the visual cortex, acute monocular deprivation leads to an increase in synaptic zinc levels within the ocular dominance columns of the deprived eye (Dyck et al., 2003). In the barrel cortex of rodents, depriving input by plucking vibrissae leads to an increase in synaptic zinc levels within the corresponding barrels in the cortex (Fig. 1A), while stimulation of vibrissae results in a decrease (Brown and Dyck, 2002, 2005). These changes are age-dependent, reflecting the decline in plasticity that occurs with aging (Brown and Dyck, 2003). Conversely, housing in enriched environments, a manipulation that robustly affects cortical plasticity, leads to an increase in this zincergic response (Nakashima and Dyck, 2008).

These alterations in synaptic zinc levels are presumed to contribute to the plastic changes that occur with sensory manipulations. Determining a locus for the experience-dependent alterations of synaptic zinc is important for assessing how these changes could affect cortical plasticity. Therefore, using electron microscopy, we assessed whether the experience-dependent regulation of synaptic zinc levels could occur as a result of a change in the number of zincergic synapses. Additionally, experience-dependent changes in synaptic zinc levels are most robustly observed in layer IV at the light microscopy level (Land and Akhtar, 1999; Quaye et al., 1999; Czupryn and Skangiel-Kramska, 2001). Given that adult cortical plasticity is more commonly observed in layers II/III and V (Fox, 2002), it was of interest to determine whether the experience-dependent regulation of synaptic zinc occurs in these layers as well.

EXPERIMENTAL PROCEDURES

Animals and tissue preparation

All procedures were authorized by the Animal Care Committee of the University of Calgary and mice were handled in accordance with the guidelines dictated by the Canadian Council for Animal

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Abbreviations: E.M.S., Electron Microscopy Sciences; LTD, long-term depression; LTP, long-term potentiation; NMDA, *N*-methyl-D-aspartic acid.

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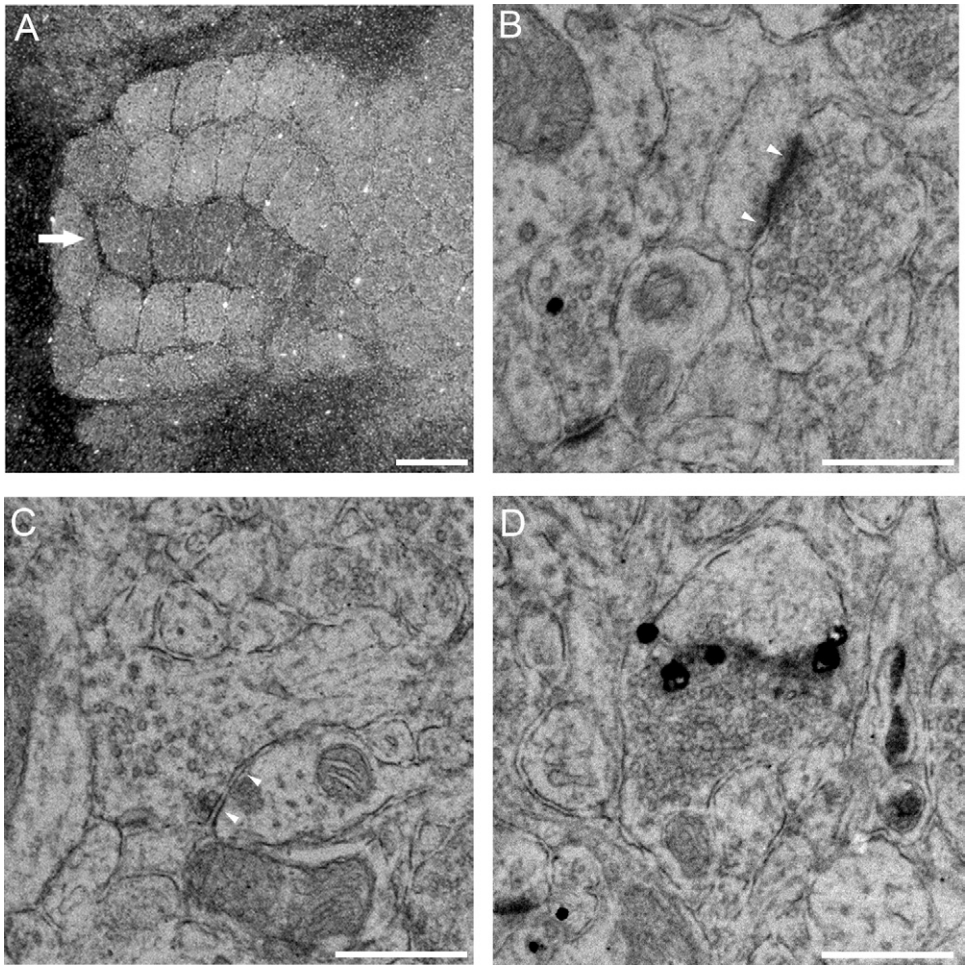

Fig. 1. Summary photomicrographs. (A) Removal of the c-row of vibrissae leads to an increase in vesicular zinc staining within the corresponding barrels (arrow) of the primary somatosensory cortex, as imaged by light microscopy. (B) Example of an asymmetric synapse (arrowheads) consisting of an electron dense postsynaptic membrane and round vesicles. A zinc-containing vesicle, as visualized by a silver precipitate, can be observed within a second synapse. (C) Example of a symmetric synapse (arrowheads) with even pre- and postsynaptic membranes and flattened vesicles. (D) Example of a synapse containing vesicular zinc, separate silver precipitates can be observed indicating the presence of several vesicles containing zinc. Scale bar for (A) is 500 μm, for (B–D) it is 500 nm.

Care. Mice were provided food and water *ad libitum* and were kept in standard laboratory housing under a 12-h light/dark cycle for the duration of the experiment.

Three male C57Bl/6 mice, 1 month of age, were used for this study. All mystacial vibrissae were pluck unilaterally under light isoflurane anesthesia, with care taken to not damage the follicle. The mice were prepared for the histochemical staining of synaptic zinc via autometallography through the injection of sodium selenite (15 mg/kg, i.p.; Sigma, ON, Canada) 48 h later. After 1 h, the mice were anesthetized with sodium pentobarbital, exsanguinated with a solution of heparin (150 U/mL; Sigma) dissolved in Sorenson's buffer, and then perfused and fixed using a 3% glutaraldehyde solution (Electron Microscopy Sciences; E.M.S., PA, USA). The brains were then removed and post-fixed in the 3% glutaraldehyde solution for 1 h. Using a vibratome (Leica VT100S, ON, Canada), 90 μm coronal sections were cut. Sections containing the posteromedial barrel subfield were stained for synaptic zinc using the R-Gent SE-LM Silver Enhancement kit (E.M.S.). After 35 min, sections were removed from the staining solution and rinsed 3×5 min in a 2.5% glutaraldehyde (E.M.S.), 0.1 M cacodylate buffer solution (E.M.S.).

Using a dissecting microscope, barrels were identified in the sections. Tissue blocks were carefully removed which contained entire barrels and the cortical layers above and below them. Blocks were cut from both the deprived and undeprived hemispheres to allow for comparison.

The tissue blocks were left overnight at 4 °C in the 2.5% glutaraldehyde, 0.1 M cacodylate buffer solution (E.M.S.). The sections were then stained in a 1% Osmium Tetraoxide (E.M.S.), 0.1 M cacodylate buffer solution for 1 h, after which they were rinsed in distilled water (2×5 min). The tissue blocks were then dehydrated in an ascending series of ethanol (50% 10 min, 70% 2×10 min, 90% 2×10 min, 95% 2×10 min, 100% 3×10 min). Following this, the tissue blocks were infiltrated with EMbed-812 resin (E.M.S.) by immersing the blocks in a 1:3 (resin:100% ethanol) solution for 1 h, a 1:1 solution for 1 h, and a 3:1 solution overnight. This was followed by 1 h in 100% resin and after replacing the solution with freshly made 100% resin, overnight at room temperature. After placing the tissue blocks in BEEM capsules filled with fresh 100% resin, polymerization was achieved by incubating the blocks at 60 °C for 24 h.

Semi-thin sections were cut and stained with Toludine Blue (E.M.S.) to allow for the precise localization of the barrel and the infra- and supragranular layers in the same vertical plane. Serial ultrathin sections (70 nm; 20–25 sections) were then cut using an ultramicrotome and collected on fomvar coated slot grids. Grids