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Zinc-enriched amygdalo- and hippocampo-cortical connections to the inferotemporal cortices in macaque monkey

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Abstract

Synaptic zinc (Zn), a co-factor in some glutamatergic synapses, has been implicated in plasticity effects, as well as in several excitotoxic and other pathophysiological conditions. In this study, we provide information about the distribution of Zn in inferotemporal cortex, a region at the interface of the visual and hippocampal networks. In brief, we found a lateral to medial increase in Zn, where TEad, a unimodal visual area, showed low levels of Zn; TEav, intermediate levels; and perirhinal cortex, a multimodal limbic area, high levels. The distribution of parvalbumin, a calcium binding protein, showed a reverse gradient to that of Zn. The neurons of origin of the Zn+ termination were identified by making intracortical injections sodium selenite (Na_2SeO_3). This substance interacts with Zn to form precipitates of ZnSe and in this form is transported retrogradely to the soma. A mixed population of labeled neurons was visualized, which included Zn+ neurons in CA1 of the hippocampus and in several amygdala subnuclei. In CA1, Zn+ neurons were restricted to the upper part of stratum pyramidale. Zn is thought to contribute to activity-dependent synaptic plasticity. The specifically high level in perirhinal cortex, and its origin from neurons in CA1 and the amygdala, may relate to cellular events involved in visual long-term memory formation.

Keywords: Parvalbumin; Gradient; Plasticity; Limbic; Theta rhythm

1. Introduction

Zinc-positive (Zn+) terminations occur widely throughout cortical areas, as has been shown in both rodent (Garrett and Slomianka, 1992; Perez-Clausell, 1996) and primates (Carmichael and Price, 1994; Franco-Pons et al., 2000; Ichinohe and Rockland, 2004). In the monkey, they are particularly dense in orbitofrontal and parahippocampal regions, and in much of the pre-Rolandic frontal cortex. In these and several other areas, a further specialization is seen in the form of distinct patches or honeycomb at the border of layers 1 and 2 (Ichinohe and Rockland, 2003, 2004). Variations in distribution of this Zn+ system are potentially important (1) as a marker of area or regional boundaries, and (2) as a clue to functional specialization. That is, Zn is believed to act in a neuromodulatory role, with significant influence on plasticity effects. It is released in an activity- and calcium-dependent fashion, and interacts with many

Abbreviations: AAA, anterior amygdaloid area; AB, accessory basal nucleus; ABmc, accessory basal nucleus, magnocellular division; ABpc, accessory basal nucleus, parvicellular division; ACo, anterior cortical nucleus; AHi, amygdalohippocampal area; amts, anterior middle temporal sulcus; Amyg, amygdala; Bi, basal nucleus, intermediate division; Bmc, basal nucleus, magnocellular division; Bpc, basal nucleus, parvicellular division; Ce, central nucleus; cs, central sulcus; EC, entorhinal cortex; En, endopiriform nucleus; Hp, hippocampus; I, intercalated nuclei; L, lateral nucleus; lf, lateral fissure; Me, medial nucleus; PAC, periamygdaloid cortex; PCo, posterior cortical nucleus; PRh, perirhinal cortex; Pi, piriform cortex; PL, paralaminar nucleus; pmts, posterior middle temporal sulcus; Pros, prosubiculum; rhs, rhinal sulcus; sts, superior temporal sulcus; TEa, anterior inferotemporal cortex; TEad, anterior dorsal inferotemporal cortex; TEav, anterior ventral inferotemporal cortex; TEO, temporal occipital cortex; TEm, area TEm; TEp, posterior inferotemporal cortex; WM, white matter

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receptors, ion channels, and neurotrophic factors (Manzerra et al., 2001; Kim et al., 2002; Rachline et al., 2005; Hwang et al., 2005; see for review: Cuajungco and Lees, 1997; Frederickson et al., 2000; Smart et al., 2004).

In this report, we provide further information about the distribution of Zn+ terminations in inferotemporal (IT) cortex, a region at the interface of the visual and hippocampal networks. In particular, we report a lateral to medial increase in Zn density, where TEad, a unimodal visual area, shows low levels of Zn; TEav, intermediate levels; and perirhinal cortex, a multimodal limbic area, high levels. A related question was to identify the neurons of origin of the Zn+ terminations. This was addressed by making intracortical injections of sodium selenite (Na_2SeO_3), a substance which interacts with Zn to form precipitates of ZnSe and in this form is transported retrogradely to the soma (Christensen et al., 1992; Casanovas-Aguilar et al., 1998, 2002). Analysis of retrogradely filled neurons is restricted to the hippocampus and amygdala in this report.

2. Materials and methods

2.1. Animals and tissue preparation

Nine adult macaque monkeys (*Macaca mulatta* and *M. fuscata*) were used (four for visualizing Zn+ terminals and five for sodium selenite injections). All experimental protocols were approved by the Experimental Animal Committee of the RIKEN Institute, and conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23; revised 1996). Every effort was made to minimize the number of animals used and any pain or discomfort experienced. Before perfusion, all animals were tranquilized with ketamine (11 mg/kg, i.m.) and deeply anesthetized with Nembutal (overdose, 75 mg/kg, i.p.).

Four monkeys were prepared for visualizing Zn+ termination, according to two perfusion methods (Danscher, 1996; Ichinohe and Rockland, 2004). (1) Two monkeys were perfused transcardially, in sequence, with saline containing 0.1% sodium sulfide (500 ml) for 5 min, and then 0.1% sodium sulfide and 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.3, 4 l) for 30 min. The brains were removed from the skull, trimmed and postfixed for 12–15 h in 4% paraformaldehyde in 0.1 M PB. Then, brains were immersed in 0.1 M PB containing 30% sucrose until they sank (2–3 days). (2) Two other animals received intravenous injection of saline with 10% sodium sulfide (200 mg/kg). Two minutes after the injection, the animals were perfused transcardially, in sequence, with saline containing 0.5% sodium nitrite, with 4% paraformaldehyde in 0.1 M PB for 30 min, and chilled 0.1 M PB with 10, 20 and 30% sucrose. There was no obvious difference in quality of staining between these two methods. All brains were coronally sectioned by frozen microtomy (at 40–50 μm thickness). Tissue was collected in repeating series of three for Zn and parvalbumin (PV) histochemistry, and for cell body staining with thionin.

2.2. Zn histochemistry

Sections were washed thoroughly with 0.1 M PB, followed by 0.01 M PB. The IntenSE M silver Enhancement kit (Amersham International; Little Chalfont, Bucks, UK) was used to intensify Zn signals (Danscher et al., 1987; De Biasi and Bendotti, 1988). A one-to-one cocktail of the IntenSE M kit solution and 33% gum arabic solution was used as a reagent. Development of reaction products was monitored under a microscope and terminated by rinsing the sections in 0.01 M PB and, subsequently, several rinses in 0.1 M PB. Selected sections were further processed for Nissl substrate using NeuroTrace 500/525 green fluorescent Nissl stain (Molecular Probes, Eugene, OR) according to the company's protocol.

In addition to Zn, these methods potentially can reveal other metals such as copper and iron (see for review, Danscher, 1996). The specificity for Zn was evaluated by several means. First, EM data (Ichinohe and Rockland, 2005a) clearly show that the silver reaction product is localized to synapses, whereas iron is reported to be localized mainly to the nucleus of neurons and glia (Yu et al., 2001), and copper, mainly to glia (Szerdahelyi and Kasa, 1986). Second, we carried out controls in rat brains perfused and reacted under the same two conditions as the monkey material. In one control, intraperitoneal injection (1000 mg/kg) of the Zn chelating agent diethyldithiocarbamate 60 min before sacrifice (as per Danscher, 1996) resulted in unstained brain sections. Similarly, pretreatment of brain sections with 0.1 M HCl (Danscher, 1996) for 30 min or 15% trichloroacetic acid (TCA; Sigma, St. Louis, MO) for 5 min (Szerdahelyi and Kasa, 1986), both known to dissolve Zn sulfide precipitates, eliminated the staining we have interpreted as owing to Zn. The TCA control was repeated in monkey tissue, with the same result. A direct control, such as staining unfixed cryostat sections with TSQ or Zinpyr-1 (Frederickson et al., 1992; Woodroofe et al., 2004) was not carried out due to the difficulty of obtaining and handling this tissue in primates.

2.3. Immunoperoxidase staining for PV

Sections were incubated for 1 h with 0.1 M phosphate buffer saline (PBS, pH 7.3) containing 0.5% Triton X-100 and 5% normal goat serum (PBS-TG) at room temperature, and then for 40–48 h at 4 °C with PBS-TG containing anti-PV monoclonal mouse antibody (Swant, Bellinzona, Switzerland; 1:50,000). After rinsing, the sections were placed in PBS-TG containing biotinylated anti-mouse IgG polyclonal goat antibody (Vector, Burlingame, CA; 1:200) for 1.5 h at room temperature. Immunoreactivity was