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Short Communication

Abnormal development of zinc-containing cortical circuits in the absence of the transcription factor *Tailless*

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Abstract

Absence of the transcription factor *tailless* (*tlx*) leads to premature laminar development and thinning of neocortex. We used zinc autometallography to determine if *tailless* deletion alters the organization of cortical circuits. In *tlx*−/− mice, layer 4 barrels, which normally lack synaptic zinc, are densely innervated by zinc-containing terminals. Furthermore, barrels with zinc inputs are constructed, in part, from zinc-sequestering neurons, a phenotype not normally found in layer 4.

Theme: Development and regeneration
Topic: Cerebral cortex and limbic system

Keywords: Barrel; Vibrissa; Whisker; Somatosensory cortex; Lamination

The mammalian *tailless* gene (*tlx*) is a forebrain-restricted transcription factor that is expressed by progenitor cells in the ventricular and subventricular zones during neurogenesis [17,18]. *Tlx* expression is essential for normal development of neocortex. In mice lacking *tlx*, cortical thickness is reduced to about 80% of that seen in wild-type (WT) mice [14]. Interestingly, superficial cortical layers are more adversely affected than deep layers 5 and 6, which appear relatively normal.

Tlx appears to regulate proliferation and timing of differentiation of progenitor cells. Around embryonic day 9.5 in *tlx*−/− mice, the cell cycle is significantly shorter than in WT littermates [21]. The accelerated proliferation leads neurons destined for a particular cortical layer to become postmitotic and differentiate precociously. Subsequently, the late progenitor cell population is prematurely depleted, around mid-gestation, resulting in more severe effects on later-born superficial layers [21]. These may be direct or indirect consequences of loss of *tlx*. Abnormalities in the number and time of differentiation of cortical neurons are likely to profoundly alter cortical circuits and their function.

In the present study, we used histochemical techniques to investigate the organization of zinc-ergic cortical circuits in the somatosensory (S1) barrel cortex of four adult *tlx*−/− mice and four of their WT littermates. We focused on S1 because of the context provided by the layer 4 whisker-related barrels [26], which are visible in mutant animals [14]. Zinc autometallography reveals a discrete population of glutamatergic, presumably excitatory synaptic terminals that are distinguished by the presence of zinc, along with glutamate, within their synaptic vesicles [3,9]. Zinc-containing synapses arise from intrinsic cortical neurons, mostly pyramidal neurons in layers 2/3 and 6, and are distributed heterogeneously among the cortical layers in a pattern that is

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highly conserved across mammals [4,5,7,8,10,12,13,23]. Localization of zinc-sequestering neurons also can be determined by autometallography (see below).

To localize zinc-containing synaptic terminals, two mice of each genotype received an intraperitoneal (IP) injection of the zinc chelator sodium selenite (20 mg/kg) from a freshly prepared stock solution and were anesthetized with IsoVet (Isoflurane; Abbot Labs, Abbot Park, IL) and killed by decapitation 1 h later. Each brain was quickly removed, encased in OCT embedding medium (Miles, Elkhart, IN) and rapidly frozen. One series of 20 μm coronal or tangential sections from each hemisphere was stained for synaptic zinc according to a modification of the Danscher method [6,8,13]. The distribution of zinc-sequestering cortical neurons was assessed in two additional mice of each strain. These mice received 12 mg/kg sodium selenite IP and were killed 24 h later. The longer survival period allows for zinc-specific retrograde transport of chelated zinc back to the neuronal somata of origin of zinc-containing synapses [23]. Sections were cut and stained as above. Sections then were counterstained with 0.1% thionin or were further dehydrated and coverslipped. Series of adjacent sections were stained for cytochrome oxidase (CO) according to previously published methods [15,25]. The care and handling of animals was approved by the University of Pittsburgh Institutional Animal Care and Use Committee and conformed to NIH guidelines.

Zinc-containing terminals are distributed in a layer specific pattern in WT and *tlx*−/− mice (Fig. 1). In both genotypes, density of synaptic zinc is high in layers 1–3 and in layer 5, intermediate in layer 6 and very low in layer 4. Staining of adjacent sections for zinc or CO shows that regions of layer 4 in S1 of WT mice stained lightly for zinc (e.g., Fig. 1A) denote hollows of cortical barrels (Fig. 1C).

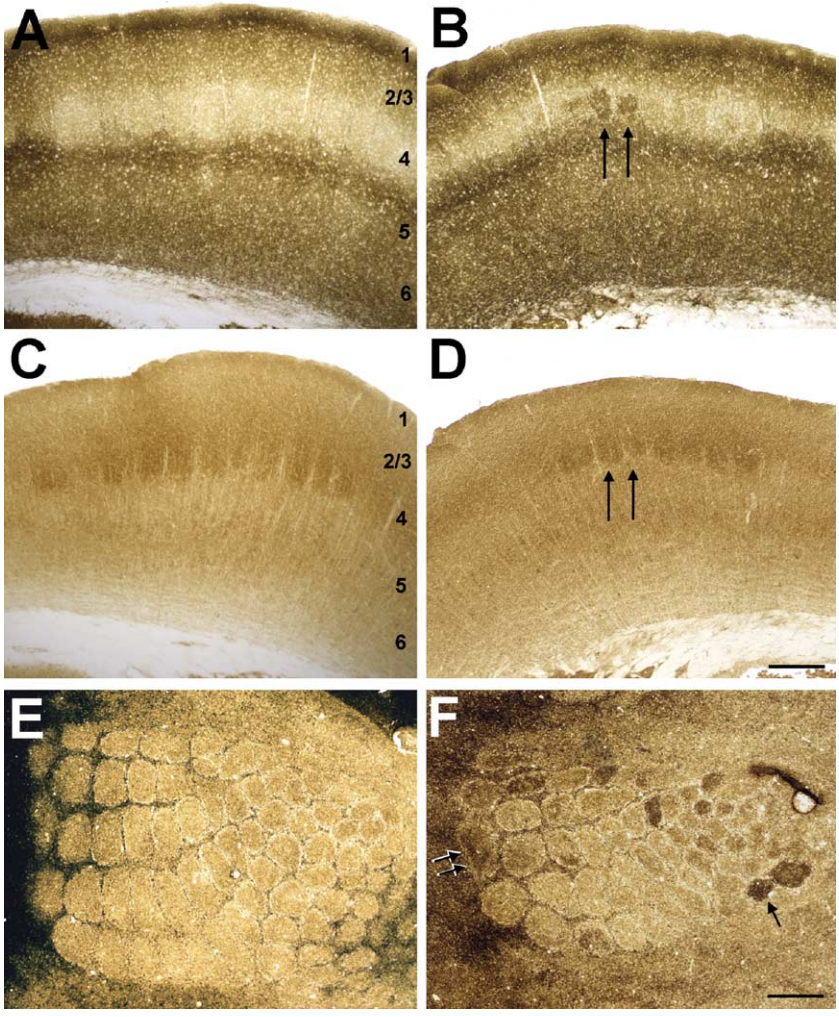

Fig. 1. Barrels in *tlx*−/− mutants contain synaptic zinc. (A, B) Zinc-stained coronal sections through S1 barrel cortex of a wild-type (A) and *tlx*−/− mouse (B). Note heterogeneous staining throughout the cortical layers (labeled 1–6). Barrels in layer 4 normally contain lowest levels of synaptic zinc, but in *tlx*−/− mice some barrels stain darkly (arrows in (B)). (C, D) CO-stained sections adjacent to those shown in panels (A) and (B), respectively. Barrels are clearly visible as dark CO patches in layer 4. Barrels indicated by arrows in panel (B) are seen also in panel (D). (E) Tangential zinc-stained section through layer 4 of a wild-type mouse showing the pattern of barrels, all of which stain lightly for zinc. (F) Tangential zinc-stained section through layer 4 of a *tlx*−/− mouse. Note that many small (arrow) and large (double arrows) barrels stain darkly; others stain lightly as in wild-type mice. Scale bar in panel (D) = 300 μm for panel (A) through panel (D); scale bar in panel (F) = 300 μm for panels (E) and (F).