Growth-Associated Protein 43 Is Located in Type I Corticothalamic Terminals in the Cat Visual Thalamus

Martha E. Bickford

Department of Anatomical Sciences and Neurobiology, University of Louisville, School of Medicine, Louisville, Kentucky 40292

Growth-associated protein 43 (GAP 43) is a presynaptic protein that has been proposed to be involved in synaptic plasticity. To determine the location of GAP 43 within the synaptic circuitry of the thalamus, immunocytochemical staining for GAP 43 was examined in a relay nucleus, the dorsal lateral geniculate nucleus (dLGN), and two association nuclei, the pulvinar nucleus and the lateral subdivision of the lateral posterior (LP) nucleus. In the dLGN, moderate neuropil staining was seen in the A laminae, and denser staining was found in the interlaminar zones and the C laminae. Uniform dense staining of the neuropil was found in both the pulvinar and LP nuclei. At the ultrastructural level, the GAP 43 staining was restricted to small-diameter myelinated axons, thin unmyelinated fibers, and small terminals that contained densely packed round vesicles (RS profiles) and made asymmetric synaptic contacts with small-caliber dendrites in the extraglomerular neuropil. The distribution of immunocytochemical label within the visual thalamus suggests that GAP 43 is confined to type I corticothalamic terminals and axons that originate from extrastriate cortical areas. These results also suggest that in both relay and association nuclei GAP 43 may be used to augment the cortical control of thalamic activity. In addition, these results underscore the distinction between the small type I corticothalamic terminals, which appear to contain GAP 43 throughout the visual thalamus, and the large type II corticothalamic terminals that, like the type II retinal terminals in the dLGN, do not contain GAP 43.

Key words: lateral geniculate nucleus; pulvinar nucleus; lateral posterior nucleus; relay; association; ultrastructure; synaptic plasticity
examined in the visual thalamus of the cat. GAP 43 staining was examined in a well characterized relay nucleus, the dorsal lateral geniculate nucleus (dLGN), and two association nuclei, the pulvinar nucleus and the lateral subdivision of the lateral posterior (LP) nucleus.

MATERIALS AND METHODS

Three adult cats were used for this study. The cats were deeply anesthetized and perfused with 4% paraformaldehyde, 4% parafomaldehyde, and 0.5% glutaraldehyde or 2% paraformaldehyde and 0.1% glutaraldehyde in 0.1M sodium phosphate buffer, pH 7.4. The brains were removed, and blocks of the thalamus were sectioned in the sagittal or coronal plane with a vibratome to a thickness of 50μm. Sections were stained with the GAP 43 antibody (mouse monoclonal; Boehringer Mannheim, Indianapolis, IN) diluted 1:1000–1:2000. Using previous immunocytochemical techniques (Patel and Bickford, 1997; Patel et al., 1999), the antibody was tagged with a biotinylated goat-anti-mouse antibody, a complex of avidin biotinylated horseradish peroxidase, and revealed with a diamino-benzidine reaction. Sections were then mounted on slides for light microscopy or processed for electron microscopy as previously described (Patel and Bickford, 1997; Patel et al., 1999).

RESULTS

As shown in Figure 1A, GAP 43 staining is denser in the pulvinar and LP nuclei than in the dLGN. At higher magnification (Fig. 1B–F), it can be seen that in all three nuclei, GAP 43 staining is confined to the neuropil. As previously noted (Baekelandt et al., 1998), in the dLGN moderate staining is seen in the A laminae, and denser staining is found in the interlaminar zones (B, C). The arrow points to an area shown at higher magnification in C. GAP 43 staining is dense in the dLGN C laminae (D) and pulvinar (E) and LPl (F) nuclei. Scale bars: A, 1 mm; B, 100 μm; C, 50 μm (also applies to D–F). OT, Optic tract.

DISCUSSION

In the visual thalamus, the morphology and synaptic arrangements of GAP 43-stained profiles are identical to the morphology and synaptic arrangements of type I corticothalamic terminals. That is, GAP 43 staining was confined to RS profiles that contacted small-caliber dendrites in the extraglomerular neuropil. The other main source of RS profiles in the visual thalamus is the cholinergic parabrachial region (PBR) of the brainstem. However, in the dLGN and pulvinar nucleus, PBR terminals primarily innervate glomeruli (Cucchiaro et al., 1988; Erisir et al., 1997; Patel and Bickford, 1997). Because GAP 43 staining was for the most part excluded from glomeruli, it is unlikely that GAP 43 is...
In the visual thalamus, GAP 43 staining is located in small terminals that contain densely packed round vesicles (RS profiles) and contact (arrows) dendrites with thick postsynaptic densities. Some RS profiles are not labeled by the GAP 43 antibody (asterisks).

A, B, GAP 43 staining in the lateral geniculate nucleus. C, E, GAP 43 staining in the lateral posterior nucleus. D, GAP 43 staining in the pulvinar nucleus. Scale bar, 0.5 µm.
contained in PBR terminals. In addition, although PBR terminals in the lateral LP nucleus are located outside of glomeruli, they make up a very small proportion of the RS terminals in this region of the thalamus (Patel et al., 1999). Because GAP 43-stained terminals are quite dense in the lateral LP nucleus, it is likely that most, if not all, GAP 43 is contained within corticothalamic axons and terminals.

The distribution of GAP 43 staining in the visual thalamus correlates well with the distribution of certain groups of corticothalamic terminals. In the dLGN, GAP 43 staining is densest in the interlaminar zones, where it has been noted that corticothalamic terminals from area 18 are most dense (Updyke, 1975; Vidnya´nszky and Hámosi, 1994). GAP 43 staining is also quite dense in the C laminae, which receive the majority of their cortical input from areas 18 and 19 (Updyke, 1975). Because not all RS profiles were labeled by the GAP 43 antibody, perhaps the reason that thalamic association nuclei are more densely stained for GAP 43 is that this protein is preferentially distributed within type I terminals from extrastriate cortical areas. In support of this idea, in the human striate cortex, GAP 43 mRNA is found only in superficial layer cells, but in the inferotemporal cortex, GAP 43 mRNA is additionally found in deeper cortical layers (Neve et al., 1988) where corticothalamic neurons are located (Gilbert and Kelly, 1975; Abramson and Chalupa, 1985).

Regardless of the precise cortical area of origin, the present results suggest that in the visual thalamus, GAP 43 is located exclusively in type I corticothalamic terminals. This distribution may account for the synaptic potentiation observed after high-frequency stimulation of the optic radiations in dLGN slices (Scharffman et al., 1990; McCormick and von Krosigk, 1992). High-frequency stimulation of corticothalamic fibers could result in the phosphorylation of the GAP 43 contained within these terminals, which might lead to the release of a larger volume of glutamate from their synaptic vesicles after subsequent stimulation. It is also interesting to note that a calcium–calmodulin-dependent protein kinase (CaM kinase II) is located postsynaptic to many, but not all, RS profiles (Liu and Jones, 1996). It has been proposed that CaM kinase II plays an important role in the process of long-term potentiation (Malenka et al., 1989). In addition, certain types of metabotropic glutamate receptors appear to be selectively associated with RS profiles (Godwin et al., 1997), and their activation can change the response mode of thalamocortical cells in the dLGN (McCormick and von Krosigk, 1992; Godwin et al., 1996). Thus, it appears that within the synaptic circuitry of the thalamus there is a precise localization of several proteins that can specifically amplify the signal transmitted by type I corticothalamic terminals.

Obviously, further experiments are necessary to determine the exact function of GAP 43 in the thalamus. However, the observed distribution of GAP 43 suggests that both relay and association nuclei may share a common mechanism to augment the cortical control of thalamic activity. Finally, these results underscore the distinction between the small type I corticothalamic terminals, which appear to contain GAP 43 throughout the visual thalamus, and the large type II corticothalamic terminals, which, like the type II retinal terminals in the dLGN, do not contain GAP 43.

REFERENCES


