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# HETEROGENEITY OF AXON TERMINALS EXPRESSING VGLUT1 IN THE CEREBRAL NEOCORTEX

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In the mammalian cerebral neocortex excitatory synaptic transmission is mediated by axon terminals forming asymmetric synapses and releasing glutamate (Glu), whereas inhibitory transmission is mediated by terminals that form symmetric synapses and release GABA (29). Both Glu- and GABA-mediated synaptic transmission in the neocortex is characterized by high variability of responses, which have a crucial role in information processing in cortical circuits (27). Among the several factors that contribute to this variability, receptor heterogeneity appears to be of paramount importance, but presynaptic factors and factors acting at the cleft also play a role (7, 10).

An essential presynaptic mechanism is neurotransmitter loading in synaptic vesicles by specific proteins named vesicular transporters. To date, three distinct vesicular glutamate transporters (VGLUTs) have been identified: VGLUT1 (5, 33), VGLUT2 (1, 3, 13, 34) and VGLUT3 (14, 18, 31). General mapping studies have shown that VGLUT1 and VGLUT2 are distributed in a mutually exclusive fashion in the adult nervous system, with VGLUT1 being expressed mainly in telencephalon and VGLUT2 in diencephalon and lower brainstem (4, 13, 16, 19, 22, 28); both transporters are associated with synaptic vesicles located in axon terminals forming asymmetric synaptic contacts with dendritic shafts and spines (4, 13, 17, 19, 21). VGLUT3 is expressed primarily in nerve fibers arising in the brainstem and is localized to both asymmetric and symmetric axon terminals (14, 31, 32).

In the last few years, we have been systematically studying the localization of the three VGLUTs in the mammalian cerebral cortex, a region where Glu plays a fundamental role in both physiological and pathophysiological conditions (9). Here, we briefly review the main features of the distribution of VGLUT1 and VGLUT2 in the cerebral cortex of adult rats (see Ref. 25 for data on the maturation of VGLUT1), with some considerations on the heterogeneity of cortical glutamatergic axon terminals. For recent data on the localization of VGLUT3 in the neocortex, see Hioki *et al.* (20) and Somogyi *et al.* (32).

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## VGLUT1 AND VGLUT2 IN THE ADULT CEREBRAL CORTEX

VGLUT1 immunoreactivity (ir) is present in all cortical layers with a laminar pattern that is particularly evident in the parietal cortex, where it is strong in layers I and II-III, followed by layers V and VI, and less intense in layer IV (Fig. 1A). In all layers, VGLUT1 ir is exclusively associated with punctate structures (puncta) of various sizes that are both distributed in the neuropil and in close apposition to unlabeled cell bodies of different size and shape (Fig. 1B). In layers II-III and V, VGLUT1-positive puncta outline the profile of pyramidal neurons and of their apical dendrites (Fig. 1B-D). Virtually all VGLUT1-positive puncta, including those outlining pyramidal cell somata and proximal dendrites, coexpress synaptophysin-1 ir, indicating that they are axon terminals (for details, see Alonso-Nanclares *et al.* [2]).

VGLUT2 ir is expressed in all cortical layers, more robustly in layers I and IV (Fig. 2A); it is exclusively associated with puncta of various sizes that are preferentially distributed in the neuropil and less frequently apposed to unlabeled cell bodies (Fig. 2B). In layer IV, VGLUT2-positive puncta outline the profiles of nonpyramidal neurons (Fig. 2C).

These findings are in line with previous reports of VGLUT1 and VGLUT2 ir in the neocortex, with two notable exceptions. First, we observed a widespread

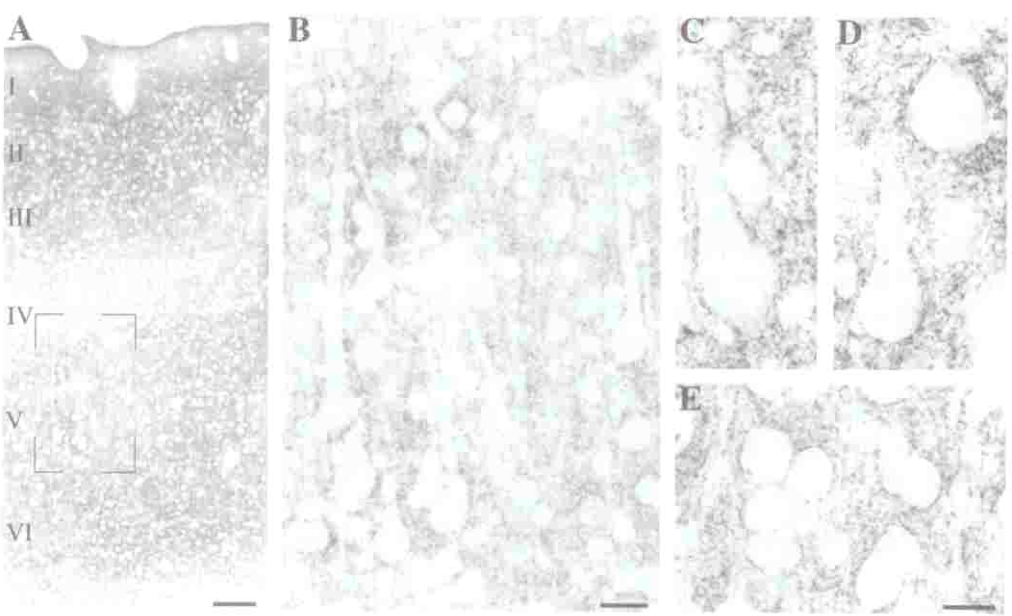


Fig. 1. - *VGLUT1 ir in rat parietal neocortex.*
A, distribution of VGLUT1 ir in parietal cortex. Roman numerals indicate cortical layers; the framed region is reproduced enlarged in B. *B*, VGLUT1 ir in punctate structures distributed in the neuropil and around unlabeled pyramidal cell somata and apical dendrites (layer V); *C* and *D*, two examples of large pyramidal cells in layer V surrounded by intensely stained VGLUT1-positive puncta. *E*, nonpyramidal cell bodies in layer VI outlined by VGLUT1-positive puncta. Calibration bar: 100 μm for A, 20 μm for B, and 10 μm for C-E. (Modified from Alonso-Nanclares *et al.* [2]).

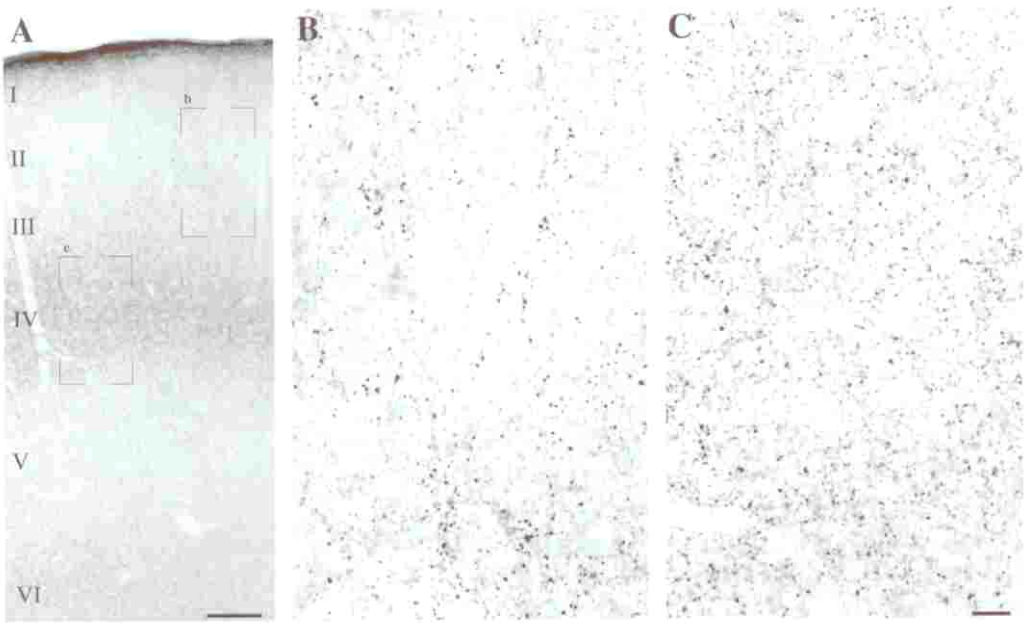


Fig. 2. - *VGLUT2 ir in rat parietal cortex.*
*A*, distribution of VGLUT2 ir in parietal cortex; Roman numerals indicate cortical layers; the framed regions b and c are reproduced enlarged in B and C. *B* and *C*, VGLUT2 ir in layers II-III (*B*) and in layer IV (*C*). Calibration bar: 100 µm for A, 20 µm for B and C.

VGLUT1-positive innervation of the somata of most cortical neurons, including pyramidal neurons. Secondly, we found VGLUT2-positive puncta in layers II-III, although they were less numerous than in layers I and IV.

## VGLUT1 AXON TERMINAL HETEROGENEITY IN THE ADULT NEOCORTEX

The extensive targeting of pyramidal cell somata, typical of GABAergic inhibitory nerve endings, is rather unexpected in glutamatergic excitatory axon terminals (30). We thus first undertook electron microscopic studies to evaluate whether perisomatic VGLUT1-positive puncta were indeed axon terminals and to assess the type of synaptic contacts they form. Using a correlative light and electron microscopic method (11), we examined several hundred VGLUT1-positive puncta throughout the neuropil of the parietal cortex and found numerous VGLUT1-positive axon terminals in direct apposition to the membrane of unlabeled neuronal cell bodies, particularly pyramidal neurons in layers II-III and V, and proximal dendrites (2). Interestingly, all labeled perisomatic axon terminals formed asymmetric synapses with adjacent dendritic shafts and spines, but not with the pyramidal cell soma to which they were contiguous (2). Next, we tested the hypothesis that some perisomatic axon terminals coexpress Glu and GABA transporters by means of confocal

microscopy double-labeling studies using antibodies to VGLUT1 and VGAT (6, 24). Preliminary results indicate that a sizeable fraction of perisomatic VGLUT1-positive axon terminals do coexpress VGAT, indicating heterogeneity in the population of VGLUT1-positive axon terminals and suggesting that in the adult neocortex some axon terminals may release both Glu and GABA (8, 12).

The presence of VGLUT2-positive puncta in layers II-III, where VGLUT1 is highly expressed, raises the possibility that in these layers some axon terminals coexpress VGLUT1 and VGLUT2. Preliminary data of our confocal microscopy double-labeling studies showed that in the cerebral cortex of adult rats some axon terminals coexpress VGLUT1 and VGLUT2, in line with the results of Li *et al.* (23) in trigeminal ganglion neurons and of Morimoto *et al.* (26) in pinealocytes. These *in vivo* observations in the adult brain, together with the recent *in vitro* demonstration that VGLUT1 and VGLUT2 are transiently coexpressed during postnatal development (15, 35), indicate that the concept of mutually exclusive expression generated by earlier studies has important exceptions and point to the existence of another subpopulation of glutamatergic axon terminals.

The results presented at this symposium and summarized in this brief overview thus suggest that terminals expressing VGLUT1 are heterogeneous both anatomically and molecularly. These studies, though still in their early stage, suggest that in the near future it may be possible to characterize the molecular organization of cortical glutamatergic axon terminals in the cerebral cortex. Although little is known of the functional consequences of the differential expression of VGLUTs, some findings suggest that it may correlate with important aspects of synaptic function, e.g. with the probability of transmitter release, which is low at synapses using VGLUT1 and high at synapses using VGLUT2 (13, 15). Since synapses with low probability of release have a higher potential for plasticity, it follows that expression of the VGLUT1 isoform may correlate with synaptic plasticity. The characterization of the molecular organization of cortical glutamatergic axon terminals may thus shed light on their differential impact on the functional state of cortical circuits.

## SUMMARY

Using immunocytochemical techniques and confocal microscopy we have studied the localization of the vesicular glutamate transporters (VGLUTs) 1 and 2 in the mammalian cerebral cortex. The cardinal observations gathered to date can be summarized as follows: 1) Many VGLUT1-positive puncta coexpressing synaptophysin-1 outline pyramidal cell somata and proximal dendrites; of these, a sizeable fraction coexpress VGAT, the vesicular transporter for GABA; 2) VGLUT2-positive puncta are also present in layers II-III and some of them coexpress VGLUT1. These findings suggest that in the cerebral cortex of adult rats axon terminals expressing VGLUT1 are heterogeneous.

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