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Neocortex and Hippocampus Contain Distinct Distributions of Calcium-Calmodulin Protein Kinase II and GAP43 mRNA

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ABSTRACT

Calcium-calmodulin protein kinase II and GAP43 are two molecules which have been linked to synaptic plasticity. Localization of mRNA for these molecules identifies the neuronal populations which have the potential to utilize these mechanisms. General descriptions for calcium-calmodulin protein kinase II or GAP43 mRNA have been previously reported. In light of recent evidence that suggests that at some sites these two molecules may interact, we sought to determine the cortical distribution in detail, and to examine the extent of overlap between neuronal populations containing each mRNA. To this end we have used in situ hybridization techniques to study the distribution of calcium-calmodulin protein kinase II and GAP43 mRNA in adjacent sections of adult rat forebrain. Overall, the distribution patterns were distinct but partially overlapping. For both calcium-calmodulin protein kinase II and GAP43, mRNA levels were highest in hippocampus, allo- and neocortex, compared to moderate to low levels in striatum and thalamic nuclei. Within the heavily labeled regions certain populations expressed both calcium-calmodulin protein kinase II and GAP43 mRNA at high levels, while other populations were selective for calcium-calmodulin protein kinase II. In the hippocampus, the stratum pyramidale of CA1-3 expressed high levels of both calcium-calmodulin protein kinase II and GAP43 mRNA. Granule cells of the fascia dentata and the stratum radiatum of CA3 both contained moderate to high levels of calcium-calmodulin protein kinase II mRNA, but near background levels of GAP43 mRNA label. Within the neocortex, deep layers were distinguished from superficial layers by their lack of calcium-calmodulin protein kinase II mRNA expression within the neuropil, and the presence of GAP43 mRNA in neurons located in layer V and the deepest part of layer VI. Thus, layer V and deep layer VI neurons showed high levels of label for both GAP43 and calcium-calmodulin protein kinase II mRNA, while neurons of superficial layers contained only calcium-calmodulin protein kinase II mRNA. These markers differentiate neuronal populations which can also be distinguished on the basis of their ability to undergo specific forms of synaptic plasticity. These different forms of plasticity may be due in part to the laminar-specific patterns of GAP43 and calcium-calmodulin protein kinase II mRNA that we have described.

Key words: cortical plasticity, synaptic modification, second-messenger systems, B-50, in situ hybridization

It is now evident that the functional organization and connectivity of much of cerebral cortex is modifiable in mature animals (Van der Loos and Woolsey, '73; Kaas et al., '83; Donoghue et al., '90). One way this may occur is through activity dependent modification of synaptic strength within cortical circuits. Although molecular mechanisms for cortical synaptic plasticity are not fully understood, several such mechanisms probably exist. For example, synaptic strength changes may occur pre- or post-synaptically and may (Harris et al., '84; Wigstrom et al., '86; Artola and Singer, '87) or may not (Harris and Cotman, '86; Komatsu et al., '91; Bear et al., '92) require N-methyl-D-aspartic acid (NMDA) receptors. Thus the form and mecha-

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nism of synaptic change may be unique to specific cortical pathways.

Calcium-calmodulin protein kinase II (CKII), one of the most abundant proteins in the brain (Kennedy et al., '83a; Goldenring et al., '84; Kelly et al., '84), and the growth-associated protein, GAP43 (also known as B50, F1, neuromodulin, or pp46), have each been implicated in mechanisms underlying transmitter release and synaptic plasticity in adult animals. Many characteristics of both CKII and GAP43 make these proteins likely participants in the ongoing modification of synapses in the mature brain. Initiation of hippocampal long-term potentiation (LTP) requires CKII activity (Malenka et al., '89; Malinow et al., '89), while the amount of GAP43 phosphorylation is correlated with the degree of potentiation produced (Lovinger et al., '85, '86; Gianotti et al., '92; see also De Graan et al., '90). Recent evidence suggests that at presynaptic locations these two proteins may in fact work together in these functions. In one scenario, phosphorylated GAP43 releases bound calmodulin (Alexander et al., '87; see reviews by Benowitz et al., '90a; De Graan et al., '90) and makes it available to activate CKII. CKII is then able to phosphorylate molecules such as synapsin I, microtubule associated protein 2 (MAP2), and tubulin (DeLorenzo, '81; Yamauchi and Fujisawa, '82; Bennett et al., '83; Goldenring et al., '83; Kennedy et al., '83b; McGuinness et al., '83; Erondu and Kennedy, '85; Shenolikar et al., '88), which influence synaptic structure and vesicular release of transmitter (synapsin I: Benfenati et al., '89; McGuinness et al., '89; tubulin: Feit et al., '71; Zisapel et al., '80). Further, autophosphorylation of CKII may permit sustained changes in the functioning of these substrates (Miller and Kennedy, '86). GAP43, which is predominantly associated with the cytoplasmic side of the membrane (Gispen et al., '85; Gorgels et al., '89), may have further actions on other membrane-associated second messenger systems (De Graan et al., '90). GAP43 levels are high throughout the developing brain; however, in the mature brain GAP43 expression is confined largely to associative regions (Benowitz et al., '88; Neve et al., '88; De la Monte et al., '89). Increased amounts of GAP43 can be induced in both the peripheral and central nervous systems under conditions of regeneration or sprouting after axonal lesions (Benowitz et al., '90a). Moreover, patterns of expression of CKII and GAP43 mRNAs in the kitten visual cortex are altered by visual deprivation and light exposure (Neve and Bear, '89). Therefore, the presence of CKII and GAP43 in cortical neurons is likely to identify those neurons with a potential to undergo synaptic modification. While general descriptions of the distribution of CKII and GAP43 mRNAs have been reported separately, the detailed cortical localization and the potential overlap of neuronal populations containing these molecules has not been studied. To identify these populations, in situ hybridization was carried out on adjacent sections of rat brain using radiolabeled probes for GAP43 and CKII mRNA. Our results show that in mature rat brain, highest levels of both CKII and GAP43 mRNA are expressed in hippocampus and neocortex; however, each region expresses a distinct distribution pattern for these two transcripts.

MATERIALS AND METHODS

Five female adult albino rats were deeply anesthetized with pentobarbital and perfused transcardially with 4% paraformaldehyde. Brains were removed, immersed for at least 24 hours in 30% sucrose for cryoprotection, and frozen sectioned at 15 μm through frontal, parietal, and temporal cortex. From these sections, we examined the cerebral cortex and diencephalon. Occipital cortex was not studied. The cDNA probe used for GAP43 was the clone pGA3A (Neve et al., '87), which includes the entire human GAP43 coding sequence. For CKII the probe used was the clone C23, which represents a rat brain cDNA that was originally isolated with a CKII antibody (R.L.N. and C.-A. Ohmstede, unpublished); sequence analysis of the clone has verified that it does encode the carboxyterminus of the alpha subunit of rat CKII. The cDNA was labeled by the random priming method, with deoxycytidine 5′-[alpha[^{35}S]thio]triphosphate, New England Nuclear, 1,000 Ci/mmol. Tissue sections were pretreated in 20 mM HCl, 0.01% Triton X-100, 1 μg/ml proteinase K, postfixed with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4), and acetylated by immersing the slides in 100 mM triethanolamine (pH 8), 0.25% acetic anhydride and stirring for 10 min. After the sections were rinsed in phosphate-buffered saline (PBS) with 2 mg/ml glycine, they were prehybridized in 50% deionized formamide, 2× SSC (NaCl and sodium citrate), 25 μg/ml yeast tRNA, 250 μg/ml salmon testes DNA, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.1% bovine serum albumin, 0.2% sodium dodecyl sulfate (SDS), 25 mM EDTA, 20 mM B-mercaptoethanol for one to several hours at 42°C. (Before prehybridization, control slides were pretreated with 100 μg/ml RNAse A at 37°C for 1 hour.) The prehybridization mix was drained from the slides, which were then incubated with hybridization buffer (prehybridization mix plus 3×10^6 cpm of probe per 100 μl) at 42°C overnight.

After hybridization, sections were washed in 2×, 1×, and 0.1× SSC for 30 min each at room temperature, then in 0.1× SSC at 42°C, 55°C, and finally 65°C. All washes contained 20 mM b-mercaptoethanol. The sections were dipped in Kodak NTB2 emulsion, and exposed at 4°C for 3 to 5 weeks. Slides were developed in Kodak D developer and fixed in Kodak fix. After developing, selected slides were counter-stained with thionin for determination of cellular versus neuropil labeling. The level of autoradiographic labeling over non-section regions of the slide was considered to represent background levels. The specificity of these probes has been previously demonstrated (Neve et al., '88; Mah et al., '92) and only background levels of grains were seen on sections treated with RNAse before in situ hybridization. In addition, Figure 4 contains insets showing labeling over subcortical white matter, which is considered to be at background levels of labeling. To compare the number of neurons within superficial versus deep neocortical layers that were heavily labeled for CKII mRNA, cell counts were made in superficial and deep (directly beneath superficial region counted) layers within somatosensory cortex. Only cells containing a nucleus were counted. In four different sections, 50 cells in II/III and 50 cells in layer V were qualitatively judged to be either heavily, moderately or lightly labeled. Cells considered heavily labeled contained approximately 50 grains or more, while lightly labeled cells contained approximately ten or fewer grains.

RESULTS

The distribution patterns of autoradiographic labeling visible at low magnification with darkfield optics were unique but partially overlapping for GAP43 and CKII

mRNA throughout the forebrain. CKII mRNA was most abundant in the hippocampus, olfactory, prelimbic, and neocortex (Figs. 1A, 2). CKII labeling in striatum was moderate, while thalamic and hypothalamic areas were generally low, although some thalamic nuclei contained moderate levels of labeling in clusters, indicative of a cellular locus of the mRNA. GAP43 mRNA labeling was highest in hippocampus (Fig. 1B) and olfactory cortex. Moderate to heavy GAP43 mRNA labeling could be found within neocortex (Fig. 1B), while striatum contained near background levels. In contrast to the CKII transcript, GAP43 mRNA levels were also robust in the amygdala, hypothalamus, and midline thalamic nuclei. Labeling patterns for each probe were consistent in all five animals.

Within the cerebral cortex, the distribution pattern of each transcript varied according to architectonic subdivisions. In the hippocampus, CKII mRNA was expressed at highest levels in CA1–CA4, as revealed by a thin layer of heavy label in CA1, a thick band in CA2 and CA3, and moderate to high labeling in CA4 and fascia dentata (Fig. 1A). This labeling was primarily contained within pyramidal and granule cell layers; however, diffuse labeling above background levels was also seen in the stratum radiatum. This neuropil labeling is presumably dendritically localized mRNA, as has been noted previously (Burgin et al., '90). The band of CKII labeling within CA1 and CA3 varied in width, corresponding to changes in the width of the pyramidal cell layer. GAP43 mRNA was more limited in its distribution compared to CKII. Heaviest grain density occurred in CA3 and CA4, with less intense labeling in CA1 and CA2, and near background levels in the fascia dentata (Fig. 1B). Differences in labeling between CA3 and CA1 lay not only in thickness of the band of labeling, but also in intensity of labeling, so that both band width and intensity were greatly decreased in CA1 relative to CA3 and CA4. GAP43 mRNA labeling was confined to stratum pyramidale, with background levels in other hippocampal layers.

Neo- and allocortical patterns were also distinctive for each probe. CKII mRNA labeling tended to be higher in tenia tecta and olfactory cortex than in neocortical areas (Fig. 2); however this appearance may have been due to the high density of cell packing found in these regions. No consistent differences were evident for the levels of CKII mRNA expression between neocortical regions, although in some sections the parietal cortex contained regions of heavy label above the level of other neocortical areas. For example, the section shown in Figure 2 contains a region of very heavy labeling within parietal cortex, while an adjacent section had no obvious irregularity in the level of labeling throughout neocortex. Regions of very high label within parietal cortex were not correlated with any particular cytoarchitectonic feature. The same general laminar distribution was observed throughout all of these cortical areas. The CKII mRNA labeling was heaviest in superficial cell layers: layers II–IV of neocortex (Fig. 3A), the pyramidal cell layer of olfactory cortex and layers II–III of cingulate cortex. Frontal, parietal, and temporal cortex displayed moderate CKII mRNA labeling in layer I, heavy to moderate labeling over most cells in layer Vb, near background levels in most of layer Va, with scattered clusters of heavily labeled neurons and moderate to light levels in layer VI. In layer II/III 88 ± 5.4% (mean ± S.D., N = 200) of neurons were heavily labeled, whereas in layer V only 47 ± 15.5% (N = 200) contained high levels of CKII mRNA. Neurons considered to be moderately labeled made up an additional 11 ± 4.8% in superficial and 37 ± 4.2% in deep layers, while all other neurons were only lightly labeled or near background levels. Although the pattern of high expression in superficial layers relative to deep layers reflects the general pattern of cell packing density, neuropil labeling formed an additional component of the expression in the superficial layers that contributed significantly to the marked difference in CKII mRNA labeling between superficial and deep layers (Fig. 4A). A dramatic decrease in neuropil labeling was evident at the upper boundary of layer V (Figs. 3A, 4B). Clusters of heavy labeling within layer Vb were confirmed to be contained primarily over neuronal somata in thionin counter-stained sections. In layer VI, moderate labeling was seen over both neurons and neuropil. No consistent differences in CKII mRNA expression beyond that described above were seen between neocortical areas.

In contrast to the heavy neocortical labeling seen for CKII mRNA, labeling of GAP43 mRNA was moderate to light throughout most of neocortex. Substantial GAP43 mRNA labeling was associated primarily with two groups of neurons: large layer V neurons, and small neurons in the deepest part of layer VI (Figs. 3B, 4C, 4D). There were also a few neurons with heavy labeling scattered within layer VI. This pattern was consistent throughout frontal, parietal, and cingulate cortex. Within olfactory cortex the pyramidal cell layer and some neurons deep to this layer contained heavy labeling. Neuropil labeling of GAP43 mRNA was near background levels in all cortical areas.

DISCUSSION

CKII and GAP43 mRNA show specific regional, laminar, and cellular labeling patterns that partially overlap (Fig. 5). Regions of the hippocampus and neocortex express both CKII and GAP43 mRNA, while other regions show specificity for either CKII or GAP43 mRNA. Within the hippocampus, CA3 neurons contain high levels of both CKII and GAP43 mRNA label. This indicates that CA3 terminals, and in particular the Schaeffer-collateral synapse in CA1, could contain both proteins. In support of this idea are reports of immunocytochemical staining for GAP43 (Benowitz et al., '88) and CKII (Ouimet et al., '84; Fukunaga et al., '88) in the region of the apical dendrites of CA1 neurons, where CA3 neurons would likely synapse. It is likely that GAP43 identified at this site is located in the terminals of CA3 neurons, since immunocytochemical staining for GAP43 combined with electron microscopy shows that within rat brain this protein is primarily localized presynaptically (Sorensen et al., '81; Gispen et al., '85; Gorgels et al., '89). By contrast, CKII has been found both postsynaptically (Ouimet et al., '84; Fukunaga et al., '88), where it may be involved in induction of long-term plasticity (Malenka et al., '89), and presynaptically (Ouimet et al., '84; Erondu and Kennedy, '85), where it, in combination with GAP43, may play a role in transmitter release (Ivins et al., '93; Dekker et al., '89) or in controlling synaptic structure. Thus at the Schaeffer-collateral synapse, CKII may be produced presynaptically, postsynaptically, or both.

In contrast to the presence of both CKII and GAP43 mRNA at the site of the Schaeffer-collateral synapse is the specificity for CKII at the site of the mossy fiber-synapse onto CA3 neurons. We have found that both fascia dentata neurons and CA3 stratum radiatum express moderate to

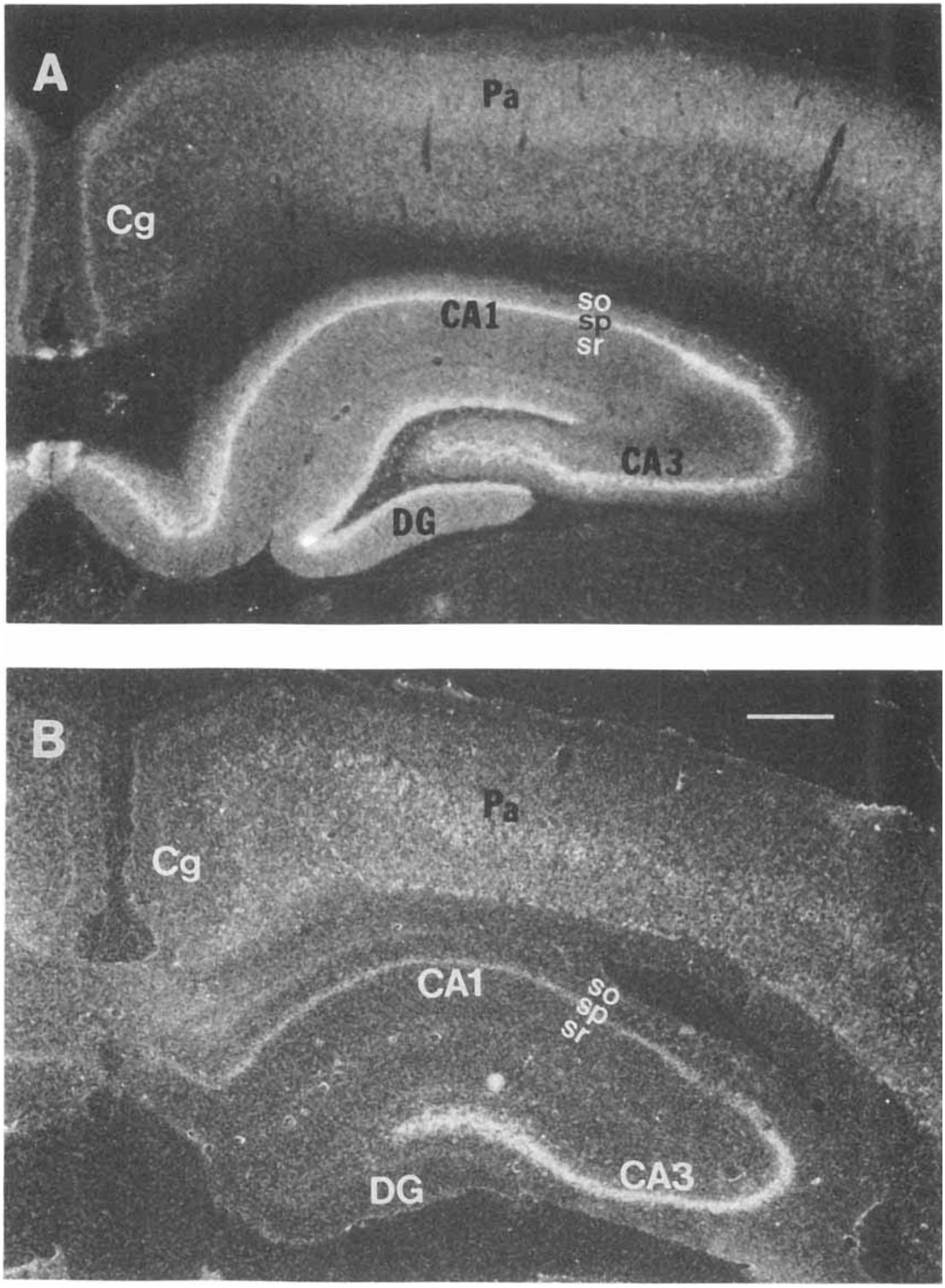

Fig. 1. Neocortical and hippocampal labeling patterns for calcium-calmodulin protein kinase II (CKII) (**A**) and GAP43 (**B**) mRNA. A: Darkfield photomicrograph of in situ hybridization of CKII mRNA. Superficial layers of neocortex and pyramidal cell layers of the hippocampus express CKII mRNA at high levels. Neuropil labeling is also high in superficial layers of neocortex and in stratum radiatum of CA3 in the hippocampus. B: Darkfield photomicrograph of in situ hybridization of GAP43 mRNA. Within neocortex, layer V and deep layer VI show heavy neuronal labeling while other layers have near background levels. In hippocampus, CA3 and CA4 contain the highest levels of label. Pa, parietal cortex; Cg, cingulate cortex; DG, dentate gyrus; so, stratum oriens; sp, stratum pyramidale; sr, stratum radiatum. Scale bar = 500 μm.

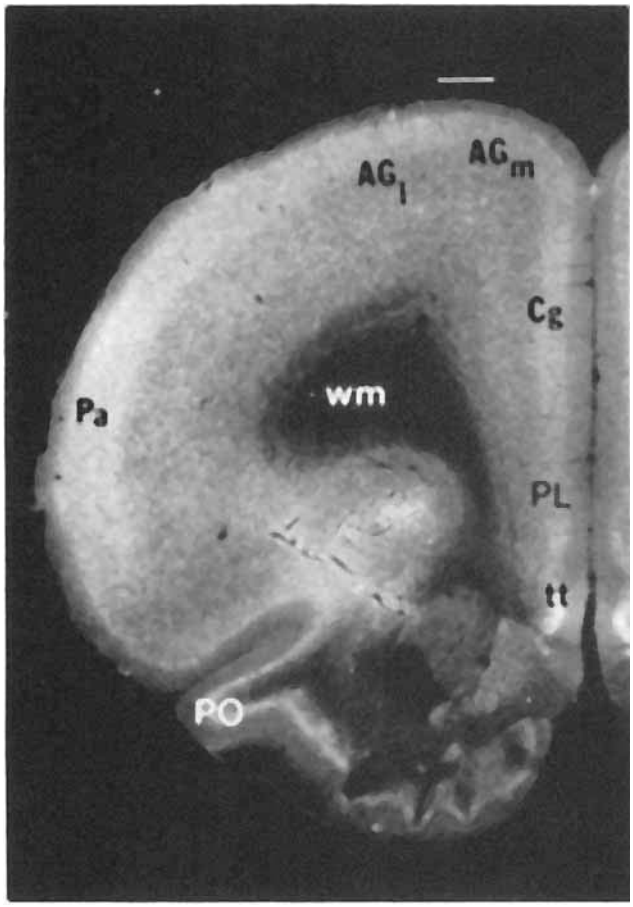

Fig. 2. CKII mRNA distribution in rostral hemisection. Laminar pattern of heavy labeling within the superficial layers was found throughout neo- and allocortex. AG_m, medial agranular cortex; AG_l, lateral agranular cortex; Cg, cingulate cortex; Pa, parietal cortex; PL, prelimbic cortex; PO, primary olfactory cortex; tt, tenia tecta; wm, white matter. Scale bar = 400 μm.

high levels of CKII mRNA but virtually no GAP43 mRNA. Previous reports of GAP43 mRNA expression within the dentate have been conflicting. De la Monte et al. ('89) reported heavy dentate labeling, while the findings of Rosenthal et al. ('87), Kruger et al. ('92), and Meberg and Routtenberg ('91) were similar to ours, in that they reported that the dentate contained little GAP43 mRNA labeling. These labeling differences may reflect differences in the type of probe and the specific protocols used. The distribution of immunocytochemical staining for the protein correlates well with projection sites for the neurons we found to contain the GAP43 mRNA. Stratum radiatum of CA3, the site of mossy fiber synapses from the dentate, contains high levels of CKII protein (Ouimet et al., '84; Fukunaga et al., '88), but low levels of GAP43 protein (Benowitz et al., '88). The high levels of CKII mRNA in stratum radiatum, which contains mostly pyramidal cell dendrites and few somata, suggest that the mRNA is dendritically localized. Very little mRNA is seen at sites which primarily contain axons (Burgin et al., '90). As Burgin et al. ('90) suggest, this dendritic localization may allow for fast turnover of the protein near the site of synapse, where CKII makes up 30 to 50% of the total post-synaptic density protein (Kennedy et al., '83a; Goldenring et al., '84; Kelly et al., '84). The differential labeling of CKII and GAP43 mRNA within CA1 and CA3 (Fig. 1) suggests that the mossy fiber synapse may possess different potential mechanisms for plasticity than does the Schaeffer-collateral synapse. How this relates to findings of different mechanisms for LTP at these two synapses (see Johnston et al., '92) is not known.

Our results confirm that CKII and GAP43 mRNA are also expressed by neocortical neurons. Two distinct populations of neurons within neocortex localize both CKII and GAP43 mRNA: Large pyramidal neurons of layer V and the small neurons in the deepest part of layer VI. The reported distribution of GAP43 protein in rat brain (Benowitz et al., '88) is somewhat more restricted than the known projections of the layer V and VI neurons which we have found express high levels of GAP43 mRNA. Benowitz et al. ('88) found dense immunocytochemical staining for GAP43 in layer I, and moderate staining in layers Va and VI. Layer V neurons send axonal collaterals within layer V, as well as to layers III and VI (Lund and Boothe, '75; Valverde, '78; Schwark and Jones, '89; and Kwon, Jacobs and Donoghue, unpublished observations). It is unclear why GAP43 protein levels are highest in some, but not all of the projection sites of GAP43 mRNA-containing layer V neurons. The layer I immunocytochemical staining could be contained either in known projections from layer VI to layer I (Fairen et al., '84) or from certain nonspecific thalamic nuclei, in which we found heavy labeling for GAP43 mRNA. Although the adult pyramidal tract as a whole shows only moderate levels of GAP43 immunoreactivity, smaller, unmyelinated axons and some small myelinated axons of the pyramidal tract have been reported to contain high levels of GAP43 (Gorgels et al., '89). Based on this finding, Gorgels et al. ('89) suggested that GAP43 plays a role in axonal growth or sprouting in the adult animal. In the brainstem GAP43 mRNA is specifically expressed at high levels in monoaminergic neurons (Bendotti et al., '91). Decreasing the presence of GAP43 has been shown to decrease dopamine (Ivins et al.,) and norepinephrine release (Dekker et al., '89). Within neocortex, where most neurons are thought to be glutaminergic, the specificity appears to be not for particular transmitter systems, but rather for a subset of cortical efferent cells (layer V and VI neurons). Some layer V neurons have axonal collaterals which travel for long distances intracortically and appear to play a role in modulation of intracortical excitability (Chagnac-Amitai and Connors, '89; Jacobs and Donoghue, '91; Connors and Amitai, '93, in press). It is not known if GAP43 plays a role in transmitter release or in sprouting mechanisms at this site.

Levels of GAP43 do not change after lesions of the pyramidal tract in adult animals, in which the lesioned axons also fail to regenerate (Kalil and Skene, '86). However, conditions which have been shown to initiate sprouting in the central nervous system, including the hippocampus of adult animals, do increase levels of GAP43 or its mRNA (Benowitz et al., '90b; Tetzlaff et al., '90; Tetzlaff et al., '91). Changes in levels of GAP43 mRNA may also be an indicator of change in protein levels (Basi et al., '87; Rosenthal et al., '87; Ng et al., '88). Existing levels of GAP43 may also allow for sprouting or other mechanisms of plasticity, under appropriate conditions, such as specific

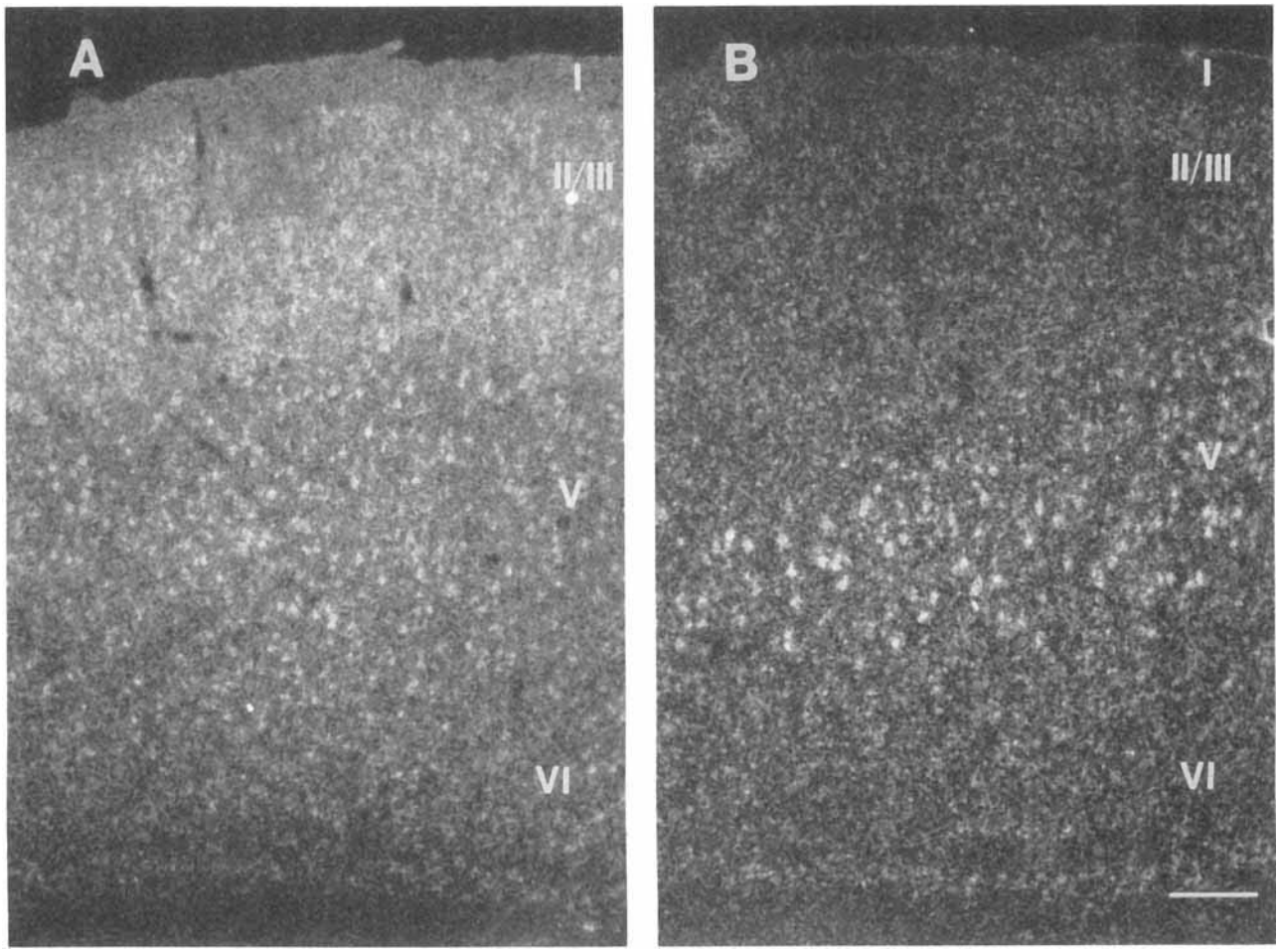

Fig. 3. Neocortical laminar labeling patterns for CKII (**A**) and GAP43 (**B**) mRNA. A: High power darkfield photomicrograph of in situ hybridization of CKII mRNA. B: High power darkfield photomicrograph of in situ hybridization of GAP43 mRNA. A and B from somato-sensorimotor cortex. Scale bar = 200 μm.

patterned activity. The fact that within neocortex layer V and deep layer VI neurons express high levels of GAP43 mRNA suggests that these neurons have the potential for a form of plasticity not found in other neocortical neurons. The possibility that neocortical layer V neurons are specialized for certain forms of plasticity is significant, since within motor cortex, layer V contains a substrate sufficient to allow for cortical map reorganization (Jacobs et al., '91; Jacobs and Donoghue, '91). This form of reorganization is long lasting (Sanes et al., '90) and therefore could depend on permanent structural changes for which the presence of growth factors would be necessary.

Neurons in superficial layers of neocortex have high levels of CKII mRNA but show relatively little labeling for GAP43 mRNA. It has previously been reported that layers II–III contain the highest levels of CKII mRNA (Burgin et al., '90). We find that high levels of CKII mRNA are expressed in layers I–IV, with a large decrease in neuropil labeling at the IV/V border. The only heavy labeling in the infragranular layers is found in neuronal somata in layer V. This pattern corresponds closely to the immunocytochemical staining for CKII (Ouimet et al., '84; Fukunaga et al., '88). The CKII immunoreactivity and mRNA label seen in neuropil of layers I–IV may be due to dendritic labeling from CKII mRNA labeled neurons lying beneath the neuropil labeling. Since most of the heavily labeled neurons were within layers II–IV, neuropil labeling is likely to be primarily derived from these neurons. However, some layer V neurons also displayed high expression of the CKII gene and may also contain high levels of dendritic labeling, specifically localized to dendritic regions contained within superficial layers. Selective localization of an mRNA to particular subcellular regions and specifically in some but not all of the dendritic tree has been demonstrated for MAP-2 in cultured neurons (Kleiman et al., '90). In addition, the mRNA for some molecules is heavily concentrated in the dendrites of neurons whose somata show only light labeling (Garner et al., '88). The laminar pattern of CKII mRNA, showing heavy neuropil labeling only in superficial layers, suggests that CKII may be more readily available at postsynaptic sites within superficial layers than in deep layers. This may then allow for functional separation of inputs to layers I–IV from those projecting to layers V and VI. As has been previously noted (Ouimet et al., '84; Vallano, '88; Fukunaga et al., '89), the laminar pattern of CKII labeling corresponds to the distribution of NMDA

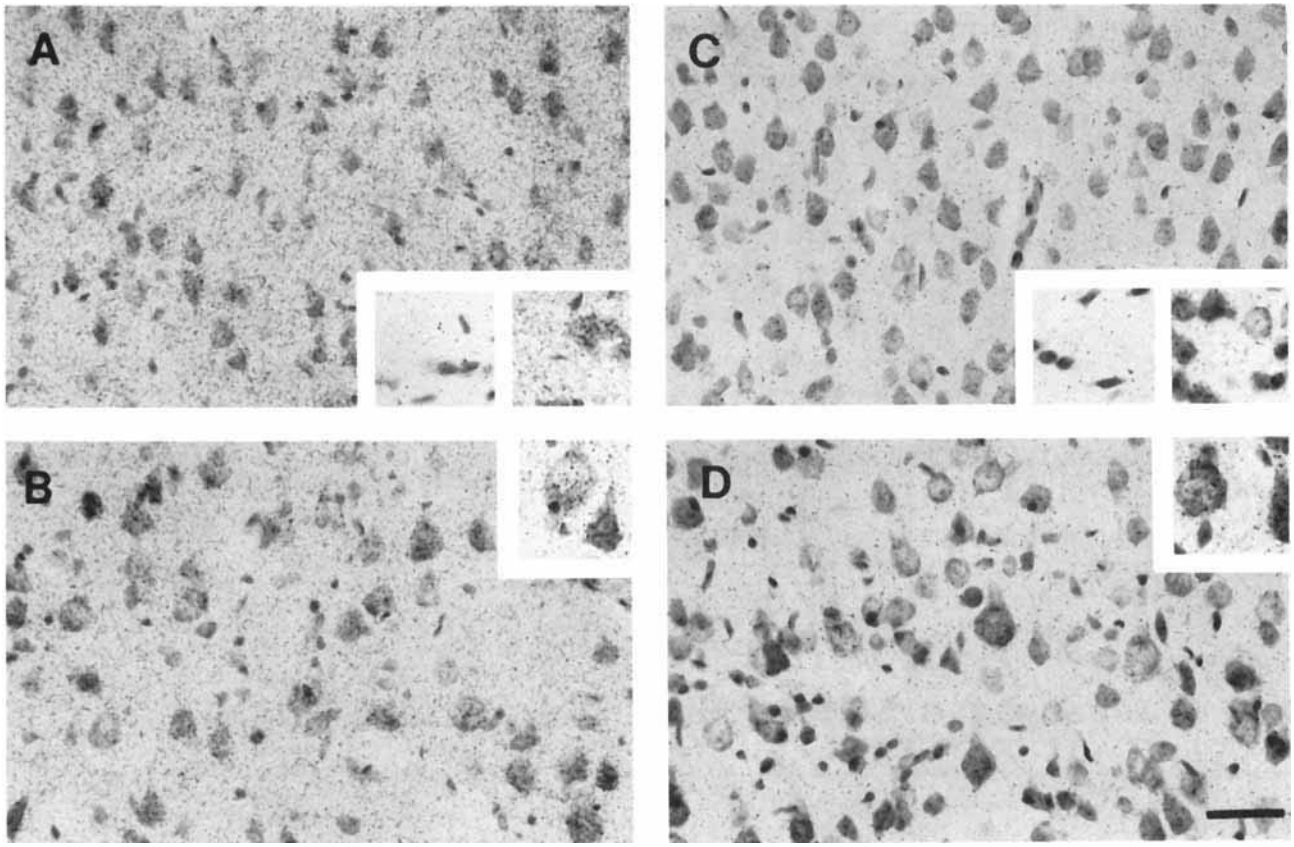

Fig. 4. High power light photomicrographs show difference between superficial and deep neocortical layers for CKII and GAP43 mRNA labeling. **A:** CKII mRNA in neocortical layers II/III. Nearly every neuron is heavily labeled. Neuropil is also heavily labeled. **B:** CKII mRNA in deep layer V of neocortex. In contrast to superficial layers neuropil is not as heavily labeled. **C:** GAP43 mRNA in neocortical layers II/III. Labeling is near background levels for both neurons and neuropil. **D:** GAP43 mRNA in layer V of neocortex. Only large layer V neurons are heavily labeled. **Insets** show same probe and lamina (for a different section) at higher magnification and also show subcortical white matter (left panel of inset in A and C), which is considered to be the level of background labeling. Scale bar = 30 μm, and 23 μm for insets.

receptor binding (Monaghan and Cotman, '85). A link between CKII and NMDA is further supported by evidence that changes in neuronal activity that evoke NMDA receptor activity lead to changes in CKII activity (Goldenring et al., '86; Anderson et al., '89; Churn et al., '89).

There appear to be distinct species differences in distribution of both CKII and GAP43 mRNAs. The pattern of CKII gene expression in monkey visual cortex is slightly different from that in rat frontoparietal cortex, with the monkey displaying lower expression of the mRNA in layer III, and higher expression in parts of layer IV of area 17 and in layer VI of area 18 (Benson et al., '91). These differences allow a distinction between layers II and III and the subdivisions of layer IV. The immunocytochemical staining for CKII in monkey closely follows this pattern of gene expression. Differential labeling within layer IV and between primary and secondary visual areas may reflect specializations in layer IV that are not apparent in rats.

By contrast, the laminar pattern of GAP43 gene expression in adult rat is dramatically different from that seen in adult human brain, in which layer II showed heavy labeling for the identical GAP43 mRNA probe (Neve et al., '88), while deeper layers showed very little labeling. A difference between species in the neocortical areal pattern of GAP43 mRNA expression is also evident. In human brain GAP43 expression is greater in association cortex than in primary sensory or motor areas (Neve et al., '88). Our results show that in the rat GAP43 is expressed at high levels in layer V throughout neocortex, including primary sensory and motor areas. These differences may reflect greater specialization in human neocortex or a change in locus of potential plasticity mechanisms.

The differential distribution of CKII and GAP43 mRNA (Fig. 5) indicates that superficial neocortical layers may contain different potential mechanisms for plasticity than do the deep layers. This suggestion is supported by physiological studies. Komatsu et al. ('88) have found that in kitten visual cortex, LTP elicited via electrical stimulation of thalamic afferents is more prominent in the superficial than in deeper layers. Similarly, Iriki et al. ('89) have found that by stimulating SI inputs to MI, LTP can be produced in the superficial layers but not in the deep layers. By contrast, Baranyi and Szente ('87) have found that by pairing intracellular depolarization and antidromic activation, a form of potentiation can be produced in layer V pyramidal tract neurons of MI. Thus, physiologically neocortical poten-

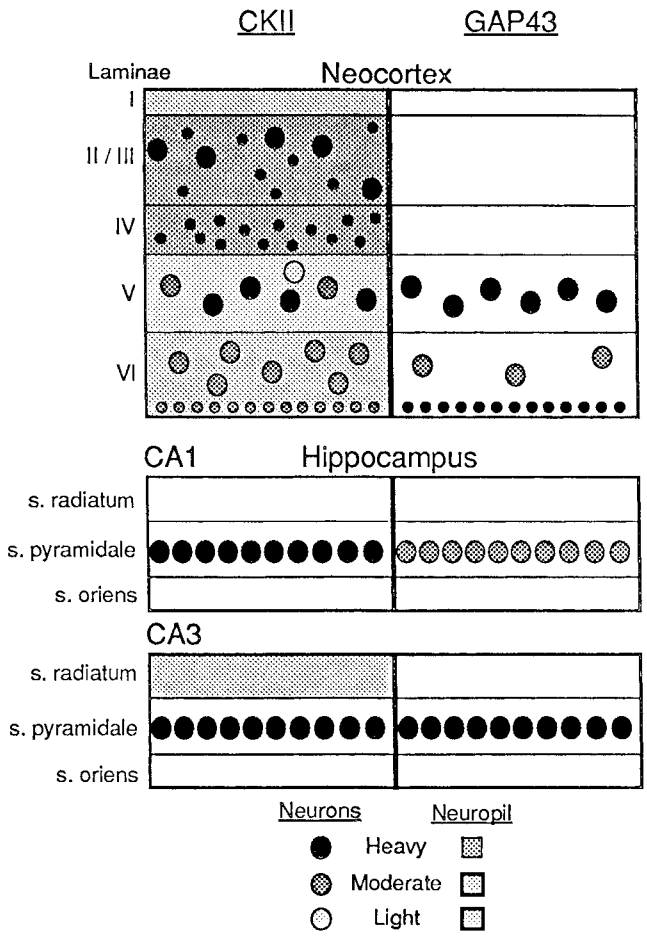

Fig. 5. Schematic diagram of laminar pattern of CKII and GAP43 mRNA distribution within neocortex and hippocampus. Circles indicate labeling of neuronal somata for either large or small cells. Background patterns represent neuropil labeling. Some cell populations showed heavy labeling for both probes, suggesting that some neurons contain both CKII and GAP43 mRNA. Within neocortex, large layer V neurons were heavily labeled for both CKII and GAP43 mRNA. Within the hippocampus, pyramidal neurons, particularly within CA3, expressed high levels of both CKII and GAP43 mRNA.

tiation or plasticity appears to require laminar-specific induction methods, which reflect distinct forms of cortical plasticity that may be related to the laminar-specific patterns of GAP43 and CKII gene expression.

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