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Regional Expression and Cellular Localization of the Na^+-Dependent Inorganic Phosphate Cotransporter of Rat Brain

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We have recently isolated and identified a brain specific Na^+-dependent inorganic phosphate (P_i) cotransporter cDNA (rBNPI) from a rat brain cDNA library (Ni et al., 1994). We now report the regional and developmental expression, as well as the cellular localization, of rBNPI mRNA in the rat brain. *In situ* hybridization histochemistry reveals that rBNPI mRNA is selectively expressed in neuron-enriched regions of the adult rat brain, such as the cerebral cortex, hippocampus, and cerebellum. Cellular localization of rBNPI transcripts reveals expression in both pyramidal and granule neurons in these regions. By contrast, little to no hybridization signal was observed in white matter-enriched areas such as the corpus callosum. The expression of rBNPI mRNA was determined during pre- and postnatal development of the rat CNS. From embryonic day 17 to early postnatal day 10 (PND 10), there is a rather widespread but diffuse pattern of rBNPI expression in brain. During late postnatal development, however, the expression of rBNPI mRNA becomes confined to discrete populations of neurons in the cerebral cortex, hippocampus, and cerebellum. Thus, rBNPI expression is developmentally regulated and abundant levels of mRNA are found in rather discrete populations of neurons in the adult rat brain. The latter suggests that rBNPI may serve to selectively regulate intracellular P_i transport in certain neurons for either metabolic and (or) signaling events.

[Key words: brain Na^+-dependent inorganic phosphate cotransporter, regional and developmental expression, phosphate transport and cellular metabolism, NMDA receptor]

Inorganic phosphate (P_i), a charged anion, essential for many cellular metabolic and signaling events, is also a constituent of virtually all cell membranes. Phosphate homeostasis in the body depends primarily on mechanisms that govern the renal excretion of P_i into the glomerular filtrate and its subsequent reabsorption against an electrochemical gradient via brush-border epithelial cells located in the proximal tubule of the kidney (Bonjour and Caverzasio 1985; Gmaj and Murer 1986; Dennis 1991). This transepithelial transport of P_i is mediated, in part, by a P_i transport system which is driven by the transmembrane Na^+ gradient across the microviller brush border membrane (Gmaj and Murer, 1986). In the kidney the Na^+-dependent P_i cotransport system is highly developed and regulated both by $[P_i]_e$ and by a variety of hormones and metabolic factors (Mizgala and Quamme 1985; Berndt and Knox 1991). Experiments using isolated kidney tubules or brush-border membranes have shown, for example, that depletion of extracellular P_i or increased circulating levels of parathyroid hormone alter the activity and expression of transporter molecules (Strickler et al., 1964; Gmaj and Murer 1986; Dennis 1991) or both. Further, chronic P_i depletion *in vivo* is associated with a significant reduction in the ATP content of many cells including polymorphonyclear leukocytes (Craddock et al., 1974), platelets (Yawata et al., 1974), and various tissues including kidney (Kreusser et al., 1978), heart (Brautbar et al., 1982), and skeletal muscle (Brautbar et al., 1983). This reduction in intracellular ATP has been shown to be a direct consequence of the decrease in intracellular P_i which occurs following P_i depletion (Kreusser et al., 1978; Brautbar et al., 1982, 1983). Moreover, differences in plasma P_i concentrations among various species have been correlated with differences in their basal metabolic rates such that higher plasma P_i concentrations occur in species that exhibit high rates of O_2 consumption and adenosine triphosphate (ATP) production (Sestoft, 1979). Similar tight coupling between P_i transport and ATP production has also been observed in cultured peripheral vagal nerves (Anner et al., 1976; Straub, 1979).

Despite a considerable body of information that has been generated on Na^+-dependent P_i cotransport in the kidney, and to a lesser degree the small intestine (for review, see Dennis 1991), little is known about Na^+-dependent P_i cotransport in other tissues—especially the CNS. In the course of studies designed to identify genes that are differentially expressed following exposure of cultured rat cerebellar granule neurons to subtoxic concentration of NMDA, we have recently cloned and characterized a brain-specific Na^+-dependent P_i cotransporter with transport kinetics similar to that reported for the kidney transporter of various species (Ni et al., 1994). Although the exact physiological significance of this transporter is unknown, we have postulated that the neuronal Na^+-dependent P_i cotransporter may serve to regulate the concentration of intracellular P_i necessary for maintaining the relatively high phosphorylation potential of neurons. We now describe the developmental and regional expression of this Na^+-dependent P_i cotransporter

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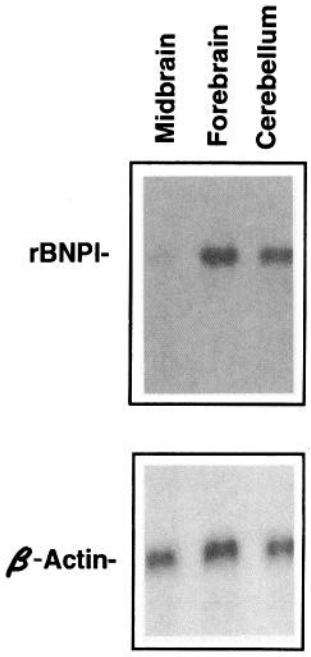

Figure 1. Northern analysis of rBNPI mRNA detected in rat brain RNA by cDNA clone rBNPI. rBNPI transcript is abundantly expressed in RNA extracted from the rat forebrain and cerebellum but relatively low levels of the transcript are found in RNA extracted from the midbrain. *A*, total RNA (20 μg per lane) from adult rat brain regions was separated on a 1.5% agarose gel containing 6% formaldehyde and blotted onto a nylon membrane (see text for details). The rBNPI cDNA probe detected one mRNA species of 2.8 kb. *Lane 1*, midbrain; *lane 2*, forebrain; *lane 3*, cerebellum. *B*, The same Northern blot as shown in *A* was stripped and reprobed with a β-actin cDNA probe as described in Materials and Methods.

(rBNPI) in rat brain using *in situ* hybridization histochemistry. Cellular localization of BNPI mRNA confirm the selective expression of transcripts in neurons of the hippocampus, cerebral cortex, and cerebellum.

Materials and Methods

Probe labeling. For Northern blotting, the rBNPI cDNA was labeled with ^{32}P dCTP using random primer labeling as described by the manufacturer (Clontech). Riboprobes were synthesized by *in vitro* transcription of the rBNPI cDNA as described by Stratagene and used for *in situ* hybridization histochemistry. Briefly, ^{35}S-labeled sense and antisense RNA probes were synthesized from rBNPI Bluescript after linearization with XbaI and BstXI, using T3 and T7 RNA polymerase, respectively. RNA probes were shortened to an average length of 150 bp by alkaline hydrolysis prior to hybridization.

Northern blot analysis. Total RNA was extracted from brain tissue (forebrain, brainstem, and cerebellum) of adult male Sprague–Dawley rats or from rat brain of different ages and separated by electrophoresis on 1.5% agarose gels containing 6% formaldehyde and blotted onto nylon membranes. Blots were hybridized at 42°C for 2 hr in buffer containing 50% formamide, 5 × SSPE, 10 × Denhardt's, 2% SDS, and 100 μg/ml salmon sperm DNA. Hybridization was carried out overnight in the same buffer containing denatured ^{32}P-labeled cDNA from clone rBNPI (Ni et al., 1994). Blots were washed at 50°C in 2 × SSC, 0.1% SDS and exposed to Kodak X-OMAT film at −70°C with Cronex Lightning Plus intensifying screen.

In situ *hybridization.* Rat brains were removed and frozen in cold 2-methylbutane (−20°C) for 1 min and stored on dry ice. Frozen sections (15 μm) were prepared on a cryostat, mounted onto poly-L-lysine-coated glass slides and dried at room temperature. Prior to hybridization, sections were warmed to 25°C, fixed in 4% formaldehyde, and immersed for 10 min in 0.25% acetic anhydride, 0.1 M triethanolamine hydrochloride, 0.9% NaCl (Ni et al., 1994). Tissue was then dehydrated through an ethanol series, delipidated in chloroform, rehydrated and air dried. The ^{35}S-labeled riboprobes were added to a hybridization buffer composed of 50% formamide, 0.3 M NaCl, 20 mM Tris-HCl (pH 8), 5 mM EDTA, 500 μg tRNA/ml, 10% dextran sulfate, 10 mM dithiothreitol, and 0.02% each of bovine serum albumin, ficoll, and polyvinylpyrrolidone (Ni et al., 1994). Coverslips were placed over sections which were first covered with hybridization buffer containing ^{35}S-labeled probe (sense probe was used for control). The slides were incubated in a humidified chamber overnight at 55°C. They were subsequently washed several times in 4 × sodium chloride–sodium citrate buffer (SSC) to remove coverslips and hybridization buffer, dehydrated, and immersed in 0.3 M NaCl, 50% formamide, 20 mM Tris-HCl, and 1 mM EDTA at 60°C for 15 min. The sections were then treated with RNase A (20 μg/ml) for 30 min at room temperature, followed by a 15 min wash in 0.1 × SSC at 55°C. Finally, the slides were air dried and apposed to Hyperfilm-beta Max (Amersham) for 2 d. For analysis at the cellular level, the slides were dipped in Kodak NTB3 nuclear emulsion, stored with desiccant at 4°C for 10 d, developed, and counterstained with hematoxylin/eosin-phloxine for microscopic evaluation.

Results

In our previous report, we described the isolation of a Na⁺-dependent P_i cotransporter cDNA (rBNPI) using a subtractive cDNA cloning strategy (Ni et al., 1994). Using Northern blot analysis (Fig. 1), we confirmed that rBNPI is abundantly expressed in RNA extracted from the rat forebrain and cerebellum but relatively low levels of the transcript are found in RNA extracted from the midbrain. We used *in situ* hybridization to more precisely localize rBNPI mRNA in regions of the rat brain. A series of coronal cryostat-prepared tissue sections (from forebrain to cerebellum) of the adult rat brain were probed with a ^{35}S-labeled antisense cRNA probe (see Materials and Methods). Following exposure to x-ray film for 2 d, hybridization with the rBNPI probe resulted in a strong hybridization signal in neuron-enriched regions of the cerebral cortex (CTX) (Fig. 2*B–D*), hippocampus (HP) (Fig. 2*C,D*), and cerebellum (CB) (Fig. 2*E,F*). A weak hybridization signal was detected in regions of the diencephalon, including the ventral posterior thalamic nucleus (VP) and medial habenular nucleus (MHb) (Fig. 2*C*). A dense hybridization signal was also observed in the cortex piriformis (PIR) (Fig. 2*B*) and the olfactorius anterior (OAL) (Fig. 2*A*). In the amygdaloid complex, the highest level of rBNPI mRNA is found in the region of the lateral amygdaloid nucleus (AL) (Fig. 2*C*). As shown in Figure 2, *E* and *F*, labeling is also prominent in the principal oculomotor nucleus (PON), entorhinal cortex (Ent), and selective neurons of the brainstem (BS). No hybridization signal was detected in the caudate putamen (CPu) and glia or myelin-enriched areas such as the corpus callosum (cc) (Fig. 2*A–D*) or the deep white matter of the cerebellum (wm) (Fig. 2*E,F*). Moreover, no hybridization signal was observed in any brain region probed with an ^{35}S-labeled sense probe (data not shown).

The localization of rBNPI transcripts was further analyzed at the cellular level at higher magnification using liquid photographic emulsion. As illustrated in Figure 3, rBNPI mRNA is wildly distributed in different layers of the cerebral cortex (II–VI) and is distinctively more abundant in layers V, VI (Fig. 3*B*) where a distinct hybridization signal is observed over both pyramidal and nonpyramidal neurons (Fig. 3*C,D*). No labeling is detected in the molecular layer (layer I) where there are no neuronal cell bodies. The hippocampus is densely labeled compared to other brain regions, where the hybridization signal is more apparent in the CA2 and CA3 regions of the hippocampus

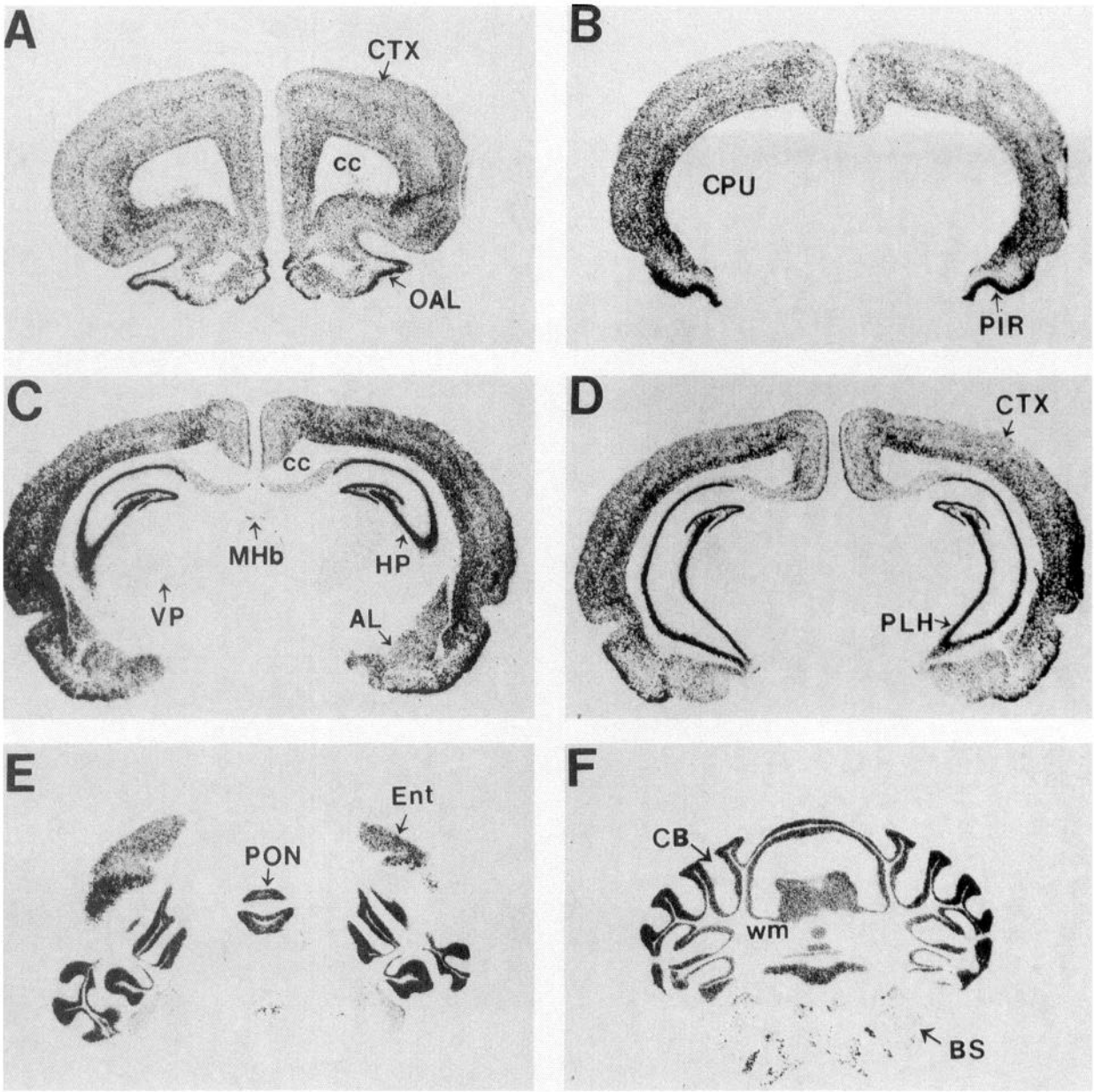

Figure 2. Distribution of rBNPI mRNA in the adult rat brain analyzed by *in situ* hybridization histochemistry. Hybridization with the rBNPI probe revealed a strong hybridization signal in neuron-enriched regions of the cerebral cortex (*CTX*) (*C, D*), hippocampus (*HP*) (*C, D*), and cerebellum (*CB*) (*E, F*). A weak hybridization signal was detected in regions of the diencephalon, including the ventral posterior thalamic nucleus (*VP*), and medial habenular nucleus (*MHb*) (*C*). Serial coronal tissue sections (from forebrain to cerebellum) of the adult rat brain were prepared using a cryostat and hybridized with a ^{35}S-labeled rBNPI antisense riboprobe. The hybridization buffer is composed of 50% formamide, 0.3 M NaCl, 20 mM Tris-HCl (pH 8), 5 mM EDTA, 500 μg tRNA/ml, 10% dextran sulfate, 10 mM dithiothreitol, and 0.02% each of bovine serum albumin, ficoll, and polyvinylpyrrolidone. The slides were incubated in a humidified chamber overnight at 55°C and then washed several times in 4 × sodium chloride–sodium citrate buffer (SSC). Sections were exposed directly to x-ray film for 2 d. The hybridization signal was visualized by bright-field microscopy.

(Fig. 4). An intense signal is observed in the pyramidal layer of the hippocampus (PLH) and the granule layer of the dentate gyrus (GLD) (Fig. 4*A,B*). The latter is most apparent over individual pyramidal and granule neurons in their respective layers (Fig. 4*C,D*). In the cerebellum, rBNPI mRNA is present in the granule cell layer (GL) (Fig. 5*A,B*), but is undetectable in the molecular layer (ML) and larger projection neurons such as Purkinje cells. rBNPI mRNA is also present in regions of the cortex piriformis (Fig. 6*A,B*) and brainstem (Fig. 6*C,D*). The hybridization signal detected by the rBNPI probe in these two regions is intense and well localized, suggesting expression in large cells, such as those in the inferior olive (IO) the gigantocellular reticular nucleus (GiV), and the intermediate reticular zone (IRt) of the brainstem (Fig. 6*C,D*).

To characterize the expression of the rBNPI mRNA during CNS development a Northern blot of rat brain RNA isolated from rats of various ages was prepared. The blot was hybridized with both a ^{32}P-labeled rBNPI and β-actin cDNA probe as described in the Materials and Methods. As shown in Figure 7, the relative abundance of the rBNPI mRNA increased during

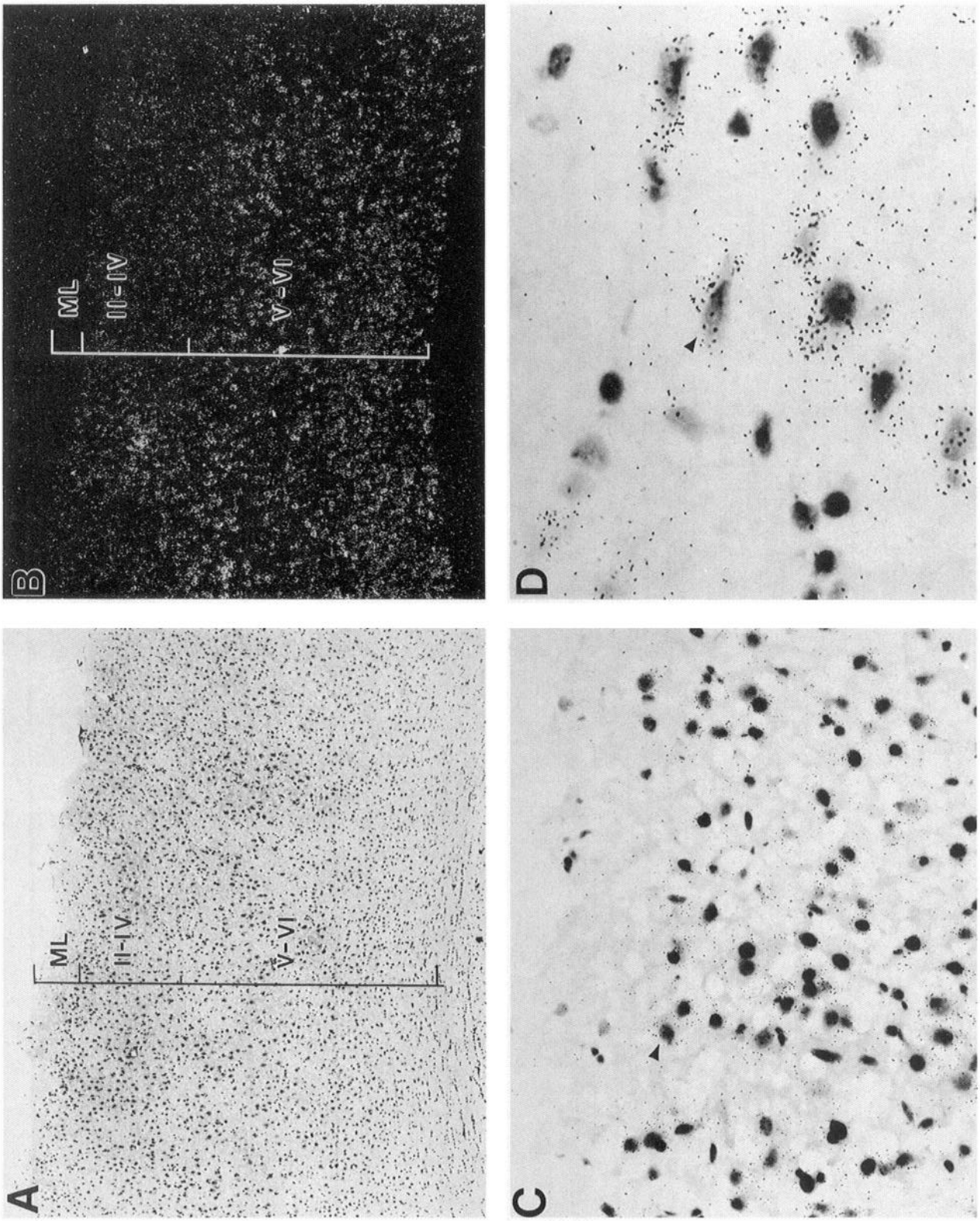

Figure 3. Cellular expression of rBNPI mRNA in layers of the cerebral cortex. Hybridization with the rBNPI antisense probe resulted in a strong hybridization signal in layers of cerebral cortex, particularly in pyramidal layer (layer V) and polymorphic layer (layer VI). The hybridization signal was localized over individual neurons in layers II–IV. Tissue sections were hybridized as described in the Figure 2 caption and stained with Mayer's hematoxylin/eosin-phloxine. The hybridization signal was visualized using bright-field (*A, C, D*) and dark-field microscopy (*B*). *A* and *B*, Cellular layers of the neocortex (14×). *C* and *D*, Pyramidal and nonpyramidal neurons in cortical layers II–IV (285×).

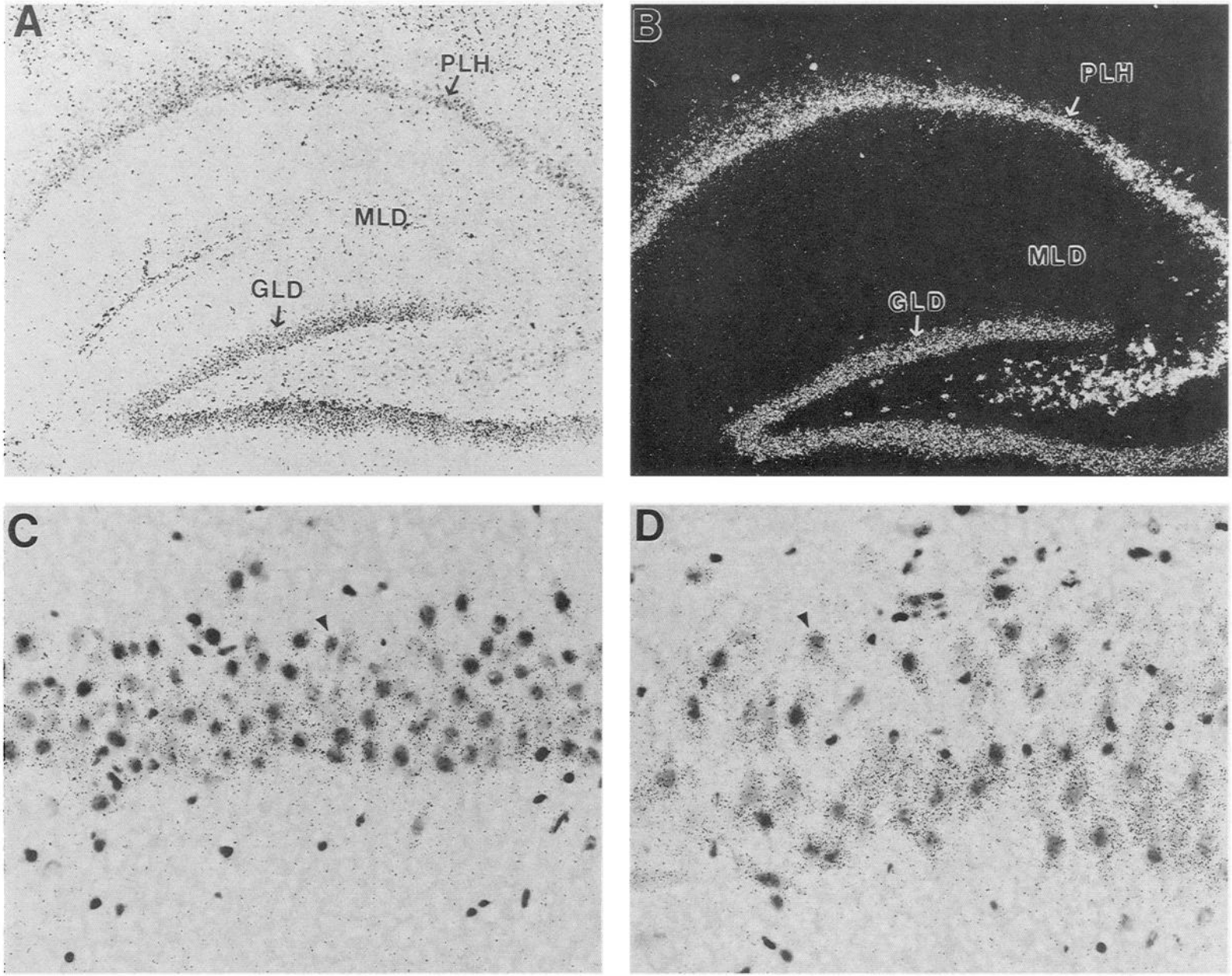

Figure 4. Cellular distribution of rBNPI mRNA in the rat hippocampus. An intense hybridization signal is observed in the pyramidal layer of the hippocampus (*PLH*) and the granule layer of the dentate gyrus (*GLD*). The signal was localized over individual pyramidal and granule neurons in their respective layers. Tissue sections were hybridized as described in the Figure 2 caption and Materials and Methods and stained with Mayer's hematoxylin/eosin-phloxine. *A* and *B*, Cellular layer of hippocampus (72×). *C*, Granule neurons of dentate gyrus (*GLD*) (142×). *D*, Pyramidal neurons of CA3 and CA4 (142×).

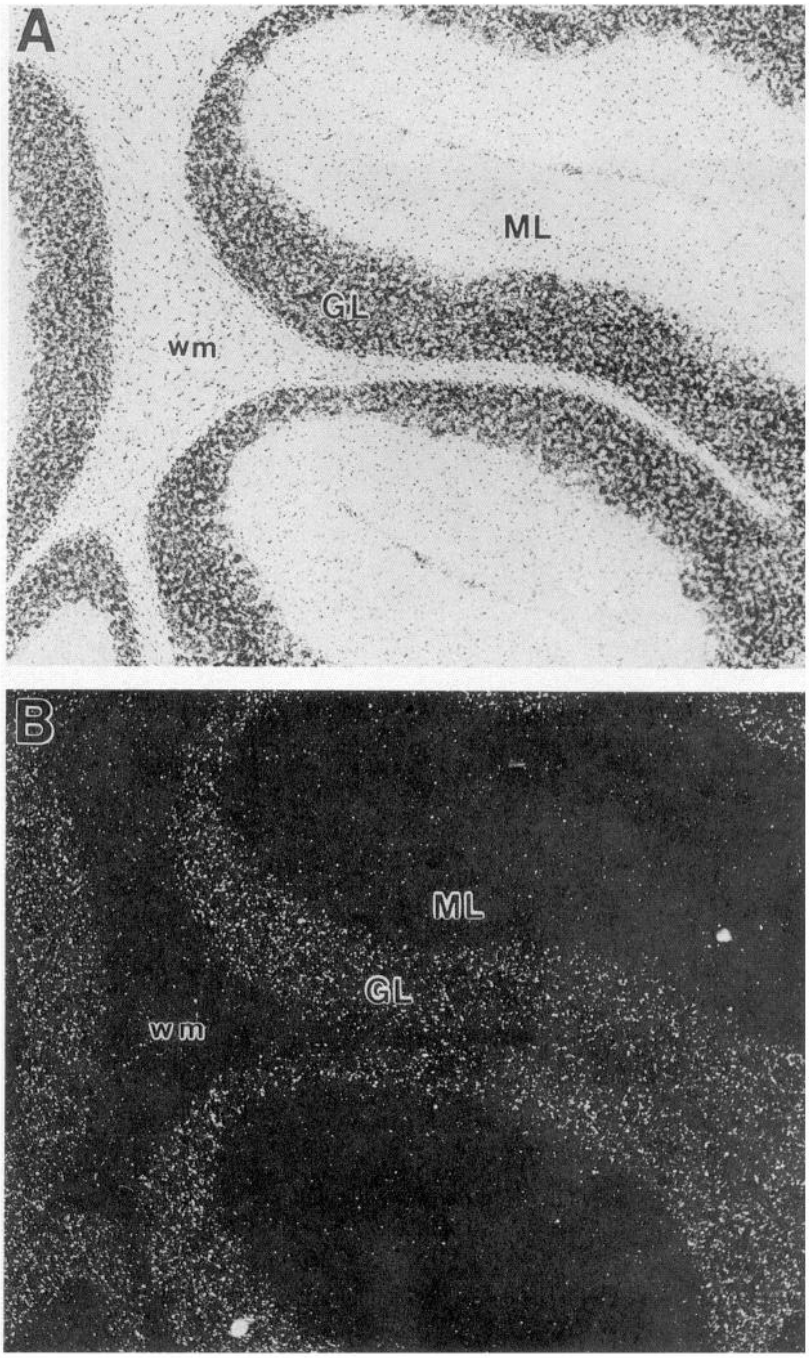

Figure 5. Expression of rBNPI mRNA in the laminar layers of the cerebellum. Dense labeling is observed in the granule layer of the cerebellum (*GL*) (69×, *A* and *B*). No hybridization signal is evident over Purkinje cells, the molecular layer (*ML*), or white matter (*wm*). The hybridization signal was visualized with bright-field (*A*) and dark-field microscopy (*B*). *In situ* hybridization histochemistry was carried out as described in the Figure 2 caption.

development (Fig. 7*A*). The β actin probe confirmed the presence of RNA in each lane. The developmental pattern of expression of rBNPI mRNA was further studied using *in situ* hybridization as illustrated in Figure 8. Both pre- and postnatal stages were examined in this study and included embryonic day 17, and PND 1, 5, 10, 21 (3 weeks), and 28 (4 weeks). The rBNPI mRNA is confined to pyramidal neurons of the hippocampus, neurons of the cerebral cortex and granule layer of the cerebellum after PND 10. By contrast, rBNPI mRNA is expressed rather ubiquitously during early neuronal development (from embryonic day 17 to PND 5) with higher levels of expression in the cortical plate (CP), ventricular zone (vz) and hippocampal formation (HP) and moderate levels in midline structures such as various thalamic and hypothalamic nuclei (tn). By PND 10, however, rBNPI mRNA is restricted in its pattern of expression, being most abundant in the cerebral cortex, hippocampus, and cerebellum. Low levels of expression are detected in midline structures.

As illustrated in Figure 9, expression of rBNPI appears to be related to the development of the cerebral cortex and hippocampal formation. The rBNPI hybridization signal is apparent at embryonic day 17 in the ventricular zone (VZ) and is more abundant in the outer layer of the cortical plate (CP) which develops in an "inside-out" pattern (Fig. 9*A–C*). By PND 1 rBNPI mRNA appears to be localized over the pyramidal cell layer of the hippocampus and the granule cell layer of the dentate gyrus. This pattern becomes sharply defined by PND 10.

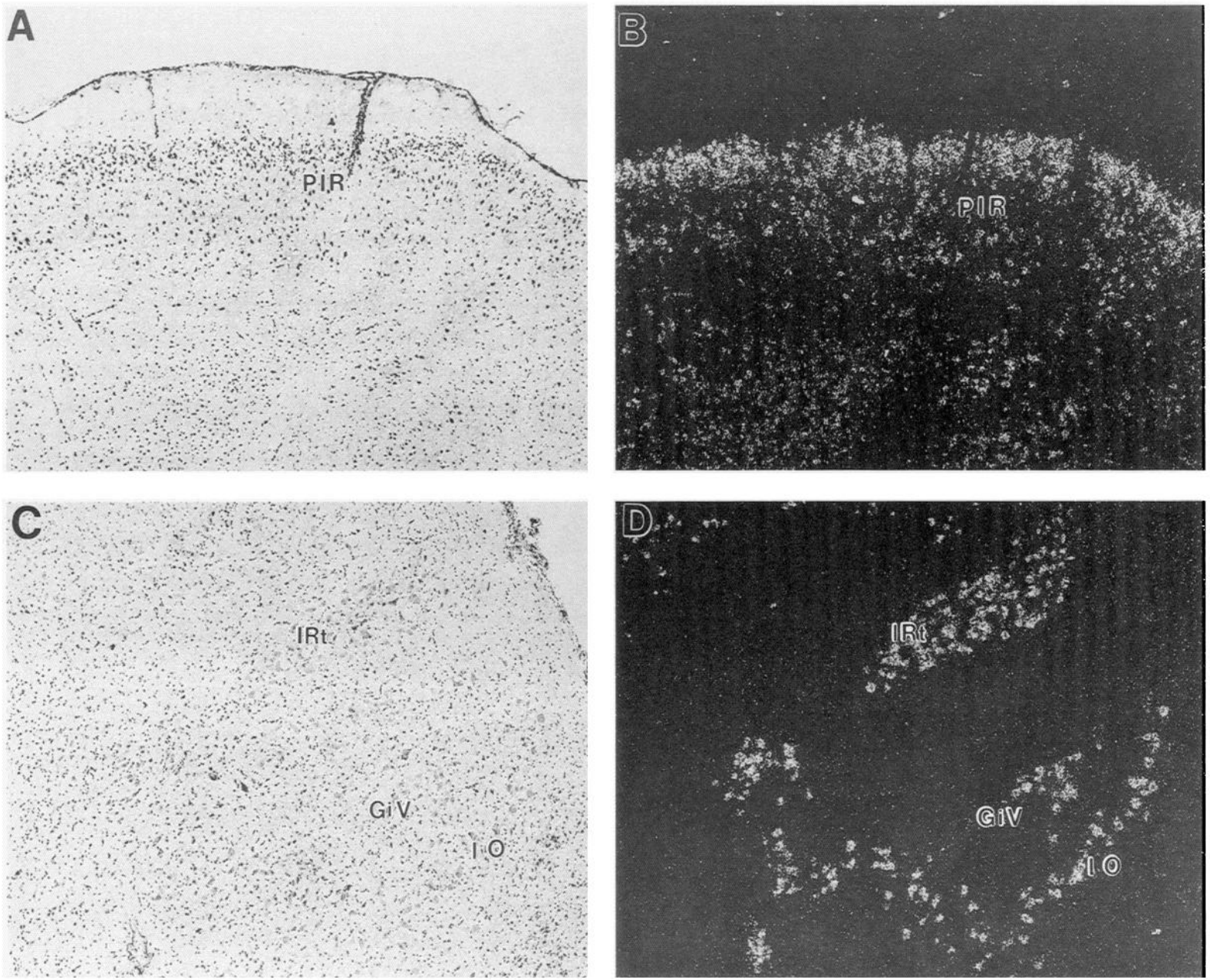

Figure 6. Cellular localization of rBNPI mRNA in the cortical piriformis and brainstem. The hybridization signal detected by rBNPI is present in very discrete population of neurons in these two regions, such as those in the inferior olive (*IO*) and the intermediate reticular zone (*IRt*), as well as in the gigantocellular reticular nucleus (*GiV*) of brainstem. *In situ* hybridization histochemistry was carried out as described in the Figure 2 caption. The hybridization signal is visualized under bright-field (*A* and *C*) and dark-field (*B* and *D*) microscopy. *A* and *B*, Cortical piriformis (60×). *C* and *D*, Brainstem (90×).

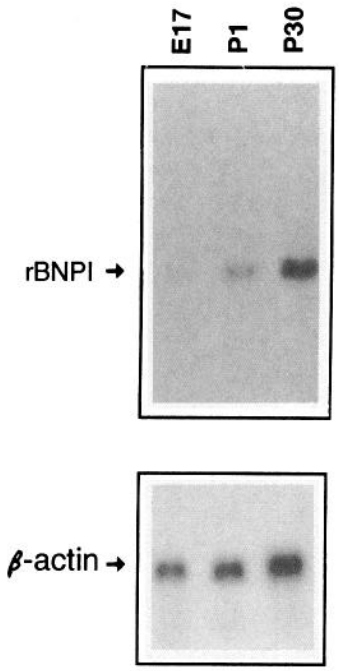

Figure 7. Developmental expression of rBNPI mRNA in the rat brain revealed by Northern blot analysis. Total RNA (20 μg per lane) was isolated from whole rat brain at various developmental stages and hybridized with a ^{32}P-labeled rBNPI riboprobe (see Materials and Methods for details). The Northern blot analysis showed that level of the rBNPI mRNA increased during brain development. *A*, Northern blot probed with the rBNPI riboprobe. *B*, The same Northern blot as shown in *A* probed with human β-actin riboprobe. *Lane 1*, Embryonic day 17 (*E17*); *lane 2*, postnatal day 8 (*P8*); *lane 3*, postnatal day 30 (*P30*).

Discussion

Despite the importance of P_i for a variety of cellular metabolic events, the precise mechanisms responsible for P_i transport in cells of the CNS are poorly understood. Our recent isolation and identification of a cDNA encoding a brain-specific Na^+-dependent P_i cotransporter (rBNPI) suggests a role for this transporter in maintaining intracellular [P_i] within the CNS (Ni et al., 1994). In the present study, we have confirmed and extended our previous findings and demonstrate a rather discrete and well-localized expression of rBNPI in regions of the adult rat brain. Moreover, it appears that rBNPI encodes both a brain-specific as well as neuron-specific Na^+-dependent P_i cotransporter (see below). A possible role for this neuronal P_i transport system in maintaining $[P_i]_i$ for a variety of intracellular metabolic and signaling events involving P_i, including ATP biosynthesis and second messenger/signaling is currently being investigated.

The expression of rBNPI mRNA in neurons of the CNS was confirmed at both the macroscopic and cellular level(s) in the hippocampus, cerebral cortex, and cerebellum. The discrete labeling patterns observed in these regions are similar to those of several neuron-specific genes such as neurofilament and calmodulin (Ni et al., 1992), and are distinctively different from those of glia-specific genes like glial fibrillary acid protein (GFAP) and S100 (Landry et al., 1989). The restricted pattern of expression and the cellular localization of rBNPI mRNA suggests that the Na^+-dependent P_i cotransporter is predominately concentrated in principle neurons of the cerebral cortex, hippocampus and cerebellum, although the significance of this discrete pattern of expression vis-à-vis the role of rBNPI in these neurons is still unclear. Since it is well established, however, that the kidney Na^+-dependent P_i cotransporter mediates, at least in part, the transepithelial transport of P_i from the tubule lumen into the systemic circulation in order to maintain total body P_i homeostasis (Dennis, 1991; Murer et al., 1991), we have speculated that rBNPI may serve a similar physiological role in regulating intraneuronal P_i levels. Inorganic phosphate is known to be a primary substrate for ATP biosynthesis and several lines of evidence suggest that reducing Na^+-dependent P_i uptake in neurons inhibits the synthesis of ATP (for review, see Straub et al., 1979). Thus, regulation of neuronal P_i uptake may serve to govern the intracellular pool of P_i necessary for maintaining the phosphorylation potential of neurons (Nestler and Greengard, 1983).

In addition to its possible role in ATP biosynthesis P_i has been shown to regulate cytosolic [Ca^{2+}] in synaptosomes (Massry et al., 1991). In this regard, intracellular P_i has also recently been reported to enhance the ATP-dependent binding of Ca^{2+} to brain microsomes, resulting in a larger intracellular pool of Ca^{2+} releasable by inositol trisphosphate (Fulceri et al., 1993). Inorganic P_i can also directly participate in the phosphorylation of Na^+/K^+ ATPase (Matsuda and Iwata, 1987), increasing enzyme activity and thus facilitating the membrane sodium pump, so important to maintaining the resting membrane potential of neurons.

Our previous work has shown that preincubation of cerebellum granule neurons with subtoxic concentrations of NMDA results in increased expression of rBNPI mRNA (Ni et al., 1994). We have also found that exposure of cultured cerebral cortical neurons to glutamate inhibits P_i uptake in these neurons (in preparation). A comparison of the pattern of expression of rBNPI with that of Ca^{2+} channel-linked NMDA receptors (NR1, NR2a, NR2b, NR2c, and NR2d) reveals similarities between rBNPI and NR2a, both of which are predominately expressed in the hippocampus, cerebral cortex, and cerebellum of the rat brain (Meguro et al., 1992; Monyer et al., 1992). Moreover, at the cellular level, both mRNAs are expressed in the same type of neurons including those in the cerebral cortex, hippocampus, and cerebellum, as well as the amygdaloid and thalamic nuclei (Monyer et al., 1992). It is conceivable, therefore, that NMDA receptors (comprised of NR2a) are expressed in neurons along with rBNPI and that P_i transport is somehow coupled to the activity of NMDA receptors in these neurons.

Analysis of the developmental pattern of rBNPI mRNA expression in the rat brain reveals two basic patterns. In the earliest stages of development rBNPI mRNA is present at E17, and is ubiquitously expressed from E17 to P10. Although this uniform expression of rBNPI is characteristic of early brain development (E17–P10), rBNPI expression is still restricted to neuron-enriched areas such as the cortical plate and ventricular zone whereas little to no mRNA is detected in glia-enriched areas such as the corpus callosum. rBNPI mRNA present in the cell-dense neuroepithelium at E17 persists in neuron-enriched areas of the mature brain such as the granule cell layer of the dentate gyrus and the cerebral cortex. The latter finding suggests that rBNPI may be playing a role in neural proliferation and maturation. At later stages of development rBNPI expression changes to a more discrete pattern beginning approximately three weeks after birth, where high levels of mRNA are observed in neurons of the hippocampus, cerebral cortex and cerebellum and lower levels in the corpus striatum

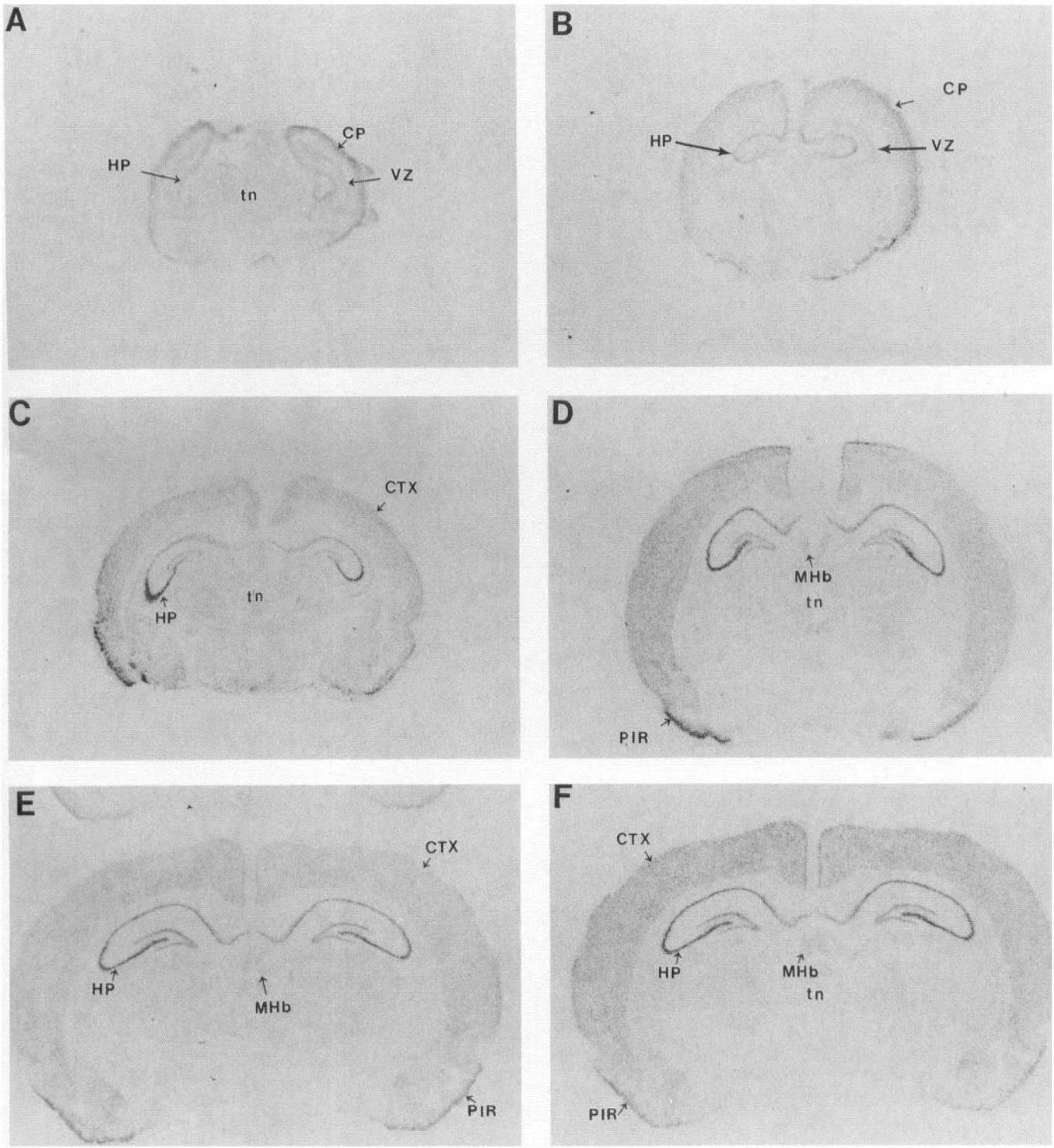

Figure 8. Expression of rBNPI mRNA during development of the rat brain. Cryostat-prepared tissue sections were hybridized with a rBNPI riboprobe and processed for *in situ* hybridization histochemistry as described in the Materials and Methods. The hybridization signal was visualized by bright-field microscopy. Six developmental stages were included: embryonic day 17 (*A*) and postnatal days 1 (*B*), 5 (*C*), 10 (*D*), 21 (*E*), 28 (*F*). Note the rather diffuse expression of rBNPI mRNA during early embryonic and postnatal stages compared to the rather discrete (well-localized) expression in the young adult brain.

and thalamus. This rather discrete pattern of expression persists in the oldest brain examined in the present study (PND 60). Interestingly, this switch from a more diffuse to discrete pattern of expression is similar to that observed for the NMDA receptor subunit NR2. NR2b is ubiquitously expressed during early brain development, and then decreases 2 weeks after birth; whereas NR2a is expressed around birth and develops a more discrete pattern of expression in the adult (Laurie and Seeburg,

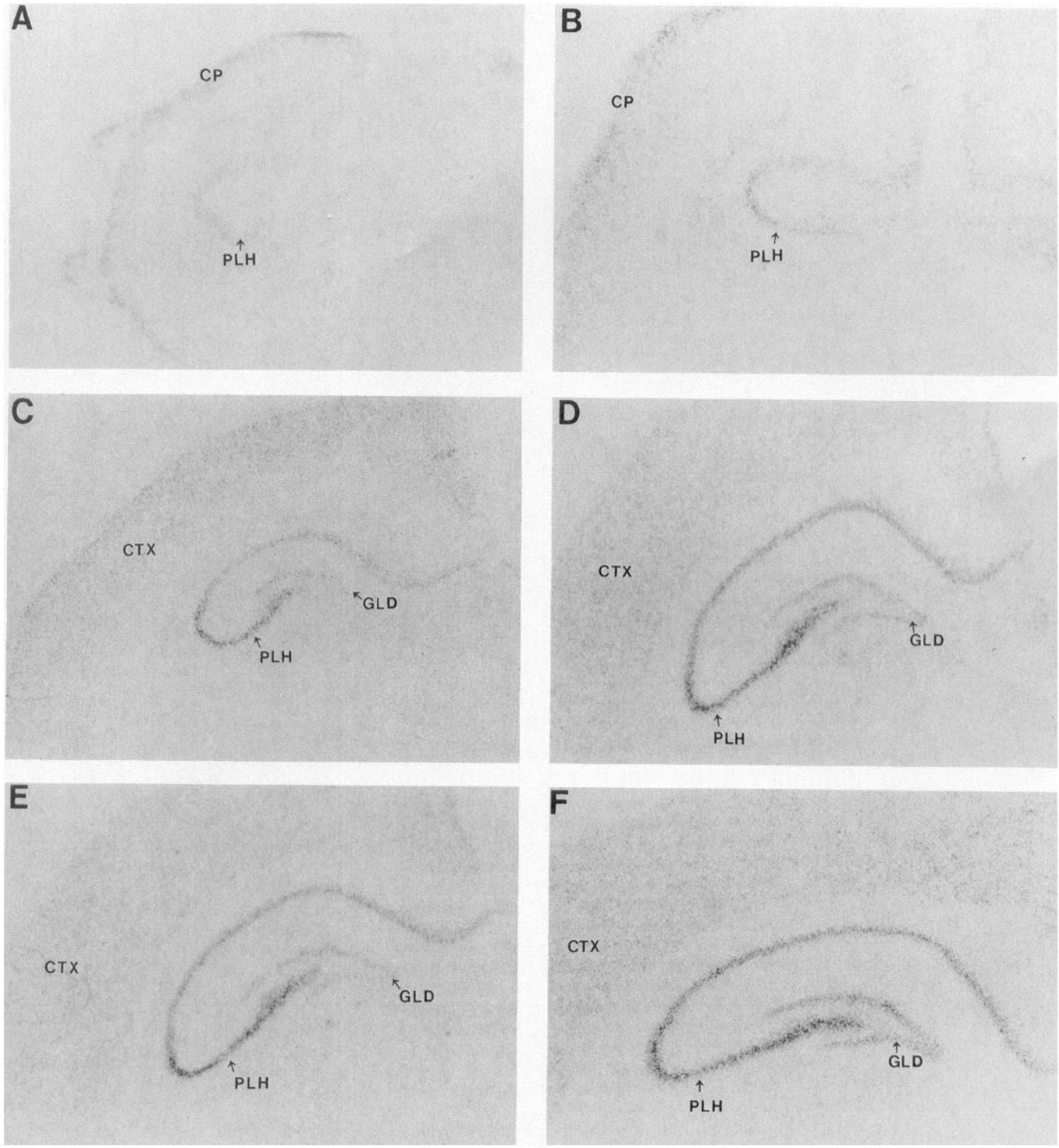

Figure 9. Expression of rBNPI mRNA in the developing hippocampus. Tissue sections were hybridized with rBNPI riboprobe as described above. The hybridization signal was visualized by bright-field microscopy. Note the neuronal expression pattern of rBNPI mRNA during the development of hippocampus formation.

1994). Whether the similarities of rBNPI and NR2 expression are simply coincidental or reflect some functional relationship between NMDA receptors and Na^+-dependent P_i transport is currently being investigated.

Finally, Brown and colleagues have recently identified and cloned a Ca^{2+}-sensing receptor from the parathyroid gland which is also expressed in brain (Brown et al., 1993). Conceivably, similar receptors for PO_4^- may be expressed within the CNS and, if so, the function of rBNPI may be similar to other neuronal Na^+-dependent neurotransmitter transporters, that is, to reduce the extracellular concentration of substrate. Thus, the discrete localization and pattern of expression of

rBNPI (which resembles that of various neurotransmitter receptors) may correspond to a similarly expressed PO_4^- receptor.

Appendix

Abbreviations

AL	lateral amygdaloid nucleus
BS	brainstem
CA1–CA4	fields 1–4 of Ammon's nucleus
CB	cerebellum
cc	corpus callosum
CNS	central nervous system
CP	cortical plate
CPu	caudate putamen
CTX	neocortex
E17	embryonic day 17
Ent	entorhinal cortex
GiV	gigantocellular reticular nucleus
GL	granule layer
GLD	granule layer of dentate gyrus
HP	hippocampus
IO	inferior olive
IRt	Intermediate reticular zone
ML	molecular layer
MHb	medical habenular nucleus
MLH	molecular layer of hippocampus
OAL	nucleus olfactorius anterior
P1	postnatal day 1
P30	postnatal day 30
PIR	piriform cortex
PLH	pyramidal layer of hippocampus
PND	postnatal development
PON	principal oculomotor nucleus
tn	thalamus nuclei
VP	ventral posterior thalamic nucleus
vz	ventricular zone
wm	white matter

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