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NEUROCHEMICAL CHARACTERIZATION OF PATHWAYS EXPRESSING PLASMA MEMBRANE MONOAMINE TRANSPORTER (PMAT) IN THE RAT BRAIN

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ABBREVIATIONS: PMAT, plasma membrane monoamine transporter; CNS, central nervous system; DA, dopamine; NE, norepinephrine; 5-HT, serotonin; OCT, organic cation transporter; PBS, phosphate buffered saline; GAD, glutamate decarboxylase; ChAT, choline acetyl-transferase; TH, tyrosine hydroxylase; HDC, histidine decarboxylase; GABA, gamma-aminobutyric acid;

Abstract Neurotransmitter transporters play an important role in the control of synaptic transmission by ensuring the clearance of released transmitters liberated in the synaptic cleft. In the case of monoaminergic neurotransmitters, this clearance is carried out by high-affinity reuptake transporters located in the plasma membrane of the presynaptic terminals. Recently PMAT, a transporter from the SLC29 (equilibrative nucleoside transporter) family, was shown to transport *in vitro* monoaminergic neurotransmitters, in particular dopamine and serotonin, nearly as efficiently as the high-affinity transporters. This transporter, well expressed in central nervous system, represents an interesting candidate for the control and modulation of aminergic pathways. We performed an extensive study of the distribution of PMAT in the rat brain. Our results highlight PMAT expression in brain regions which play a pivotal role in significant central nervous system functions and human neuropathologies. Using *in situ* hybridization immunohistochemistry co-labeling, PMAT mRNA was found in various neuron subtypes, including glutamatergic neurons of the hippocampus, mitral cells of the olfactory bulbs and GABAergic neurons in the substantia nigra pars reticulata and hypothalamus. Paradoxically, rat PMAT mRNA was found in some but not all monoaminergic nuclei. It was on the contrary predominantly expressed in major cholinergic groups throughout the brain, including brainstem motor nuclei, components of the basal forebrain cholinergic system and cholinergic interneurons of the striatum. These systems, implicated in locomotion, associative and spatial memory and reward-related learning, are disrupted at early stages of Parkinson's and Alzheimer's disease. Taken together, our observations support a role for PMAT in monoamine uptake in cholinergic neurons. Further studies are needed to test this hypothesis and explore the role of PMAT in catecholamine control of cholinergic pathways.

In the central nervous system (CNS), monoaminergic neurotransmission supports major physiological and behavioral functions, including locomotor activity, reward,

attention and mood. An important level of control of the action of monoaminergic neurotransmitters at the synapse is their rapid reuptake into the presynaptic terminals by high-affinity transporters located at the plasma membrane. These high-affinity transporters include the dopamine (DAT), the serotonin (SERT) and the norepinephrine (NET) transporters, which belong to the solute carrier 6A (SLC6A) family and are major targets for psychostimulants, antidepressants and gates for certain neurotoxins (Giros and Caron, 1993; Torres et al., 2003).

Whereas the role of these high-affinity transporters in monoamine neurotransmission is well understood, several lines of evidence support the existence in the CNS of other monoamine transport systems, whose function is still unclear. Early transport studies in cortical, striatal and accumbal tissues or synaptosomal preparations identified components with low-affinity kinetics for the accumulation of dopamine (DA) and norepinephrine (NE) (Hendley et al., 1970; Burrows et al., 1981; Mireylees et al., 1986). Furthermore, investigation of the transport and clearance kinetics of catecholamines in the brains of rat and normal, DAT or NET knock-out mice using specific inhibitors established the existence of heterologous DA and NE uptake, which cannot be merely accounted for by the high-affinity transporters (Elsworth et al., 1993; Wayment et al., 2001) (Mundorf et al., 2001; Moron et al., 2002). These low-affinity systems could contribute to catecholamine uptake in brain regions where the high-affinity transporters are underrepresented. They differ in their basic properties from the SLC6A family, and on the contrary share some of the characteristics of Uptake₂, a sodium- and chloride-independent, low-affinity, high-capacity catecholamine removal system found in peripheral tissues with sympathetic innervation (Iversen, 1965; Hendley et al., 1970; Wilson et al., 1988).

Different categories of transporters have been proposed as candidates for low-affinity monoamine uptake in the brain, including the organic cation transporter (OCT) family (Grundemann et al., 1998; Vialou et al., 2004; Amphoux et al., 2006) and the plasma monoamine transporter (PMAT). PMAT, a transporter initially identified as an equilibrative nucleoside transporter (ENT4/slc29a4), was recently shown to function as a polyspecific organic cation transporter and transport *in vitro* aminergic neurotransmitters with affinities falling in an intermediate range between the affinities of the high-affinity transporters and Uptake₂. This transporter can transport DA and serotonin (5-HT) relatively efficiently, with an affinity in the 100 micromolar range, and to some extent histamine, epinephrine and NE, albeit with less affinity (Engel et al., 2004; Engel and Wang, 2005). Its activity does not rely on the presence of sodium or chloride, and is insensitive to some of the inhibitors of the SLC6A monoamine transporters family, but moderately sensitive to decynium-22, an Uptake₂ inhibitor.

The present study establishes the anatomical distribution of PMAT in rat brain using double *in situ* hybridization/immunohistochemistry labeling. Contrary to expectations, PMAT expression was not restricted to monoaminergic pathways, but predominant in cholinergic systems throughout the brain, supporting a role for PMAT in catecholamine control of cholinergic pathways.

EXPERIMENTAL PROCEDURES

Animals

Animals were housed in an animal-care facility with a 12 h light/dark cycle with freely available food and water. Animal care and maintenance were in accordance with the guidelines of the European Convention for the Protection of Vertebrate Animals.

In situ hybridization

Brains from adult (350 g) male Sprague-Dawley rats were quickly dissected and frozen at -30°C in 2-methylbutane. Sagittal or coronal frozen sections (16 μm) were thaw-mounted onto poly-L-lysine-coated glass slides (Superfrost Plus, Menzel Glaser, Fisher Scientific, Braunschweig, Germany) and stored at -80°C . Oligonucleotide probes (45-mer) were designed to detect specifically mRNAs encoding rat PMAT. Oligonucleotides (sense and antisense) were located respectively at nucleotide positions 227-271, 651-695 and 1152-1196 on predicted rat cDNA (accession number XM_221940). *In situ* hybridization was carried out as described previously (Amphoux et al., 2006). The probes were 3' end-labeled with [α - ^{35}S]dATP (370 GBqmmol; Amersham Biosciences, Piscataway, NJ) using terminal deoxynucleotidyl transferase (Roche Applied Biosystem, Nonnenwald, Germany) at a specific activity of 5×10^8 dpm/ μg . Sections were fixed in 3.7% formaldehyde in phosphate buffered saline (PBS) for 1 hour, washed in PBS, rinsed in water, dehydrated in 70% ethanol and air-dried. Hybridization was carried out at 42°C for 16 h in hybridization medium (Helios Biosciences, Créteil, France) containing a mixture of labeled PMAT sense or antisense oligonucleotides ($4 \cdot 10^5$ cpm/100 μl each). Sections were washed to a final stringency of 0.5X SSC (7.5 mM sodium citrate, 75 mM NaCl, pH 7.0) at 53°C , dehydrated in ethanol, air-dried and exposed to Biomax MR film (Kodak, Rochester, NY) for 6 weeks,

or dipped in photographic emulsion (IlfordK5, Kodak, Rochester, NY) and exposed for 4-6 weeks. Following development, sections were counterstained with hematoxylin-eosin, dehydrated through graded ethanol and mounted under coverslips with Eukitt mounting medium (Electron Microscopy Sciences, Freiburg, Germany).

Antibodies

Antibodies directed against glutamate decarboxylase (GAD), choline acetyltransferase (ChAT) and tyrosine hydroxylase (TH) were from Chemicon International (Temacula, CA). Antibodies against histidine decarboxylase (HDC) were from Insight Biotechnology Limited (Wembley, UK). Biotinylated horse anti-mouse and goat anti-rabbit IgGs were from Jackson ImmunoResearch (West Grove, PA) and Sigma (St Louis, MO), respectively.

***In situ* hybridization immunohistochemistry co-labeling**

For double labeling experiments, brains were processed for *in situ* hybridization as above. After the last wash step, sections were fixed in 4% paraformaldehyde in PBS and preincubated in PBS containing 6% goat serum and 0.1% triton (except for ChAT for which triton was omitted). Sections were next incubated with antibodies directed against either GAD, ChAT, TH or HDC (at dilutions 1/1500, 1/500, 1/250 and 1/2000 respectively) in the same buffer, processed with biotinylated anti-mouse or anti-rabbit IgG antibodies and ABC reagents (Vector Laboratories, Burlingame, CA) and submitted to emulsion autoradiography as above.

RESULTS

PMAT is expressed in several regions of the rat brain

To investigate the regional distribution of PMAT in the adult rat brain, we explored the expression pattern of the corresponding mRNA in brain sagittal and coronal sections using a mixture of three specific radiolabeled oligonucleotides. As shown on film autoradiography, PMAT was found highly expressed in a few discrete regions, including olfactory bulbs (Fig 1A and C), cerebellum (Fig 1A to D, J and K), dentate gyrus of the hippocampus (Fig 1A to D, G and H), diagonal band of Broca in the medial septum (Fig B and E), locus caeruleus (Fig J), motor nuclei in the mesencephalon and rhombencephalon, shown here for facial nucleus (7) (Fig 1B), dorsal motor nucleus of vagus (10) and hypoglossal (12) nucleus (Fig 1A, C and K), and choroid plexus (Fig 1B and F).

Several other regions showed substantial labeling, notably, in the telencephalon, the piriform, lateral entorhinal, insular, ectorhinal, temporal association, retrosplenial granular b and frontal cortices (Fig 1D to H), the CA1 region of the hippocampus (Fig 1B, D, G and H) and the amygdala, with a strong signal in the lateral amygdaloid nucleus (Fig 1G). Labeling was also detected throughout the basal ganglia, in the striatum (Fig 1B, D to F), lateral globus pallidus (Fig 1D and F) and substantia innominata (Fig 1E and F). In the diencephalon, several nuclei were found to express PMAT, in particular, in the thalamus, the medial habenula (Fig 1G), and the anterodorsal (Fig 1B and F), anteromedial and reticular thalamic nuclei (Fig 1F), and in the hypothalamus, the dorsomedial (Fig 1G), lateral mammillary (Fig 1H), tuberomammillary (Fig 1A and C), arcuate (Fig 1A), circular (Fig 1F) and supraoptic (Fig 1F) nuclei. In the rhombencephalon, the dorsal raphe appeared clearly labeled (Fig 1A and I) and intense labeling was found in several motor nuclei like the motor

trigeminal (Mo5) (Fig 1B and J) and the medial vestibular nuclei (MVe) (Fig 1A and C). In these experiments, oligonucleotide probes tested independently showed identical but weaker hybridization patterns than those obtained with a mixture of probes and no labeling could be detected with the corresponding sense probes (Fig. 1L).

These *in situ* hybridization experiments revealed that PMAT mRNA is expressed in brain regions and nuclei composed of various neuron subtypes, in particular cholinergic, glutamatergic, GABAergic and noradrenergic cells. In selected highly-expressing regions, we further explored the phenotype of the PMAT-expressing cells on counterstained emulsion-dipped sections using *in situ* hybridization coupled with immunohistochemistry.

PMAT is highly expressed in glutamatergic neurons

Confirming the expression profile obtained by *in situ* hybridization film autoradiography, analysis of emulsion-dipped sections showed that PMAT mRNA was highly expressed in several categories of glutamatergic neurons, including the mitral cell layer in the olfactory bulb (Fig 2A), the granular cells of the dentate gyrus of the hippocampus (Fig 2B) and the granular layer of the cerebellum (Fig 2C). In addition, a high level of PMAT mRNA was also found in a non-neuronal cell type, the choroid plexus (Fig 2D).

PMAT is expressed in the main cholinergic groups of the brain

In situ hybridization/immunohistochemistry co-labeling experiments revealed a predominant expression of PMAT in cholinergic neurons in several areas of the brain, as suggested by film autoradiography. In the hindbrain, the large majority of the neurons of motor nuclei (oculomotor, facial, ambiguus, hypoglossal, dorsal motor of vagus) express a high level of PMAT mRNA. This is illustrated here for the dorsal motor

nucleus of vagus (10) (Fig 2E, H and I) and hypoglossal nucleus (12) (Fig 2E, J and K) with counterstained emulsion-dipped sections and co-labeling with antibodies directed against ChAT. In the striatum, where PMAT mRNA exhibited a peculiar speckled distribution by film autoradiography, analysis of counterstained sections showed that the silver grains are confined to large multipolar cells, which we tentatively identified based on morphological criteria as cholinergic interneurons (Fig 2F and L). Double-labeling experiments with antibodies directed against ChAT (Fig 2M) confirmed that these PMAT-positive cells are cholinergic, while most adjacent GABAergic cells, labeled with antibodies directed against GAD, do not express PMAT mRNA (Fig 2N). This localization of PMAT mRNA in cholinergic cells was also observed in the shell of the nucleus accumbens (Fig 2O). Similarly, the intense and punctuate labeling in diagonal band of Broca in medial septum (Fig 2G and P) could be attributed to a fraction of the cholinergic neuron population (Fig 2Q), with a few GABAergic neurons in this region also expressing PMAT mRNA (Fig 2R). PMAT mRNA was also found expressed in large ChAT-positive cells scattered throughout the basal ganglia, the substantia innominata and the lateral and ventral globus pallidus (not shown).

PMAT is found in some but not all aminergic pathways

Since PMAT has been shown to transport monoamines with some degree of efficacy in heterologous systems, we searched for expression of the corresponding mRNA in aminergic pathways. Contrarily to expectations, PMAT mRNA was poorly expressed in the main dopaminergic groups A9 and A10. It was clearly undetectable in the ventral tegmental area and in the substantia nigra pars compacta. In the substantia nigra pars reticulata, however, where some level of expression had been found, silver grains were found on dopaminergic neurons, labeled by TH antibodies (Fig 2S), as well as on most

but not all GABAergic neurons, labeled by GAD antibodies (Fig 2T). Other aminergic regions were also investigated by the same approach. Labeling of noradrenergic neurons using TH antibodies allowed us to demonstrate that most neurons of the locus coeruleus express PMAT (Fig 2U). Similarly, PMAT expression was detected in most histaminergic neurons of the tuberomammillary nucleus, identified with antibodies against HDC (Fig 2V). Although the arcuate nucleus and raphe nucleus were found positive for PMAT mRNA by autoradiography (Table 1), the nature of the neurons expressing PMAT in these regions could not be determined with certainty, due to the lower sensitivity of the co-labeling approach. These results, as well as for other regions expressing PMAT mRNA at a lower level, are recapitulated in Table 1.

DISCUSSION

Two categories of transporters have been proposed to carry out low-affinity transport of monoaminergic neurotransmitters in the CNS, the PMAT and the OCT families. Both these families of organic cation transporters show a broad specificity of substrates, and a relative efficacy in the transport of aminergic neurotransmitters, sharing the overall properties of Uptake₂, a catecholamine removal system found in peripheral tissues as well in the brain. Central and peripheral low-affinity catecholamine uptake systems differ however in some of their properties, in particular in their sensitivity to corticosterone and O-methylated catecholamines (Trendelenburg, 1988; Friedgen et al., 1996). In agreement with what has been described for low affinity transport in rat brain (Burrows et al., 1981; Wilson et al., 1988) and contrary to OCTs (Wu et al., 1998; Hayer-Zillgen et al., 2002), PMAT is sensitive to only millimolar concentrations of corticosterone (Engel et al., 2004).

Our study demonstrates that PMAT exhibits a discrete expression pattern and is found in a wide variety of areas and neuron subtypes throughout the brain. This expression profile is difficult to reconcile with the pharmacological profile defined previously for human PMAT. Although PMAT has been shown to transport monoamines, and preferentially serotonin and dopamine, the rat ortholog studied here is restricted to only some aminergic pathways, and completely absent from dopaminergic neurons of the substantia nigra pars compacta and the ventral tegmental area. On the contrary, it is highly expressed in the principal cholinergic groups of the brain, in glutamatergic neurons such as mitral cells and granule cells of dentate gyrus, and GABAergic neurons in the substantia nigra pars reticulata and the septum. The initial pharmacological characterization of human PMAT by Engel and co-authors

(2004) demonstrates that neither acetylcholine nor GABA interact significantly with this transporter. If we assume that the substrate specificity is conserved between human and rat species, our observations argue strongly against a main role for PMAT in monoamine uptake the nerve endings of aminergic neurons.

Reuptake transporters are usually located on the presynaptic nerve terminals or surrounding glia. For amino acid transporters, a few reports show they can occasionally be found on post-synaptic neurons. These examples include the excitatory amino acid transporter EAAC1, which has been detected on the cell bodies and dendrites of some non-glutamatergic cells like Purkinje cells of the cerebellum (Rothstein et al., 1994), and the GABA transporter GAT1, found on the non-GABAergic pyramidal cells of the cortex (Minelli et al., 1995). High-affinity monoamine transporters, on the other hand, have not been detected on post-synaptic neurons. Detailed immunohistochemical studies of the PMAT protein are required to determine its precise subcellular localization in cholinergic neurons. Depending on this localization, PMAT could exert a direct effect on the modulation of the activity of cholinergic neurons, by controlling the input they receive from monoaminergic afferents, in particular DA or 5HT afferents, thereby constituting a new modulatory mechanism for aminergic neurotransmission. Consistent with this hypothesis, all the regions that express PMAT have been shown to receive a dense aminergic innervation. Trigeminal, facial and hypoglossal motor nuclei in the brainstem have been shown to receive serotonergic and/or noradrenergic afferents (Grzanna et al., 1987; Li et al., 1993). Cholinergic interneurons in the striatum are flooded with dopamine liberated by the projections from substantia nigra pars compacta. Cholinergic neurons of the basal forebrain complex (BFC) have also been shown to be innervated by catecholaminergic (Zaborszky and Cullinan, 1996) and serotonergic

(Khateb et al., 1993) afferents. In these regions, PMAT thus appears in a strategic position to participate in catecholamine-acetylcholine interactions. The two latter systems in particular are implicated in subtle CNS functions. Cholinergic striatal interneurons play an important role in the inhibition of DA-induced behaviors like locomotion (Kaneko et al., 2000) and reward-related learning (Kitabatake et al., 2003), while cholinergic neurons of the BFC are involved in associative learning and spatial memory (Berger-Sweeney et al., 1994; Gutierrez et al., 1997). The interaction between aminergic and cholinergic systems plays an important part in the etiopathology and therapy of several neurologic and psychiatric disorders. Loss of basal forebrain cholinergic neurons is a consistent feature of the early stages of Alzheimer's disease, along with the disruption of the structure and connectivity of several cortical regions and their 5-HT and NE innervation (Coyle et al., 1983). In Parkinson's disease, whose hallmark is the degeneration of dopaminergic neurons of the substantia nigra pars compacta, extranigral damage has been shown to occur early in several brain areas, including cholinergic motor nuclei and magnocellular nuclei of basal forebrain (Braak et al., 2003). These profound cholinergic deficiencies contribute to the cognitive symptoms found in both degenerative diseases, such as memory impairment and dementia, and most therapies aiming to restore cognitive function in AD, and to a lesser extent in PD, target cholinergic systems (Bosboom et al., 2004; Youdim and Buccafusco, 2005). In this context, PMAT may constitute a promising target for the development of novel therapeutic agents.

Exploration of PMAT mRNA expression in aminergic pathways revealed it is scarcely expressed in the main dopaminergic areas. It went undetectable in the ventral tegmental area and in the substantia nigra pars compacta. It was however significantly expressed in dopaminergic neurons of the substantia nigra pars reticulata, and in

noradrenergic cells of the locus coeruleus. In noradrenergic and to some extent dopaminergic pathways, PMAT could thus complement the respective high-affinity transporters, the NET and the DAT, to ensure reuptake of neurotransmitters into the pre-synaptic terminals. Interestingly, PMAT mRNA was also expressed in histaminergic neurons of the tuberomammillary nuclei. For histamine, contrarily to DA, 5-HT and NE, no high-affinity transporter has been reported, consistent with the lack of high-affinity uptake for this neurotransmitter. In histaminergic pathways, PMAT could therefore be an essential element for the clearance of histamine and the regulation of histaminergic neurotransmission, in spite of its low-affinity.

Finally, an alternative function for PMAT was recently disclosed by the identification of adenosine as a selective substrate for PMAT at acidic pH (Barnes et al., 2006). This observation opens the possibility that this transporter could also contribute to adenosine transport in particular physiological conditions like acidosis induced by ischemia, and/or in acidic cellular compartments such as lysosomes or endosomes. Accordingly, several PMAT expressing regions contain high levels of adenosine (Braas et al., 1986). Taken together, the substrate selectivity profile and complex anatomical distribution of PMAT mRNA in the brain argue in favor of its participation in several distinct neurotransmission pathways and physiological responses. The thorough re-examination of the pharmacological properties of PMAT in different pH conditions, as well as the detailed study of its regional and subcellular localization, should contribute to clarifying these questions.

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Legends to figures

Figure 1. Regional distribution of PMAT mRNA in adult rat brain determined by *in situ* hybridization. Autoradiograms of sagittal (A to D) and coronal (E to L) brain sections labeled with a mixture of oligonucleotide antisense (A to K) or sense (L) probes specific of PMAT. Cb, cerebellum; DR, dorsal raphe nucleus; 3, facial motor nucleus; Dk, nucleus of Darkschewitsch; DG, dentate gyrus of hippocampus; Mi, mitral cell layer of olfactory bulbs; Arc, arcuate nucleus; DM, dorsomedial hypothalamic nucleus; TM, tuberomammillary nucleus; MVe, medial vestibular nucleus; 10, dorsal motor nucleus of vagus; HDB, horizontal limb nucleus of diagonal band of Broca; 7, facial nucleus; Mo5, motor trigeminal nucleus; ChP, choroid plexus; CPu, striatum; CA1, pyramidal cell layer of hippocampus; AD, anterodorsal thalamic nucleus, PVN, paraventricular hypothalamic nuclei; Pir, piriform cortex; LGP, lateral globus pallidus; VDB, vertical limb nucleus of diagonal band of Broca; SI, substantia innominata; AM, anteromedial thalamic nucleus; Rt, reticular thalamic nucleus; Cir, circular nucleus; SON, supraoptic nucleus; MHb medial habenula; LaDL, lateral amygdaloid nucleus, dorsolateral part; BLA, basolateral amygdaloid nucleus; CeL, lateral division of central amygdaloid nucleus; DM, dorsomedial hypothalamic nucleus; SNr; substantia nigra, pars reticulata; SuM, supramammillary nucleus; LM, lateral mammillary nucleus; LC, locus coeruleus; 12, hypoglossal nucleus.

Figure 2. Distribution of PMAT in specific neuronal subpopulations, determined by *in situ* hybridization and immunohistochemistry. High magnification light or dark-field photomicrographs show localization of PMAT mRNA in the mitral cell layer in the olfactory bulb (A), granular cells of dentate gyrus of the hippocampus (B), granular layer of the cerebellum (C), choroid plexus (D), dorsal motor nucleus of vagus (E and

H), hypoglossal nucleus (E and J), striatum (F and L) and diagonal band of Broca in septum (G and P) (hematoxylin/eosin counterstain); colocalization of PMAT mRNA with ChAT in the dorsal motor nucleus of vagus (10) (I) and hypoglossal motor nucleus (K); with ChAT (M) but not GAD (N) in the striatum; with ChAT in the nucleus accumbens (O); with ChAT (Q), but rarely GAD (R) in the diagonal band of Broca; with TH (S) and occasionally with GAD (T) in the substantia nigra pars reticulata; with TH in the locus coeruleus (U); with HDC in the tuberomammillary nucleus (V). ; Mi, mitral cell layer of olfactory bulbs; DG, dentate gyrus of hippocampus; Cb, cerebellum; ChP, choroid plexus; ; 10, dorsal motor nucleus of vagus; 12, hypoglossal nucleus; CPu, striatum; HDB, horizontal limb nucleus of diagonal band of Broca; SI, substantia innominata; Acb, nucleus accumbens; SNr; substantia nigra, pars reticulata; LC, locus coeruleus; TM, tuberomammillary nucleus. Scale bars: 20 μm (A to D, L to R and V), 100 μm (E to G); 50 μm (H to K), 10 μm (S to V).

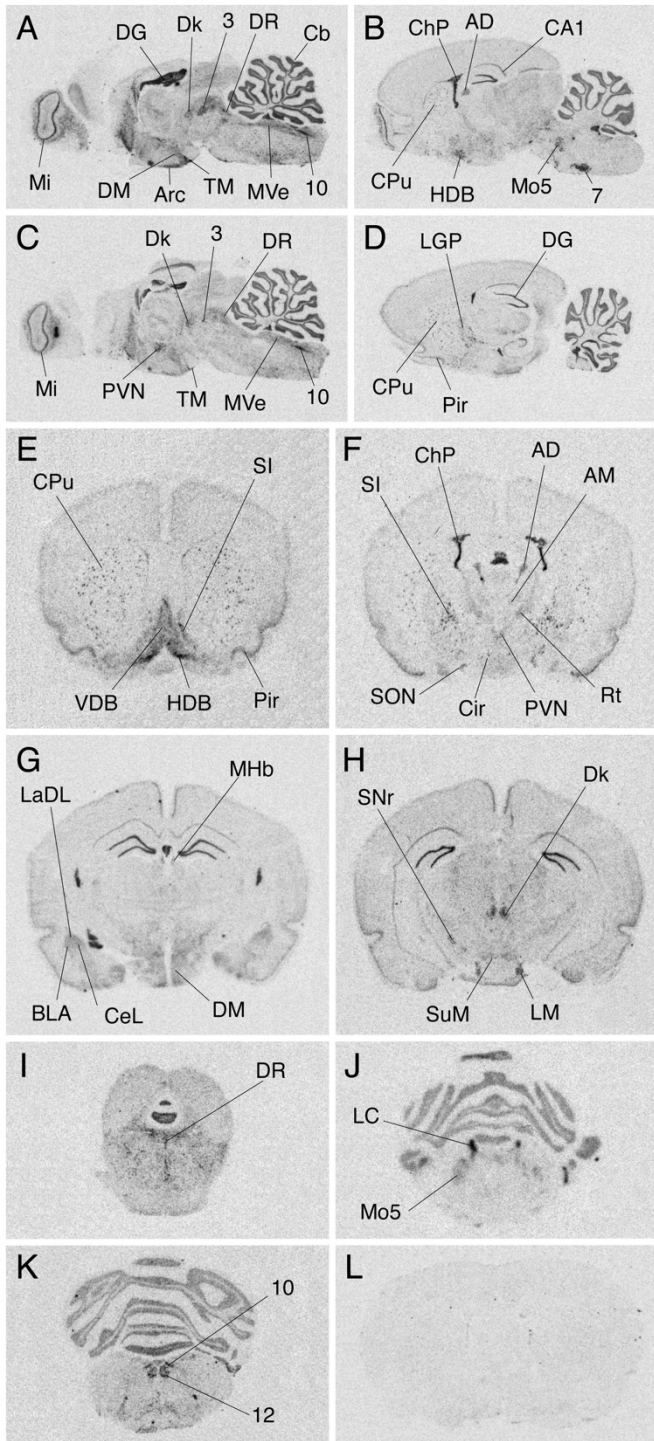


Fig. 1

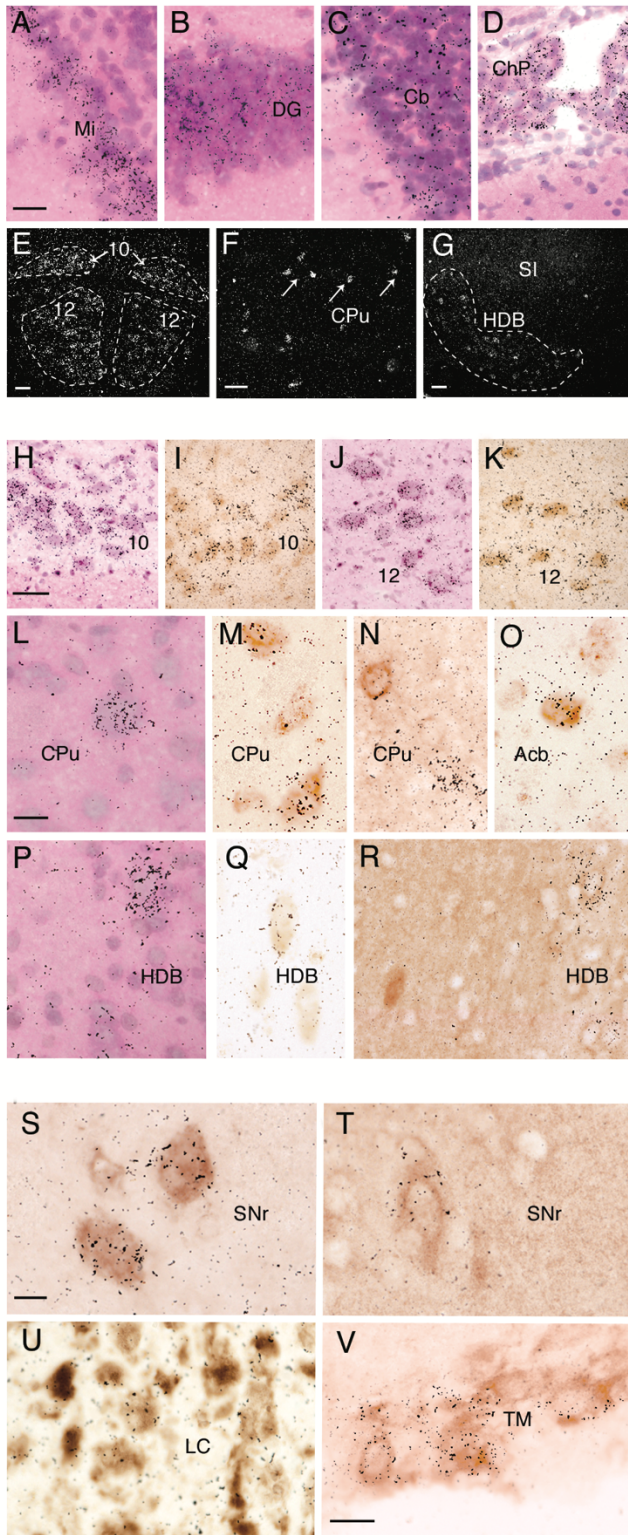


Fig. 2

Table 1. Relative density of PMAT messenger RNA in various regions of the rat brain

| Brain area | PMAT mRNA density | Brain area | PMAT mRNA density |
|---|-------------------|--|-------------------|
| Telencephalon | | Diencephalon (continued) | |
| Olfactory bulbs | | Hypothalamus | |
| Mitral cell layer | +++ | Posterior hypothalamic area | + |
| External plexiform layer | + | Lateral hypothalamic area | + |
| Ependyma and olfactory ventricle | +++ | Dorsal hypothalamic area | ++ |
| Cortex | | Dorsomedial hypothalamic nucleus, diffuse part | +++ |
| Frontal | + | Ventromedial hypothalamic nucleus | + |
| Piriform | +++ | Paraventricular hypothalamic nucleus | ++ |
| Insular | ++ | Lateral mammillary nucleus | +++ |
| Retrosplenial granular b | ++ | Supramammillary nucleus, lateral and medial part | ++ |
| Ectorhinal | ++ | Tuberomammillary nucleus | +++ |
| Perirhinal | ++ | Arcuate hypothalamic nucleus | +++ |
| Lateral entorhinal | +++ | Circular nucleus | +++ |
| Temporal, association area | ++ | Supraoptic nucleus | +++ |
| Hippocampus | | Mesencephalon | |
| Dentate gyrus | ++++ | Periaqueductal gray | + |
| CA1 field | ++ | Posterior pretectal nucleus | + |
| CA3 field | + | Nucleus of Darkschewitsch | +++ |
| Amygdala | | Substantia nigra pars reticulata | ++ |
| Lateral amygdaloid nucleus | +++ | Red nucleus, magnocellular part | ++ |
| Basolateral amygdaloid nucleus | ++ | Oculomotor nucleus | ++ |
| Cortical amygdaloid nucleus (C2 and C3) | ++ | Oculomotor nucleus, parvicellular part | ++ |
| Septum | | Supraoculomotor central gray | ++ |
| Lambdoid septal zone | +++ | Rhombencephalon | |
| Vertical limb of the diagonal band | +++ | Dorsal raphe nucleus | +++ |
| Horizontal limb of the diagonal band | ++++ | Pontine raphe nucleus | +++ |
| Medial septal nucleus | +++ | Laterodorsal tegmental nucleus | ++ |
| Basal ganglia | | Raphe obscurus nucleus | ++ |
| Caudate putamen | +++ | Motor trigeminal nucleus | +++ |
| Lateral globus pallidus | +++ | Central gray, alpha, beta and gamma part | ++ |
| Median preoptic nucleus | ++ | Facial nucleus | +++ |
| Medial preoptic area | ++ | Locus coeruleus | +++ |
| Substantia innominata | +++ | Medial vestibular nucleus | ++ |
| Ventral pallidum | + | Pedunculopontine tegmental nucleus | +++ |
| Diencephalon | | Ambiguous nucleus | ++ |
| Thalamus | | Hypoglossal nucleus | +++ |
| Medial habenular nucleus | ++ | Dorsal motor nucleus of vagus | +++ |
| Anterodorsal thalamic nucleus | +++ | Paramedian reticular nucleus | ++ |
| Anteromedial thalamic nucleus | ++ | Granular layer of cerebellum | +++ |
| Reticular thalamic nucleus | ++ | Choroid plexus | +++ |
| Zona incerta | ++ | | + |
| Intermediodorsal thalamic nucleus | + | | |
| Centrolateral thalamic nucleus | + | | |
| Paracentral thalamic nucleus | + | | |
| Central medial thalamic nucleus | ++ | | |
| Rhomboid thalamic nucleus | ++ | | |
| Ventral reuniens thalamic nucleus | + | | |

Rating scale: +++ high density, ++ moderate density, + low density of PMAT mRNA.

