

## A Mapping Study of Phosphodiesterase10A mRNA Expressing Neurons in Rat Brain

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### Abstract

Phosphodiesterase (PDE) 10A mRNAs were expressed in neurons primarily in forebrain structures, especially olfactory bulb, cerebral cortex, caudate-putamen and hippocampus, and in the cerebellum. All the same type of neurons in morphology were, however, not always positive in only nuclei or particular regions. The wide-spread but distinctive distribution of PDE10A neurons suggests that the degradation enzyme of cyclic nucleotide second messengers exerts regulatory roles in the intracellular signaling pathways of particular neurons or neuronal groups in rat brain.

**Key words :** Phosphodiesterase, Intracellular signal transduction  
cAMP, cGMP, *In situ* hybridization

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## Introduction

PDE10A is a recently cloned gene family of the PDE superfamily<sup>1-5)</sup> which comprises 11 families. PDE10A, or cGMP-binding cAMP PDE and cAMP-inhibited cGMP PDE, catalyzes the hydrolysis of both cAMP and cGMP playing pivotal roles in a variety of intracellular signaling pathways. In human two alternative splice variants of PDE10A have been isolated (PDE10A 1<sup>2,4)</sup> and PDE10A 2<sup>3,4)</sup> and exhibit similar substrate properties for  $K_m$  values of cAMP and cGMP<sup>3)</sup>. As for structural properties of the isoforms, the PDE10A 2 possesses a consensus sequence for phosphorylation by cAMP- and cGMP-dependent protein kinases (PKA and PKG) and is actually phosphorylated by PKA, whereas PDE10A 1 contains putative phosphorylation sites for protein kinase C but not for PKA<sup>2)</sup>. Of two isoforms, PDE10A 2 variant is preferentially expressed in several adult and fetal tissues<sup>3)</sup>, although the human PDE10A isoforms are differentially expressed in various tissues<sup>2-4)</sup>.

In rat 5 splice variants of PDE10A have been reported to consist of two major forms (PDE10A 2 and PDE10A 3) and three minor forms (PDE10A 4-6) in a rat brain cDNA library, but another splice variant corresponding to human PDE10A 1 has not been so far identified<sup>1)</sup>. Since sequence variations of rat PDE10A 2-6 are restricted to the N-terminal region, the putative cGMP-binding and the catalytic domains remain unchanged in all the splice variants. Rat PDE10A isoforms correspond to human PDE10A 2 in structural and enzymatic properties, and their N-terminal variations may be correlated with the tissue-specific expression. Northern blot hybridization studies have demonstrated intense signals of PDE10A transcripts (about 9,100~9,500 bases in length) in the caudate-putamen and at decreased intensity in other brain regions in rat<sup>6,7)</sup> and in human<sup>2)</sup>, although the preliminary data have shown the restricted localization of PDE10A neurons in the caudate-putamen and olfactory tubercle<sup>1)</sup>. The present *in situ* hybridization study, thus, investigates PDE10A mRNA expression in normal adult rat brain.

## Methods and Protocols

*In situ* hybridization (ISH) was performed using a 45-mer synthetic oligonucleotide on 15- $\mu$ m thick cryostat sections of paraformaldehyde-fixed brain (Sprague-Dawley male rat, postnatal 10 weeks). The protocol of ISH is essentially same to our previous studies<sup>6,8,9)</sup>. The antisense probe was complementary to a sequence of the catalytic domain of rat PDE10A 2-6 isoforms (GenBank Accession # AB027155, NT 1969-2013). The probes were 3'-end-labeled with digoxigenin-conjugated dUTP and the hybrids were detected with the anti-digoxigenin antibody and alkaline phosphatase detection system (Roche Diagnostics Inc.). Specificity of hybridization signals has been established by Northern blot hybridization (about 9,100-base long mRNA)<sup>6,7)</sup>, and is also verified by no specific signal in hybridization experiments with 100-fold excess amount of non-labeled antisense probes, labeled sense probes, or RNase treatment prior to hybridization. The distribution of PDE10A neurons were depicted on selected schemes from rat brain atlas<sup>10)</sup>.

## Results and Discussion

Neurons expressing PDE10A mRNAs were distributed in various regions of rat brain (Figs. 1 and 2), but not restricted within the caudate-putamen and olfactory tubercle as described previously.<sup>1)</sup> A large number of PDE10A neurons were localized in the layer VI of the cerebral cortex and caudate-putamen (or striatum). Many positive neurons were found in various brain regions; external plexiform and internal granule cell layers of the olfactory bulb, anterior olfactory nucleus, olfactory tubercle, piriform cortex, layer II and III of the cerebral cortex, dentate gyrus and fields CA1 to CA3 of the hippocampus, amygdaloid nuclear complex, and granule cell layer of the cerebellum. A few PDE10A neurons were also scattered in the periglomerular region of the olfactory bulb, accessory olfactory nucleus, tenia tecta, layer IV of the cerebral cortex, accumbens nucleus, globus pallidus, septum, thalamic nuclei, hypothalamus, habenular nucleus (medial and lateral), superior and inferior colliculi, molecular and Purkinje-cell layers of the cerebellum (but no Purkinje cell was positive), deep cerebellar nuclei,

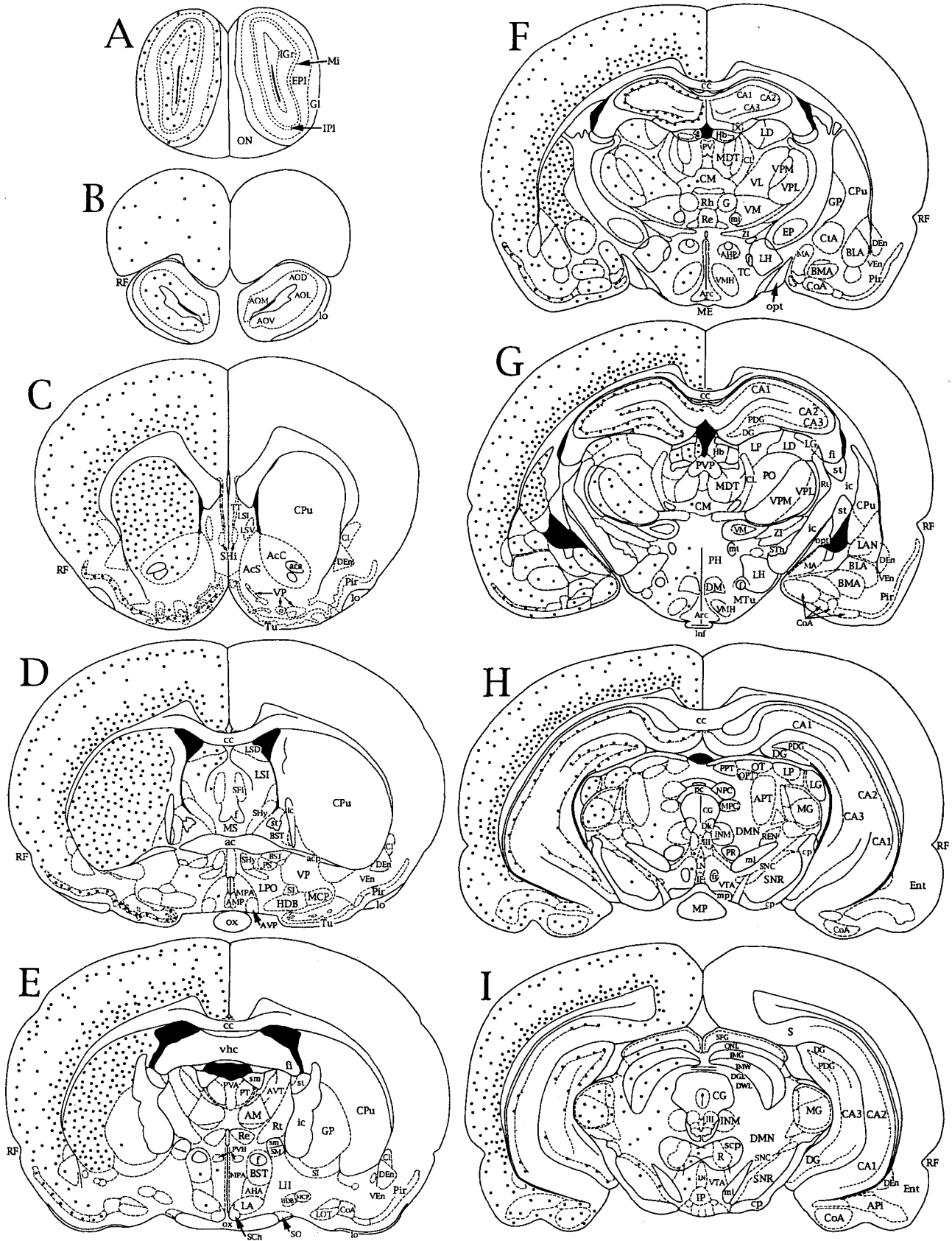


Fig. 1 Schematic chartings of PDE10A mRNA expressing neurons in selected frontal planes of rat brain<sup>10)</sup>. The distribution of PDE10A neurons were depicted by dots on the left side of figures. Rostral (A) to caudal (I).

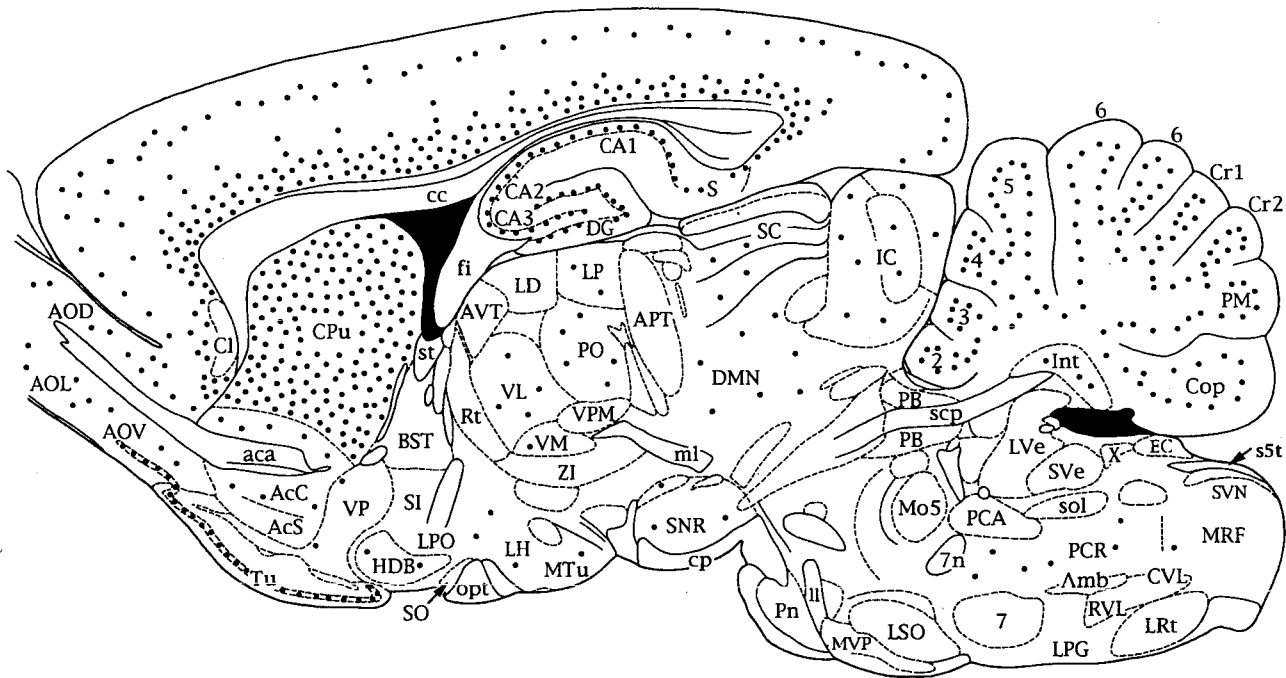


Fig. 2 Schematic charting of PDE10A mRNA expressing neurons in a selected parasagittal plane of rat brain<sup>10)</sup>. The distribution of PDE10A neurons were depicted by dots.

and reticular formation of the brain stem (Figs. 1 and 2).

The wide-spread but distinctive distribution of PDE10A neurons in rat brain is supported by Northern blot and dot blot hybridization studies in rat<sup>6, 7)</sup> as well as human brain<sup>2)</sup>. The abundance of PDE10A neurons in the caudate-putamen coincides with the Northern blot hybridization signals<sup>1, 2, 6, 7)</sup>. In addition, PDE10A neurons are conspicuous in several regions, such as central olfactory structures, cerebral cortex, hippocampus, and cerebellum, despite the low intensity of *in vitro* hybridization signals even when poly(A)<sup>+</sup> RNA was analyzed<sup>6, 7)</sup>. This disparity may be ascribed to the relative density of PDE10A neurons in single nuclei or regions; for instance, in the cerebral cortex individual PDE10A neurons may express the transcripts at a relatively high level but the concentration of PDE10A transcripts in a unit volume handled for *in vitro* study may be diluted with those derived from a large number of different types of cortical neurons.

The cellular localizations of PDE10A transcripts showed the regional overlapping with neurons expressing other PDEs (PDE 1 A<sup>11)</sup>, PDE 1 B<sup>11, 12)</sup>, PDE 1 C<sup>13)</sup> and PDE 2 A<sup>14)</sup>); in particular, olfactory bulb, cerebral cortex, caudate-putamen, hippocampus and cerebel-

lum. The PDE 1 isoforms, as well as PDE10As, catalyze the hydrolysis of both cAMP and cGMP, but unlike PDE10As, they are activated by calmodulin and Ca<sup>2+</sup>. The activities of PDE 1 As and PDE 1 Bs are modulated by phosphorylation by PKA and calmodulin-dependent protein kinase II, respectively. The cAMP and cGMP hydrolytic activities by PDE 2 As, unlike PDE10As, are stimulated by allosteric binding of cGMP under normal substrate condition. In addition, several studies on PDEs have reported that the other PDE genes (PDE 3, PDE 5, PDE 7, PDE 8 and PDE 9) are also expressed in brain tissues. These studies provide a propulsive force to the present topographic mapping of PDE10A neurons, and also encourage investigating correlation between neuronal morphologies and different types of PDEs.

### Acknowledgements

The authors are grateful to Dr. Thomas E. Finger of the University of Colorado for valuable comments and suggestions on the manuscript.

## Abbreviation for Figure 1 and 2

2-6; cerebellar lobules, 7; facial nucleus, 7n; facial nerve, III; oculomotor nucleus, ac; anterior commissure, aca & acp; anterior and posterior parts of anterior commissure, AcC & AcS; core and shell of accumbens nucleus, AHA & AHP; anterior and posterior parts of anterior hypothalamic area, AM; anteromedial thalamic nucleus, Amb; ambiguous nucleus, AMP; anterior medial preoptic nucleus, AOD; AOL; AOM & AOV; dorsal; lateral; medial and ventral parts of anterior olfactory nucleus, APi; amygdalo-piriform transition, APT; anterior pretectal nucleus, Arc; arcuate nucleus, AVP; anteroventral preoptic nucleus, AVT; anteroventral thalamic nucleus, BLA; basolateral amygdaloid nucleus, BMA; basomedial amygdaloid nucleus, BST; bed nucleus of stria terminalis, CA 1-3; fields CA 1-3 of Ammon's horn, cc; corpus callosum, CG; central gray, Cl; Claustrum, CL; centrolateral thalamic nucleus, CM; central medial thalamic nucleus, CoA; cortical amygdaloid nucleus, Cop; copula pyramis, cp; cerebral peduncle, CPu; caudate-putamen, Cr 1 & Cr 2; crus 1 and 2 ansiform lobule, CtA; central amygdaloid nucleus, CVL; caudoventrolateral reticular nucleus, DEn; dorsal endopiriform nucleus, DG; dentate gyrus, Dk; nucleus Darkschewitsch, DM; dorsomedial hypothalamic nucleus, DMN; deep mesencephalic nucleus, EC; external cuneate nucleus, Ent; entorhinal cortex, EP; entopeduncular nucleus, EPI; external plexiform layer of olfactory bulb, f; fornix, fi; fimbria hippocampi, fr; fasciculus retroflexus, G; gelatinosus thalamic nucleus, Gl; glomerular layer of olfactory bulb, GP; globus pallidus, Hb; habenular, HDB; horizontal limb of diagonal band, ic; interanal capsule, IC; inferior colliculus, IF; interfascicular nucleus, IGr; internal granular layer of olfactory bulb, Inf; infundibular stem, INM; interstitial nucleus of medial longitudinal fasciculus, Int; interposed cerebellar nucleus, IP; interpeduncular nucleus, IPI; internal plexiform layer of olfactory bulb, LA; lateroanterior hypothalamic nucleus, LAN; lateral amygdaloid nucleus, LD; laterodorsal thalamic nucleus, LG; lateral geniculate nucleus, LH; lateral hypothalamic area, ll; lateral lemniscus, LN; linear nucleus of raphe, lo; lateral olfactory tract, LOT; lateral olfactory tract nucleus, LP; lateral posterior thalamic nucleus, LPG; lateral

paragigantocellular nucleus, LPO; lateral preoptic area, LSD; LSI & LSV; dorsal; intermediate and ventral parts of lateral septal nucleus, LRt; lateral recicular nucleus, LSO; lateral superior olive, LVe; lateral vestibular nucleus, MA; medial amygdaloid nucleus, MCP; magnocellular preoptic nucleus, MDT; mediodorsal thalamic nucleus, ME; median eminence, MG; medial geniculate nucleus, Mi; mitral cell layer of olfactory bulb, ml; medial lemniscus, Mo 5; motor trigeminal nucleus, mp; mammillary peduncle, MP; medial mammillary nucleus, MPA; medial preoptic area, MPC; magnocellular nucleus of posterior commissure, MRF; medullary reticular field, MS; medial septal nucleus, mt; mamillothalamic tract, MTu; medial tuberal nucleus, MVP; medioventral periolivary nucleus, NPC; posterior commissure nucleus, ON; olfactory nerve layer of olfactory bulb, opt; optic tract, OPT; olivary pretectal nucleus, OT; optic tract nucleus, ox; optic chiasm, PB; parabrachial nucleus, pc; posterior commissure, PCA; parvocellular reticular nucleus alpha, PCR; parvocellular reticular nucleus, PDG; polymorph layer of dentate gyrus, PH; posterior hypothalamic area, Pir; piriform cortex, Pn; pontine nucleus, PO; posterior thalamic nuclear group, PPT; posterior pretectal nucleus, PR; prerubal field, PS; parastrial nucleus, PT; paratenial thalamic nucleus, PV; paraventricular thalamic nucleus, PVA & PVP; anterior and posterior parts of paraventricular thalamic nucleus, PVH; paraventricular hypothalamic nucleus, R; red nucleus, Re; reunions thalamic nucleus, REN; retrothomoid nucleus, RF; rhinal fissure, Rh; rhomboid thalamic nucleus, Rt; reticular thalamic nucleus, RVL; rostroventrolateral reticular nucleus, s 5 t; spinal trigeminal tract, S; subiculum, SC; superior colliculus (DGL & DWL; deep gray and white layers, IMG & IMW; intermediate gray and white layers, ONL; optic nerve layer, SFG; superficial gray layer), SCh; suprachiasmatic nucleus, scp; superior cerebellar peduncle, SFi; septofimbrial nucleus, SHi; septohippocampal nucleus, SHy; septohypothalamic nucleus, SI; substantia innominata, sm; stria medullaris thalami, SM; nucleus stria medialis, SNC & SNR; compact and reticular parts of substantia nigra, SO; supraoptic nucleus, sol; nucleus of solitary tract, st; stria terminalis, STh; subthalamic nucleus, SVe; spinal vestibular nucleus, SVN; spinal trigeminal nucleus, TC; tuber cinereum,

TT; tenia tecta, Tu; olfactory tubercle, VEn; ventral endopiriform nucleus, vhc; ventral hippocampal commissure, VL; ventrolateral thalamic nucleus, VM; ventromedial thalamic nucleus, VMH; ventromedial hypothalamic nucleus, VP; ventral pallidum, VPL; ventral posterolateral thalamic nucleus, VPM; ventral posteromedial thalamic nucleus, VTA; ventral tegmental area, X; vagal motor nucleus, ZI; zona incerta.

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受付日 2002年12月2日