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Distribution of Neuropeptide S Receptor mRNA and Neurochemical Characteristics of Neuropeptide S-Expressing Neurons in the Rat Brain

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ABSTRACT

Neuropeptide S (NPS) and its receptor (NPSR) constitute a novel neuropeptide system that is involved in regulating arousal and anxiety. The NPS precursor mRNA is highly expressed in a previously undescribed group of neurons located between the locus coeruleus (LC) and Barrington's nucleus. We report here that the majority of NPS-expressing neurons in the LC area and the principal sensory trigeminal nucleus are glutamatergic neurons, whereas many NPS-positive neurons in the lateral parabrachial nucleus coexpress corticotropin-releasing factor (CRF). In addition, we describe a comprehensive map of NPSR mRNA expression in the rat brain. High levels of expression are found in areas involved in olfactory processing, including the anterior olfactory nucleus, the endopiriform nucleus, and the piriform cortex. NPSR mRNA is expressed in several regions mediating anxiety responses, including the amygdaloid complex and the paraventricular hypothalamic nucleus. NPSR mRNA is also found in multiple key regions of sleep neurocircuitries, such as the thalamus, the hypothalamus, and the preoptic region. In addition, NPSR mRNA is strongly expressed in major output and input regions of hippocampus, including the parahippocampal regions, the lateral entorhinal cortex, and the retrosplenial agranular cortex. Multiple hypothalamic nuclei, including the dorsomedial and the ventromedial hypothalamic nucleus and the posterior arcuate nucleus, express high levels of NPSR mRNA, indicating that NPS may regulate energy homeostasis. These data suggest that the NPS system may play a key role in modulating a variety of physiological functions, especially arousal, anxiety, learning and memory, and energy balance. J. Comp. Neurol. 500:84–102, 2007.

Indexing terms: G protein-coupled receptor; in situ hybridization; expression pattern; locus coeruleus; glutamate; corticotropin-releasing factor; acetylcholine; GABA

Neuropeptide S (NPS) and its receptor (NPSR) form a newly discovered neuropeptide system. In the rat, NPS is encoded by a precursor protein of 89 amino acids, containing a typical amino-terminal signal peptide. The immature NPS peptide sequence is preceded by a pair of basic amino acids (Lys-Arg) that are presumably cleaved by prohormone convertases to release the 20-amino-acid-long peptide (primary structure of rNPS: SFRNGVGSGVKKTSFRRAKQ). Pharmacologically, NPS activates NPSR at low nanomolar concentration, induces mobilization of intracellular Ca^{2+}, increases intracellular levels of cAMP, and stimulates phosphorylation of mitogen-

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activated protein kinase (Xu et al., 2004b; Reinscheid et al., 2005). Physiologically, central administration of NPS in rodents induces hyperlocomotion, increases arousal and wakefulness, and suppresses all stages of sleep. NPS administration also reduces anxiety-like behaviors in a battery of four different tests that measure innate fear or responses to novelty in rodents (Xu et al., 2004b). Anatomically, we have shown that NPS precursor mRNA is expressed only in several discrete regions in the rat brain. The dorsomedial hypothalamus and amygdala contain scattered NPS precursor mRNA signals. Strong expression of NPS precursor mRNA was observed in three brainstem regions, including the principle sensory trigeminal nucleus, lateral parabrachial nucleus, and locus coeruleus (LC) area. The NPS-expressing neurons in the LC area are localized ventromedial to the LC and caudolateral to Barrington's nucleus, a neighboring nucleus of LC that is often referred to as the *pontine micturition center*. The unique anatomical pattern of this group of NPS-expressing neurons reveals a previously unmapped population of cells between Barrington's nucleus and the LC. In our previous study, we have demonstrated that neither noradrenaline (NA), the major neurotransmitter synthesized in LC neurons, nor corticotropin-releasing factor (CRF), the major peptidergic transmitter in Barrington's nucleus, is expressed in the NPS-synthesizing cell cluster in the pontine brainstem (Xu et al., 2004b). Thus, the identity of any classical neurotransmitters in this novel group of neurons in the LC area is still unknown. It is well established that neuropeptides usually do not exist alone as neurotransmitters but colocalize with classical neurotransmitters or with other neuropeptides and may play important roles in modulating the activities of these neurotransmitters/neuromodulators (Hokfelt et al., 2000).

It has been reported that γ-aminobutyric acid (GABA)-ergic neurons (Aston-Jones et al., 2004), cholinergic neurons (Sutin and Jacobowitz, 1988; Rizvi et al., 1994), and glutamatergic neurons (Stornetta et al., 2002; Varoqui et al., 2002; Moriyama and Yamamoto, 2004) are present in LC and the surrounding peri-LC area. In this study, we therefore determined the chemical neuroanatomy of NPS-expressing cells in the rat brainstem, by using glutamate acid decarboxylase 67 (GAD67; Lauterborn et al., 1995), choline acetyltransferase (ChAT; Lauterborn et al., 1993), and vesicular glutamate transporters (VGLUTs; Smith et al., 2001; McCullumsmith and Meador-Woodruff, 2003) as markers for GABAergic, cholinergic, and glutamatergic neurons, respectively. This study will reveal for the first time the neurochemical profiles of NPS-producing neurons and the neurotransmitters/neuromodulators that may be coreleased with NPS peptide. Identification of coexisting transmitters in NPS-producing neurons will

Abbreviations

3V	third ventricle
4V	fourth ventricle
ac	anterior commissure
aca	anterior commissure, anterior part
ACo	anterior cortical amygdaloid nucleus
AH	anterior hypothalamic area
AMV	anteromedial thalamic nucleus, ventral part
AON	anterior olfactory nucleus
Apir	amygdalopiriform transition area
aq	aqueduct
Arc P	arcuate hypothalamic nucleus, posterior part
CA1	CA1 region of the hippocampus
Cb	cerebellum
cc	corpus callosum
Cg1	cingulate cortex, area 1
Cl	claustrum
CLi	caudal linear nucleus of raphe
CM	central medial thalamic nucleus
cp	cerebral peduncle
DEn	dorsal endopiriform nucleus
DG	dentate gyrus
DMH	dorsomedial hypothalamic nucleus
DpG	deep gray layer of superior colliculus
DR	dorsal raphe
DTM	dorsal tuberomammillary nucleus
En	endopiriform nucleus
f	fornix
HDB	nucleus of the horizontal limb of the diagonal band
Hpx	hippocampus
I	intercalated nuclei of amygdala
IAM	interanteromedial thalamic nucleus
IPR	interpeduncular nucleus, rostral part
LC	locus coeruleus
LENt	lateral entorhinal cortex
LH	lateral hypothalamic area
LPB	lateral parabrachial nucleus
LPO	lateral preoptic area
LV	lateral ventricle
M2	motor cortex 2
MeAD	medial amygdala, anterodorsal part
MePD/V	medial amygdala, posterodorsal/ventral
MnR	median raphe nucleus
MPT	medial pretectal nucleus
OPT	olivary pretectal nucleus
opt	optical tract
ox	optic chiasm
PAG	periaqueductal gray
PaMP	medial parvicellular paraventricular hypothalamic nucleus
PaPo	paraventricular hypothalamic nucleus, posterior part
PaS	parasubiculum
PaV	paraventricular hypothalamic nucleus, ventral part
pc	posterior commissure
PeF	perifornical nucleus
PH	posterior hypothalamic nucleus
Pir	piriform cortex
PLi	posterior limitans thalamic nucleus
PMCo	posteromedial cortical amygdala
PMD	premammillary nucleus, dorsal part
PMV	premammillary nucleus, ventral part
Post	postsubiculum
Pr5	principle sensory 5 nucleus
PrC	precommissural nucleus
PrS	presubiculum
PT	paratenial thalamic nucleus
PtA	parietal association cortex
PVP	paraventricular thalamic nucleus, posterior
Re	reuniens thalamic nucleus
Rh	rhomboid thalamic nucleus
RSA	retrosplenial agranular cortex
S	subiculum
SCO	subcommissural organ
scp	superior cerebellar peduncle
SG	suprageniculate thalamic nucleus
SNCD	substantia nigra, compact part, dorsal tier
SuG	superficial layer of superior colliculus
Tg/PnO	tegmental area/pontine reticular nucleus, oral part
VLPO	ventrolateral preoptic nucleus
VMH	ventromedial hypothalamic nucleus
VMHDM	ventromedial hypothalamic nucleus, dorsomedial part
VTA	ventral tegmental area
VTM	ventral tuberomammillary nucleus
Xi	xiphoid thalamic nucleus

help to develop new hypotheses about anatomical connections of NPS neurons and possible regulatory roles of the NPS system.

Our previous study has demonstrated several representative regions with high levels of NPS receptor mRNA expression, such as anterior olfactory nucleus, motor cortex 2, amygdala, and hypothalamic regions (Xu et al., 2004b). However, the complete expression pattern of NPS receptor mRNA in the rat brain has not been described in detail. To explore the distribution of NPS receptor mRNA in the rat central nervous system, we describe in this paper a comprehensive anatomical mapping of NPSR mRNA in rat brain. Insofar as central administration of NPS potently regulates anxiety and arousal, it is important to map the anatomical substrates of these effects and investigate interactions of the NPS system with other neurotransmitter systems that are important in modulating vigilance and anxiety. Furthermore, these studies will also provide an anatomical basis for hypotheses about other physiological functions of the NPS system.

MATERIALS AND METHODS

Animals

Brain tissues from adult male Sprague-Dawley rats (250–300 g) were used for in situ hybridizations. The animals were housed under a 12-hour light-dark cycle, with lights on at 6:00 AM. Food and water were available ad libitum. The experimental procedures were approved by the Institutional Animal Care and Use Committee and are consistent with federal regulations and guidelines for experimentation on animals.

Tissue preparation

Briefly, animals were killed by decapitation, and their brains were quickly removed and frozen in methyl butane cooled to –20°C for 1 minute. Brains were stored at –80°C until sectioning. Brains were sectioned coronally into 20-μm slices with a cryostat set at –19°C and thaw-mounted on Vectabond-coated glass slides, followed by fixation in 4% paraformaldehyde, 0.1 M phosphate-buffered saline (PBS), pH 7.4, for 1 hour. Slides were rinsed briefly in 0.1 M phosphate buffer, followed by distilled water, then dried at room temperature and stored at –80°C.

Probe synthesis

A 184-bp BamHI-NotI fragment of the rat NPS precursor cDNA (corresponding to nucleotides 92–276) was generated by PCR and subcloned into pBluescript SK (Stratagene, La Jolla, CA). Antisense or sense riboprobes were generated by using T7 or T3 RNA polymerase, respectively. A 326-bp NotI-BamHI fragment of the rat NPS receptor cDNA (corresponding to nt 408–734) was cloned into the same vector. Antisense or sense riboprobes of NPS receptor were generated by using T3 or T7 RNA polymerase, respectively. Radiolabeled probes were prepared by transcription in the presence of ^{35}S-UTP (Amersham, Arlington Heights, IL). The labeled probes were separated from unincorporated nucleotides on Sephadex G-50 columns (Roche Applied Science, Indianapolis, IN).

For double in situ hybridization, antisense and sense riboprobes of ChAT, GAD67, CRF, VGLUT1, VGLUT2, or VGLUT3 were labeled with digoxigenin (DIG) using a DIG RNA labeling kit (Roche Applied Science, Indianapolis, IN). A 432-bp fragment of rat ChAT cDNA (corresponding to nucleotides 268–699) was cloned into pBluescript SK. Antisense or sense DIG-labeled ChAT cRNAs were synthesized with T7 or T3 RNA polymerase after HindIII or PvuII digestion of the pBSChAT vector, respectively (Lauterborn et al., 1993). A fragment corresponding to nucleotides 1,324–1,683 of cat glutamic acid decarboxylase (GAD)-67 cDNA was subcloned into pBluescript SK. Antisense or sense DIG-labeled GAD67 cRNAs were synthesized with T7 or T3 RNA polymerase after BamHI or PvuII digestion of pBSGAD, respectively (Lauterborn et al., 1995). CRF riboprobes were synthesized as described previously (Xu et al., 2004b). A 529-bp fragment corresponding to nucleotides 953–1,481 of human VGLUT1 cDNA was subcloned into pCR-Blunt II TOPO (Invitrogen, Carlsbad, CA). Antisense or sense riboprobes of DIG-labeled VGLUT1 were synthesized with SP6 or T7 RNA polymerase after XhoI or HindIII digestion of the vector, respectively (Smith et al., 2001; McCullumsmith and Meador-Woodruff, 2003). A fragment corresponding to nucleotides 211–800 of rat VGLUT2 cDNA was subcloned into pBluescript SK. Antisense or sense riboprobes of VGLUT2 were synthesized with T7 RNA polymerase or T3 RNA polymerase after BamHI or EcoRI digestion of the vector, respectively (Smith et al., 2001; McCullumsmith and Meador-Woodruff, 2003). A fragment corresponding to nucleotides 826–1,673 of human VGLUT3 cDNA was subcloned into pCR-Blunt II TOPO. Antisense or sense DIG-labeled VGLUT3 riboprobes were synthesized with SP6 or T7 RNA polymerase after XhoI or SpeI digestion of the vector, respectively (McCullumsmith and Meador-Woodruff, 2003). Vectors containing VGLUT1, VGLUT2, and VGLUT3 were generously provided by Dr. James H. Meador-Woodruff (University of Michigan).

In situ hybridization

Sections for in situ hybridization were processed as previously described (Clark et al., 2001). First, sections were prehybridized, followed by proteinase K (0.75 μg/ml) treatment for 10 minutes; acetylated; and dehydrated through increasing concentrations of ethanol and then air dried. Next, hybridization buffer [50% formamide, 10% dextran sulfate, 0.3 M NaCl, 0.02% polyvinylpyrolidone, 0.02% bovine serum albumin, 0.02% Ficoll, 10 mM dithiothreitol (DTT), 10 mM Tris, pH 8.0, 1 mM EDTA, pH 8.0, and 500 μg/ml tRNA] including ^{35}S-labeled antisense or sense riboprobe of NPS precursor or NPSR (1×10^7 cpm/ml) was applied to the slides and incubated overnight at 60°C. Sections were then washed four times with 4× SSC; treated with 20 μg/ml RNaseA for 30 minutes at 37°C; and washed with 2× SSC/1 mM DTT, 1× SSC/1 mM DTT, 0.5× SSC/1 mM DTT for 5–10 minutes each, followed by a 30 minutes wash in 0.1× SSC/1 mM DTT at 68°C. Slides were dehydrated by ethanol and air dried. The processed slides were then exposed to β-Max film (Kodak, Rochester, NY) for 6 days to check for positive hybridization signals. Finally, sections were dipped with NTB-2 emulsion (Kodak) and stored at 4°C for 6 weeks. Slides were then developed with Kodak D-19, followed by rapid fixative. Cresyl violet was used to counterstain the sections. Slides were then coverslipped and analyzed under a microscope.

Double in situ hybridization

Double in situ hybridization was carried out as described previously (Clark et al., 2001). Briefly, stock concentrations of DIG-labeled ChAT, GAD, or VGLUT riboprobes were determined by using dot blot hybridization, followed by optical density measurements. To determine the optimal working concentrations for the various riboprobes, each riboprobe was diluted serially with hybridization buffer and hybridized to test sections first. For each probe, one concentration giving the strongest signal at minimal background was chosen for the experimental sections. For experimental sections, a combination of isotopic and colorimetric in situ hybridization was used. First, sections were prehybridized as described above. Then, DIG-labeled riboprobes of ChAT (0.4 mg/ml) or GAD67 (0.4 mg/ml) or a combination of riboprobes for all three VGLUTs (each at 0.15 mg/ml) were mixed with ^{35}S-labeled NPS precursor riboprobe (1–2 $\times$ 10^7 cpm/ml) in hybridization buffer and incubated with sections at 60°C overnight. After hybridization, sections underwent the same posthybridization process as described above. Final stringent washes were for 30 minutes in 0.1 $\times$ SSC at 68°C for GAD67 and ChAT riboprobes or 0.3 $\times$ SSC at 58°C for VGLUTs. After incubation with 0.1$\times$ SSC or 0.3$\times$ SSC at room temperature, sections were rinsed in Genius Buffer 1 (GB1; 100 mM Tris-HCl, 150 mM NaCl, pH 7.5) for 1 minute and then incubated with blocking buffer containing GB1, 0.25% Triton X-100, 5% nonfat dry milk for 1 hour at room temperature. Alkaline phosphatase-conjugated sheep anti-DIG antibody (Roche Diagnostics, Indianapolis, IN) was diluted 1:5,000 in blocking solution, and 1 ml was applied to each slide, followed by incubation at 37°C for 3 hours. Tissues were then washed and incubated with color substrate nitroblue tetrazolium salt (0.5 mg/ml) and 5-bromo-4-chloro-3-indolyl phosphate (0.187 mg/ml) in GB3 (100 mM Tris-HCl, 100 mM NaCl, 50 mM $MgCl_2$, pH 9.5) in the dark for 12–16 hours at RT. Sections were washed again and air dried overnight before being exposed to film as described above. After the films were developed, slides were dipped in 0.3% parlodin dissolved in isoamylacetate for 5 seconds and dried overnight. Finally, slides were dipped in photoemulsion and processed as described above.

Reference sections

A series of cryostat sections of rat brain tissue adjacent to the sections used for in situ hybridizations was collected and mounted on coated microscope slides as described above. Sections were stained with cresyl violet, rinsed, and then coverslipped.

Data analysis

Sections were qualitatively analyzed with a microscope with both light- and darkfield condensers (Axioskop 40; Carl Zeiss, Goettingen, Germany). Images were captured with Spot camera software version 2.2.2 (Diagnostic Instruments, Sterling Heights, MI), and the digital images were optimized for image resolution in Adobe Photoshop 7.0. Anatomical structures were identified according to adult rat brain atlases (Paxinos, 1986; Swanson, 1999). The level of expression was scored as follows: ++++, very strong expression; +++, strong expression; ++, moderate expression; +, widely scattered to low expression; –/+, scattered expression slightly above background; –, no expression.

RESULTS

Neurochemical characteristics of NPS-expressing neurons in the brainstem

Double-label in situ hybridization demonstrated that the NPS-expressing neurons in the LC area do not express GAD67 mRNA (Fig. 1A,B), suggesting that these neurons do not produce GABA. Very small numbers of NPS mRNA-positive neurons in the lateral part of this cluster are coexpressing ChAT mRNA (Fig. 1C), indicating the presence of acetylcholine. The vast majority of NPS-expressing neurons in the locus coeruleus area coexpress VGLUTs mRNA (Fig. 1D), suggesting that these cells are glutamatergic neurons. Small numbers of NPS mRNA neurons in the LC area do not express tyrosine hydroxylase, GAD67, ChAT, or VGLUTs mRNA, indicating that neurotransmitters other than noradrenaline, GABA, acetylcholine, and glutamate may be present (Fig. 1B–D). Taken together, our data indicate that NPS-expressing neurons in the LC area are predominantly glutamatergic, with a few cholinergic neurons found in the lateral part of this brainstem structure. Another brainstem area with strong expression of NPS precursor mRNA is the principle sensory trigeminal nucleus, which is known to contain a high density of glutamatergic neurons. As shown in Figure 1E,F, many NPS-expressing neurons of the principle sensory nucleus also coexpress VGLUTs mRNA, indicating that these neurons use glutamate as neurotransmitter. The third brainstem structure with a high number of NPS precursor-expressing cells is the lateral parabrachial nucleus. By using double in situ hybridization for NPS precursor and CRF mRNA, we found that many of the NPS-positive neurons in the lateral parabrachial nucleus also coexpress CRF (Fig. 1G,H). Control hybridizations with NPS precursor sense probes showed no signals in any sections analyzed (data not shown), indicating sufficient stringency of hybridization conditions and specificity of the signals obtained with the antisense probes.

NPS receptor mRNA distribution

Regional expression of NPSR mRNA was studied by in situ hybridization with ^{35}S-labeled riboprobes. No detectable signal was found in sections hybridized with the sense probe. In contrast to NPS precursor mRNA, NPSR mRNA is found widely expressed in many brain regions. A summary of NPSR mRNA distribution in various rat brain regions is shown in Table 1.

Telencephalon. High levels of NPSR mRNA expression were found in the anterior olfactory nucleus, including medial, ventral, and posterior parts (Fig. 2A,B). Strong expression of NPSR mRNA was observed in endopiriform nucleus and deep layers of the piriform cortex in olfactory regions, and the dense labeling extended caudally throughout the entire cortical distribution of these two regions (Figs. 2A–D, 3E, 5A–F). There was no detectable signal in the main olfactory bulb (Table 1).

Strong NPSR mRNA expression was found in several distinct areas of the cerebral cortex. Densely labeled cells expressing NPSR mRNA were observed in motor cortex 2 and parts of the cingulate cortex 1, but signals were absent from cingulate cortex 2 (Fig. 3A,B). Retrosplenial

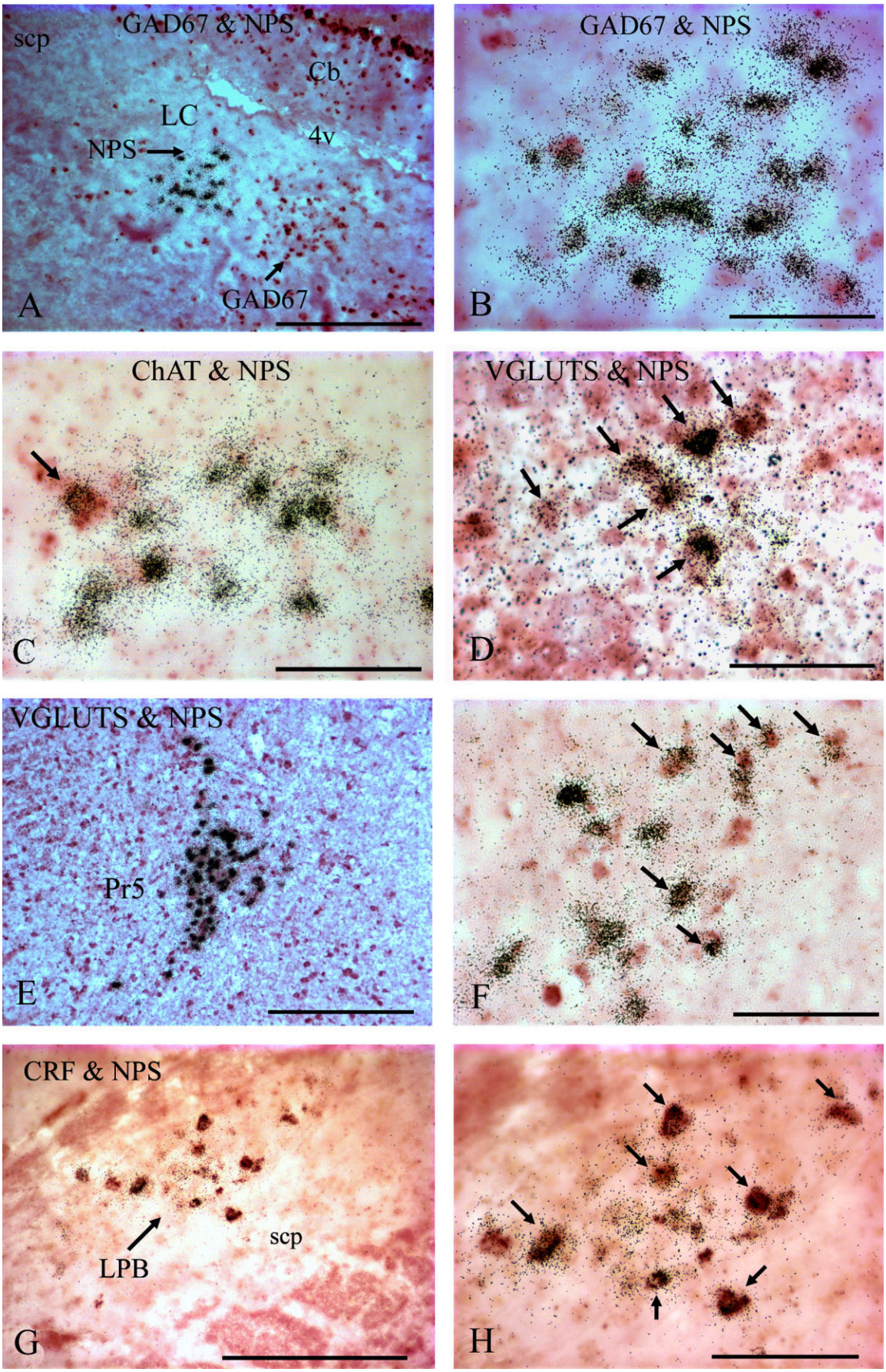
scp
GAD67 & NPS
Cb
LC
4v
NPS
GAD67
A
GAD67 & NPS
B
ChAT & NPS
C
VGLUTS & NPS
D
VGLUTS & NPS
Pr5
E
F
CRF & NPS
LPB
scp
G
H

Figure 1

TABLE 1. Summary of Regional Expression of NPSR mRNA in the Rat Brain

Region	NPSR mRNA	Region	NPSR mRNA
Olfactory		*Thalamus*	
Main bulb	–	Anteroventral/dorsal thalamic n.	–
Anterior olfactory n.	++++	Interanteromedial thalamic n.	++
Olfactory tubercle	+/++	Paraventricular thalamic n.	+/++
Endopiriform n.	++++	Reuniens thalamic n.	++
Piriform cx	+++/++++	Rhomboid thalamic n.	++
Cerebral cortex		Precommissural n.	++/+++
Orbital cx	+	Subcommissural organ	++++
Claustrum	++/+++	Paraventricular thalamic n. posterior	++/+++
Frontal cx	–/+	Central medial thalamic n.	++
Insular cx	+	Central lateral thalamic n.	+/–
Motor cx 1	+	Ventral anterior/lateral thalamic n.	–
Motor cx 2	+++	Ventral posteromedial/lateral thalamic n.	–
Cingulate cx 1	++	Posterior limitans thalamic n.	++
Cingulate cx 2	–	Suprageniculate thalamic n.	+++
Somatosensory cx	+/++	Medial geniculate body	+/++
Parietal cx	++	Lateral geniculate body	+/–
Occipital cx	–/+	*Hypothalamus*	
Temporal cx	+	Medial preoptic n.	–/+
Retrosplenial agranular cx	+++	Lateral preoptic n.	++
Retrosplenial granular cx	–	Ventrolateral preoptic n.	+
Lateral entorhinal cx	++/+++	Anterior hypothalamic area	+++
Amygdala and extended amygdala		Paraventricular hypothalamic n.	++/+++
Anterior cortical amygdaloid n.	+++	Posterior hypothalamic area	+++
Posteromedial cortical amygdala n.	++/+++	Lateral hypothalamus area	++/+++
Posterolateral cortical amygdala n.	–/+	Perifornical n.	++
Medial amygdala n.	++/+++	Dorsomedial hypothalamic n.	+++
Intercalated n. of amygdala	+++/++++	Ventromedial hypothalamic n.	+++/++++
Basolateral amygdala n.	–/+	Posterior arcuate hypothalamic n.	+++
Basomedial amygdala n.	–/+	Dorsal premammillary n.	+++
Lateral amygdala n.	–/+	Ventral premammillary n.	+++
Central amygdala n.	–	Ventral tuberomammillary n.	++/+++
Amygdalopiriform trans. area	++/+++	Dorsal tuberomammillary n.	+++
Amygdalohippocampal area	+/++	*Brainstem*	
Bed n. stria terminalis	+	Medial pretectal n.	+++
Hippocampal formation		Olivary pretectal n.	+++
CA1	–	Superficial/deep gray layer superior colliculus	++
CA2	–	Inferior colliculus	+/–
CA3	–	Periaqueductal gray	++/+++
Dentate gyrus	–	Dorsal raphe n.	+
Septal hippocampal	–	Caudal linear raphe n.	++
Subiculum	++++	Median/pontine raphe n.	+/++
Presubiculum	++++	Ventral tegmental area	++
Postsubiculum	++++	Substantia nigra pars compacta	++
Parasubiculum	++++	Paranigral n.	+/++
Basal ganglia and septum		Interpeduncular n.	++
N.accumbens shell/core	–	Pontine reticular n, oral part	++
Caudate putamen	–	Locus coeruleus	–
Globus pallidus	–/+	Barrington's n.	–
Lateral/medial septal n.	–/+	Lateral parabrachial n.	+
N. horiz limb diagonal band, medial part	++	Intermediate reticular n.	+
Magnocellular preoptic n.	–/+	Lateral reticular n.	+
		Cerebellum	–

Fig. 1. Neurochemical profiles of NPS-expressing neurons in the rat brainstem. A–D: NPS-expressing cell cluster in the locus coeruleus (LC) area. **A:** Representative section showing hybridization with antisense riboprobes for GAD67 (purple) and NPS precursor (black grains) mRNAs in the LC area. Locations of the fourth ventricle (4V), cerebellum (Cb), and superior cerebellar peduncle (scp) are indicated. **B:** Higher magnification of the cluster of NPS-expressing neurons in A, showing no colocalization of the hybridization signals. **C:** Adjacent section of the LC area hybridized with antisense riboprobes for NPS precursor (black grains) and ChAT (purple) mRNA. A single cell coexpressing NPS precursor and ChAT mRNA is indicated by an arrow. **D:** Section hybridized with antisense riboprobes for VGLUTs 1–3 (purple) and NPS precursor (black grains) mRNA. Arrows indicate cells coexpressing VGLUTs and NPS precursor mRNAs. **E,F:** Coexpression of VGLUTs (purple) and NPS precursor (black grains) mRNAs in neurons of the principle sensory 5 nucleus (Pr5). F is an adjacent section of the same area, and arrows indicated double-labeled cells. **G,H:** Coexpression of CRF (dark brown) and NPS precursor (black grains) mRNAs in cells of the lateral parabrachial nucleus (LPB). Arrows in H indicate double-labeled cells, and the location of the superior cerebellar peduncle (scp) is given as a landmark. Sections are oriented with medial areas on the right and lateral areas on the left. Scale bars = 250 μm in A,E,G; 100 μm in B–D,F,H.

agranular cortex contained neurons strongly expressing NPSR mRNA, and the labeling extended into the adjacent parietal association cortex (Fig. 3C,D). Neurons in deep layers of the amygdalopiriform transition area also express high levels of NPSR mRNA (Fig. 3E,F). Scattered cells with moderate to sparse labeling were observed in other cortical areas, including ventral and lateral orbital cortex; motor cortex 1; insular cortex; parts of cingulate cortex 1 in the parietal cortical region; somatosensory cortex, including the barrel fields; temporal association cortex; and auditory cortex (Table 1).

In the hippocampal formation and parahippocampal regions, prominent expression of NPSR mRNA was observed in the dorsal subiculum (Fig. 4A–D). Densely labeled cells were also observed in both superficial and deep layers of postsubiculum (Fig. 4C,D). Presubiculum and parasubiculum also display substantial signals (Fig. 4E,F). However, no hybridization signals were found in hippocampus proper, including CA1–3 regions and the dentate gyrus (Fig. 4A–D, Table 1). Deep layers of the lateral entorhinal cortex also express moderate to high levels of NPSR mRNA (Fig. 4E,F).

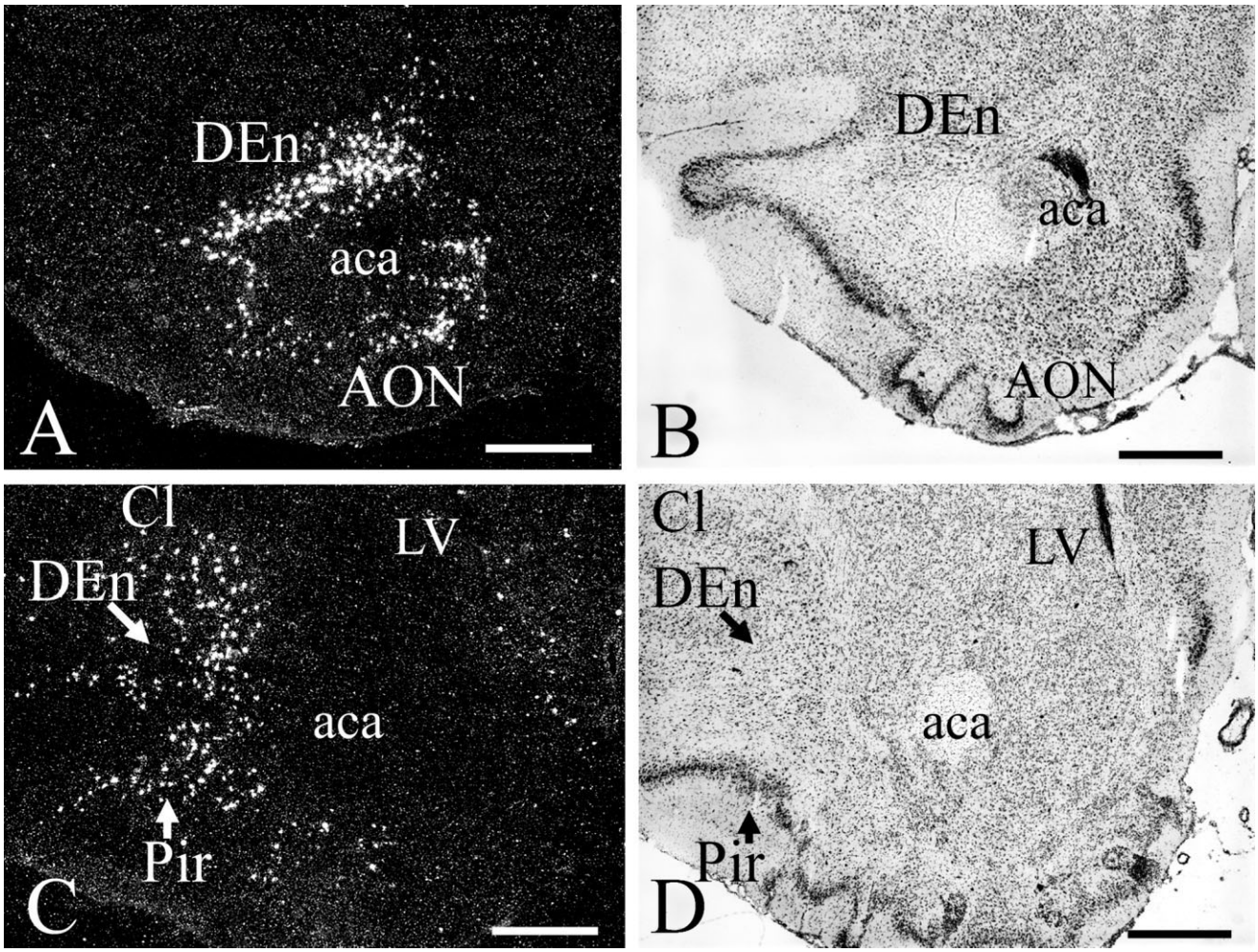

Fig. 2. Distribution of NPSR mRNA in olfactory regions. **A,C:** Darkfield images of sections hybridized with antisense ^{35}S-labeled riboprobes for NPSR mRNA. **B,D:** Brightfield images of adjoining sections illustrated in A,C counterstained with cresyl violet, respectively. For abbreviations see list. Scale bar = 500 μm.

The amygdaloidal complex shows strong to moderate signals of NPSR mRNA expression in several divisions. High levels of expression are evident in neurons within the medial (Fig. 5A–F), anterior cortical (Fig. 5A–D), posteromedial cortical (Fig. 5E,F) and intercalated (Fig. 5A–D) nuclei of the amygdala. No significant expression was observed in basolateral or lateral amygdala, except for a few scattered neurons (Fig. 5A–F, Table 1).

Septal and basal forebrain regions generally express low levels of NPSR mRNA. The magnocellular preoptic nucleus shows only weakly labeled cells. Scattered neurons in the bed nucleus of the stria terminalis contain low levels of NPSR mRNA. Only scant signals were found in lateral and medial septum (Table 1). Basal ganglia, including caudate, putamen, and globus pallidus, show little hybridization signal. No NPSR mRNA expression was observed in the nucleus accumbens (Table 1).

Diencephalon. In the thalamus, moderate to high levels of NPSR mRNA expression were found in various areas. Multiple midline thalamic nuclei show significant levels of expression, such as interanteromedial (Fig. 6A,B), rhomboid (Figs. 6A,B, 8A,B), reuniens (Fig. 6A,B), anteromedial (Fig. 8A,B), and xiphoid (Fig. 8A,B) thalamic nuclei. Substantial hybridization signals were observed in the precommissural nucleus, subcommissural organ, and paraventricular thalamic nucleus (Fig. 6C–F). A moderate level of expression was also found in the posterior limitans thalamic nucleus (Fig. 6G,H). Substantial NPSR mRNA expression was detected in scattered neurons of the medial geniculate body, with labeling extending to a cluster of cells in the suprageniculate thalamic nucleus (Fig. 9C,D). Dispersed, moderately labeled cells are observed in the intralaminar nuclei (Fig. 6G,H). The major sensory relay nuclei of the thalamus, such as ventral anterior, ventral lateral and ventroposteromedial/lateral thalamic nuclei, do not express NPSR mRNA (Table 1).

In preoptic regions, NPSR mRNA is expressed mainly in the lateral preoptic area. Moderate labeling was as-

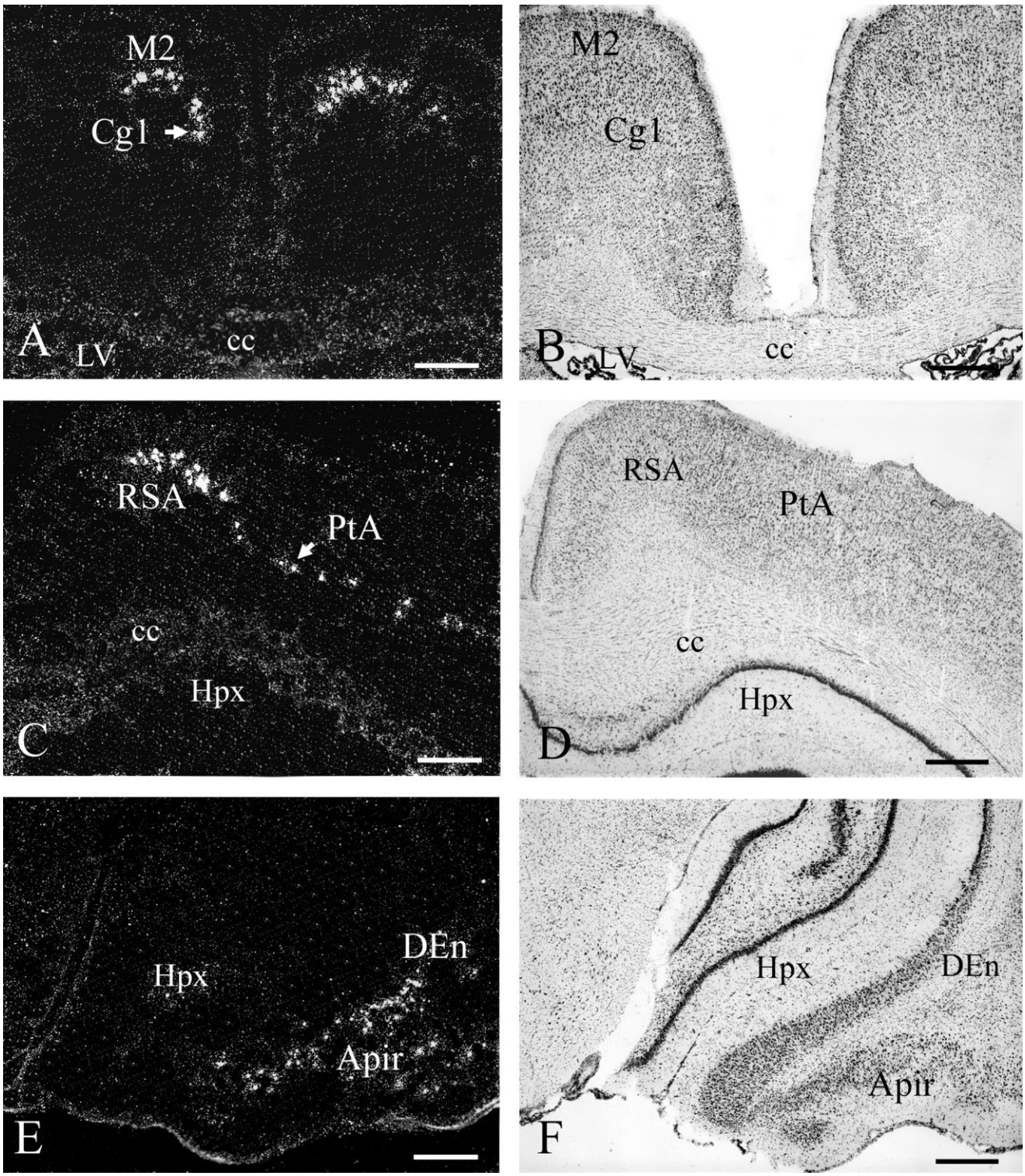

Fig. 3. Expression of NPSR mRNA in cortical regions. **A,C,E:** Darkfield images of sections hybridized with antisense ^{35}S-labeled riboprobe for NPSR mRNA. **B,D,F:** Brightfield images of adjoining sections shown in A,C,E counterstained with cresyl violet. For abbreviations see list. Scale bar = 500 μm.

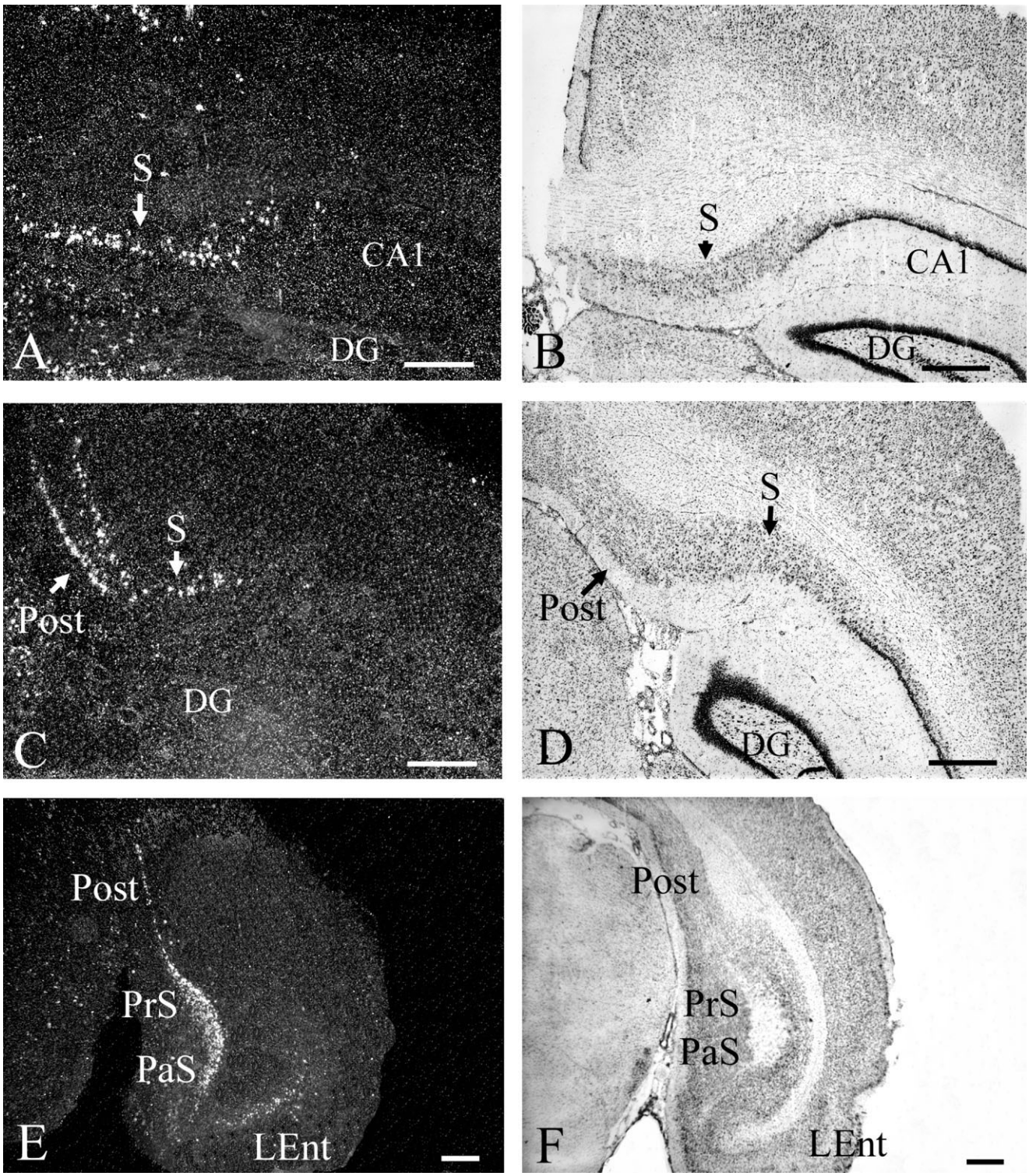

Fig. 4. Expression of NPSR mRNA in hippocampal formation and parahippocampus. **A,C,E:** Dark-field images of NPSR mRNA expression. **B,D,F:** Brightfield images of adjacent sections shown in A,C,E counterstained with cresyl violet. For abbreviations see list. Scale bar = 500 μm.

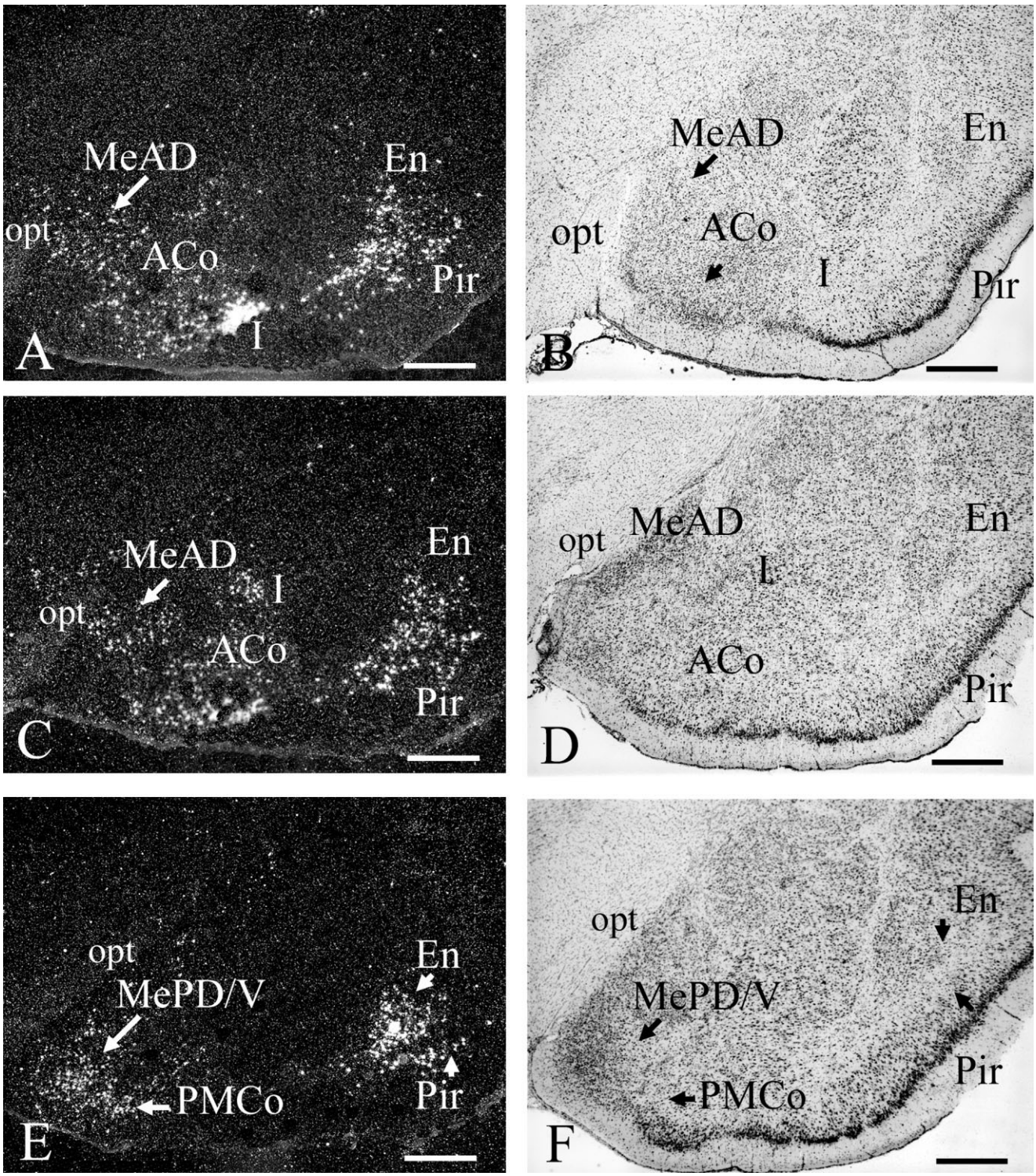

Fig. 5. NPSR mRNA is expressed in multiple nuclei of the amygdala complex. **A,C,E:** Darkfield images of NPSR mRNA expression in amygdala complex. **B,D,F:** Brightfield images of adjoining sections shown in A,C,E counterstained with cresyl violet. For abbreviations see list. Scale bar = 500 μm.

sociated with scattered neurons of the lateral preoptic area and a cluster of neurons at the medial end of the nucleus of the horizontal limb of the diagonal band (HDB; Fig. 7A,B), whereas, more caudally, this cluster is found at the interface of the ventrolateral preoptic nucleus and the medial part of HDB (Fig. 7C,D).

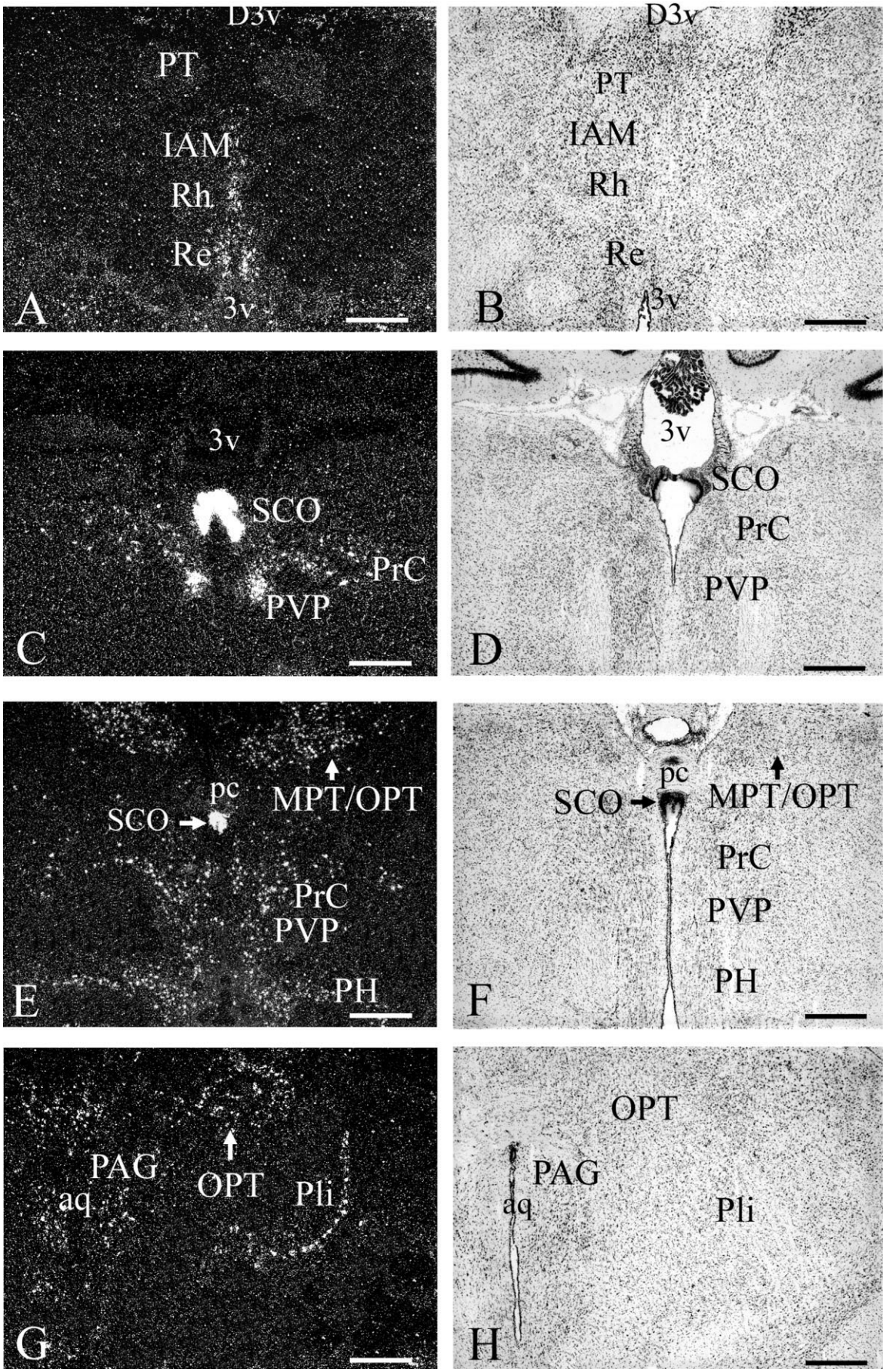

Fig. 6. Expression of NPSR mRNA in thalamus and pretectal area. **A,C,E,G:** Darkfield images of sections hybridized with antisense ^{35}S-labeled riboprobe for NPSR mRNA. **B,D,F,H:** Brightfield images of adjacent sections shown in A,C,E,G counterstained with cresyl violet. For abbreviations see list. Scale bar = 500 μm.

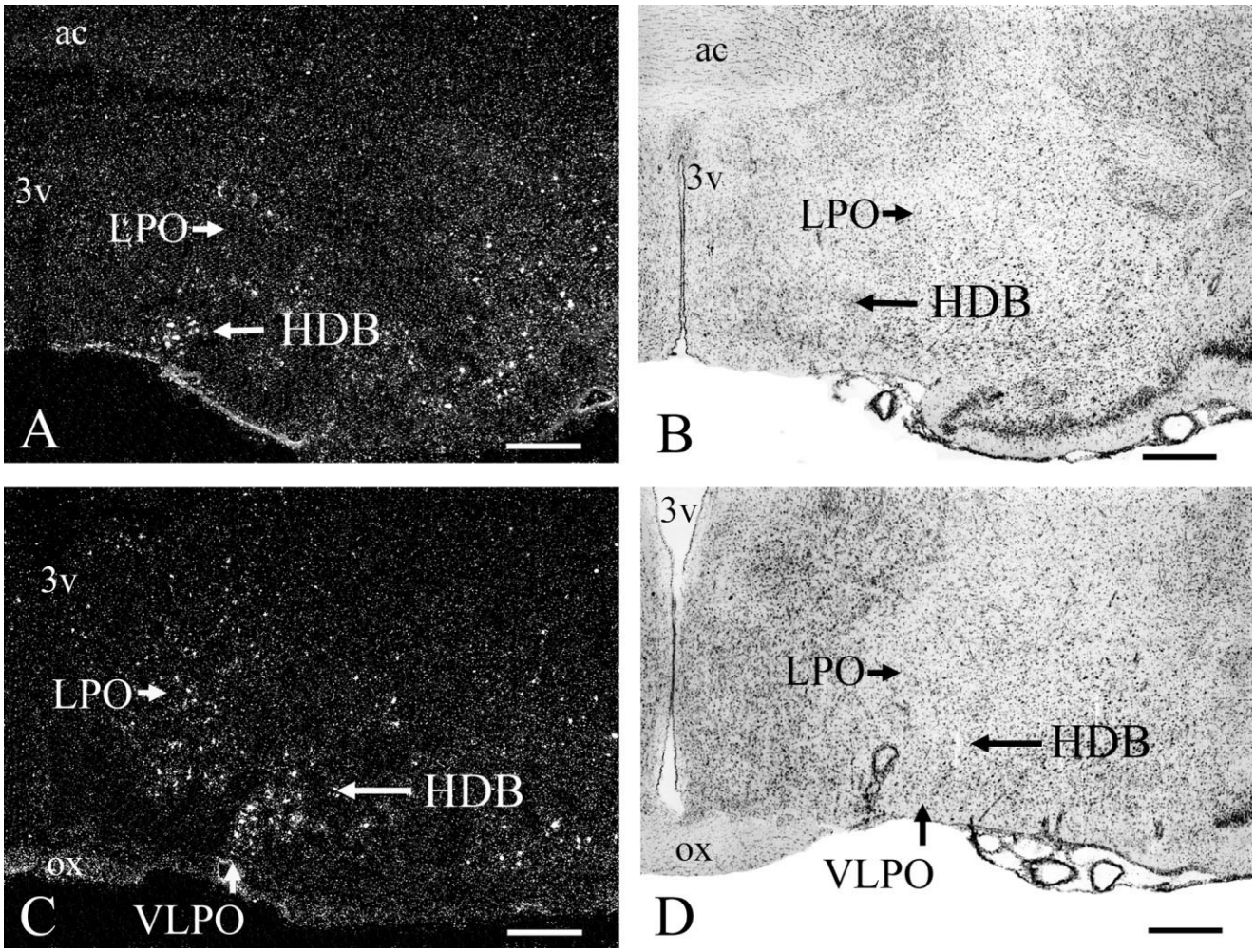

Fig. 7. Expression of NPSR mRNA in preoptic area. **A,C:** Darkfield images of sections hybridized with antisense ^{35}S-labeled riboprobes for NPSR mRNA; **B,D:** Brightfield images of adjacent sections shown in A,C counterstained with cresyl violet. For abbreviations see list. Scale bar = 500 μm.

NPSR mRNA signals were localized within multiple hypothalamic areas and nuclei. Moderate to strong levels of expression were found in many neurons of the dorsal hypothalamus, including the paraventricular hypothalamic nucleus (Fig. 8A–D). Abundant signals were observed in the anterior (Fig. 8A,B), posterior, and lateral hypothalamic areas, including the perifornical nucleus (Figs. 6E,F, 8A,B,E,F). Dorsomedial and ventromedial hypothalamic nuclei express high levels of NPSR mRNA (Fig. 8C–F). The tuberomammillary area contains significant amounts of NPSR mRNA, and many neurons in the posterior arcuate hypothalamic nucleus were well labeled (Fig. 8G,H). High levels of expression were found in both the dorsal and the ventral premammillary nucleus and in the dorsal tuberomammillary nucleus (Fig. 8G,H). Scatted neurons in the ventral tuberomammillary nucleus also contain significant levels of NPSR mRNA (Fig. 8G,H). No hybridization signal was found in medial and lateral mammillary nuclei or in the supramammillary nucleus (Table 1).

Brainstem. NPSR mRNA was abundant in the medial pretectal nucleus and olivary pretectal nucleus in pretectum regions (Fig. 6E–H). Moderate hybridization signals also labeled neurons in superficial and deep layers of the superior colliculus (Fig. 9A,D). The inferior colliculus contained only weak NPSR expression. Many neurons in the periaqueductal gray (PAG), especially the medial PAG, were labeled with moderate intensity (Fig. 9A,B,E,F).

Scattered neurons in the caudal linear nuclei of raphe contained moderate NPSR mRNA signals (Fig. 10A,B). The dorsal raphe nucleus displays only weak hybridization signals (Fig. 9E,F). Weak to moderate levels of expression were also found in scattered neurons of the median (Fig. 10E,F) and pontine raphe (Table 1). A few positive neurons with moderate intensity of signals were identified in the ventral tegmental area and substantia nigra pars compacta (Fig. 10C,D). Pontine tegmental regions in general contain scatted NPSR mRNA expression. The paranigral and interpeduncular nuclei of the pons show moderate to weak NPSR signals (Fig. 10A,B). Moderate signals were also observed in a few neurons in the oral part of the pontine reticular nucleus (Fig. 10E,F). Weak expression of NPSR mRNA was found in the lateral parabrachial nucleus as well as in the ventral, external,

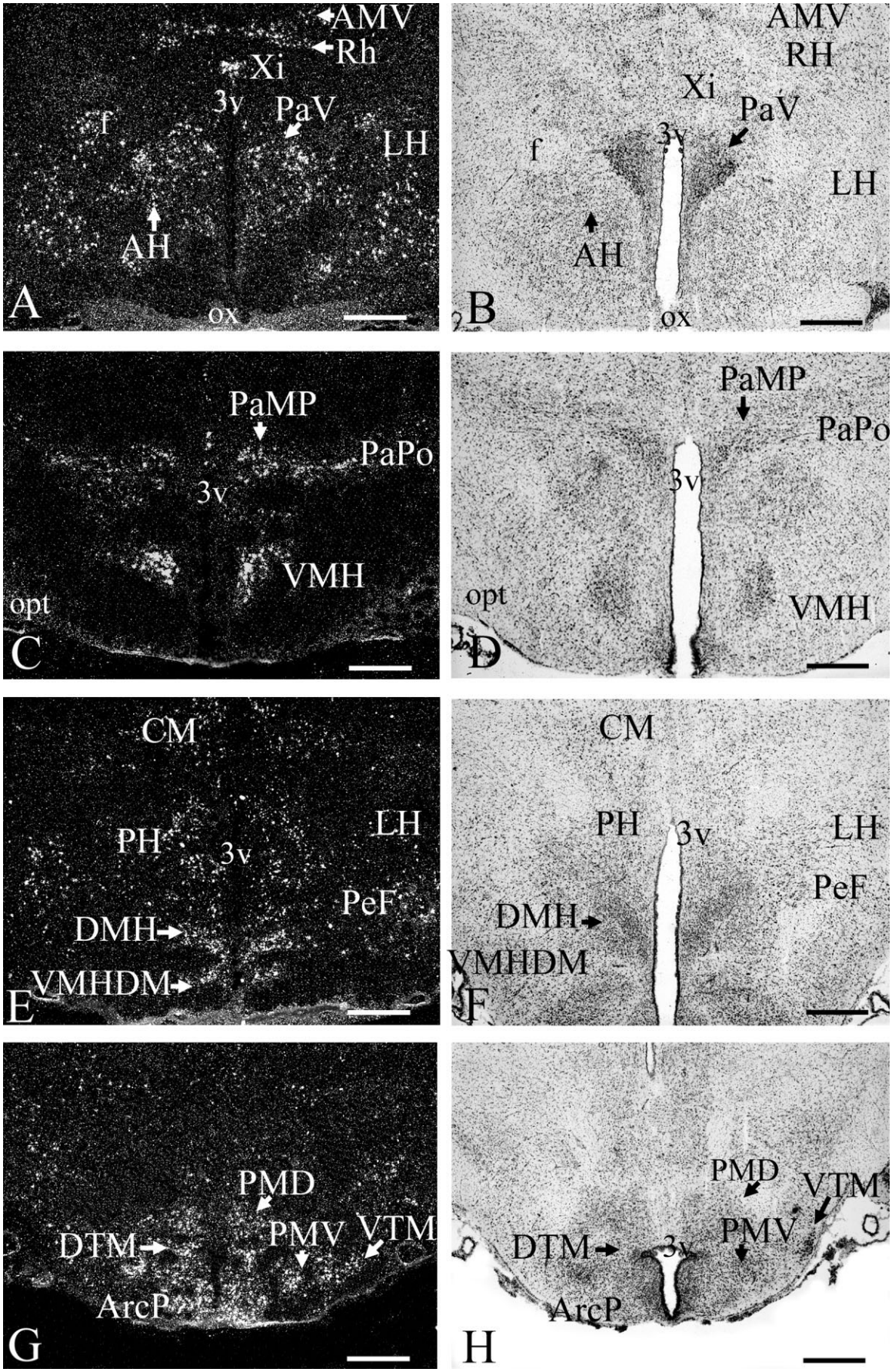

Fig. 8. Distribution of NPSR mRNA in hypothalamus and surrounding thalamic regions. **A,C,E,G:** Darkfield images of rat hypothalamus area showing NPSR mRNA expression. **B,D,F,H:** Brightfield images of adjoining sections shown in A,C,E,G counterstained with cresyl violet. For abbreviations see list. Scale bar = 500 μm.

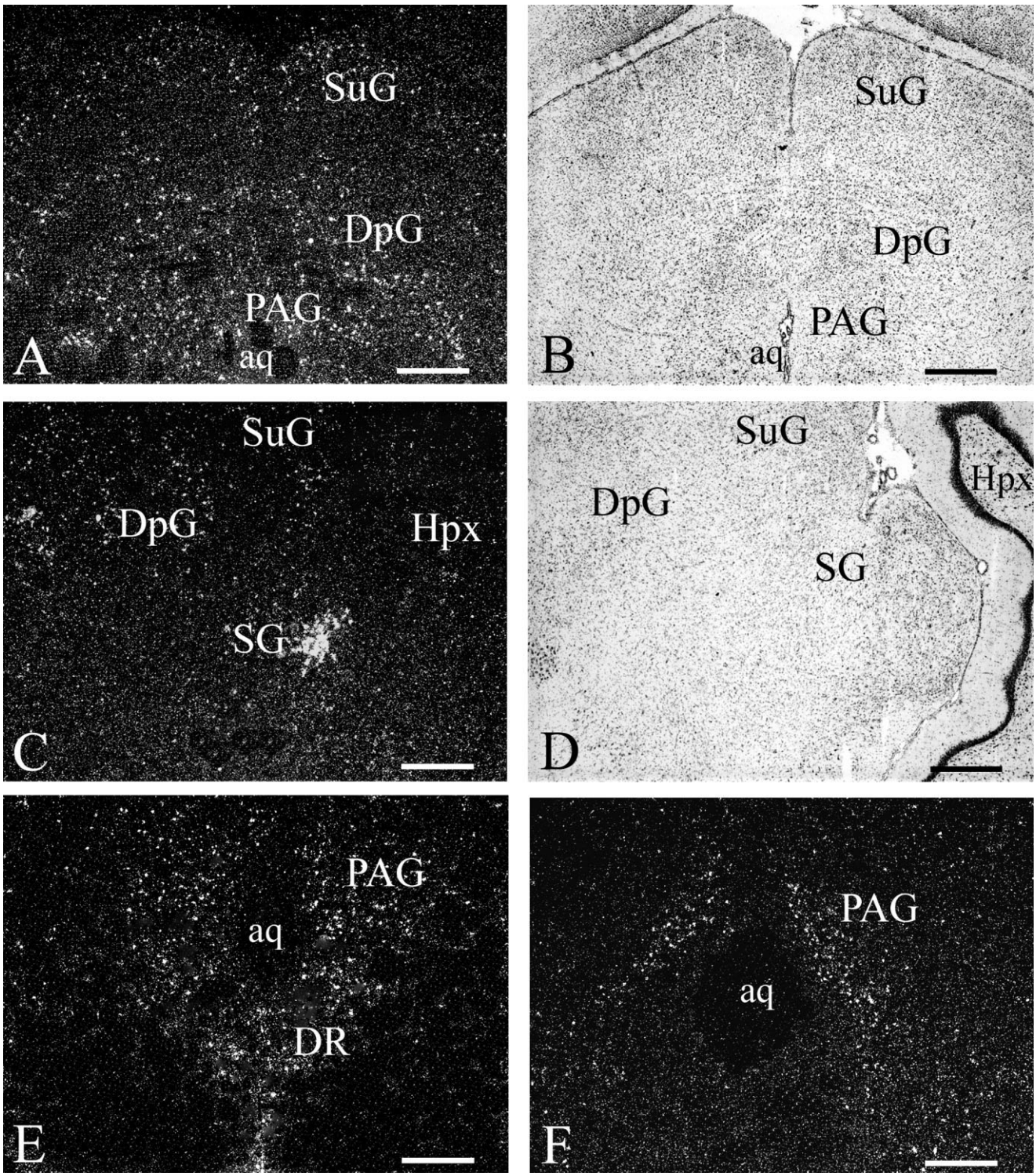

Fig. 9. Expression of NPSR mRNA in midbrain regions. **A,C,E,F:** Darkfield images of rat midbrain sections showing NPSR mRNA expression. **B,D:** Brightfield images of adjacent sections shown in A,C counterstained with cresyl violet. For abbreviations see list. Scale bar = 500 μm.

and dorsal part of medial parabrachial nuclei (Table 1). A few neurons in facial nucleus express weak to moderate signals (Table 1). Scattered neurons in intermediate and lateral reticular nuclei show weak NPSR mRNA signals. Neurons of LC proper and surrounding pericoerulear areas did not show measurable hybridization signals of

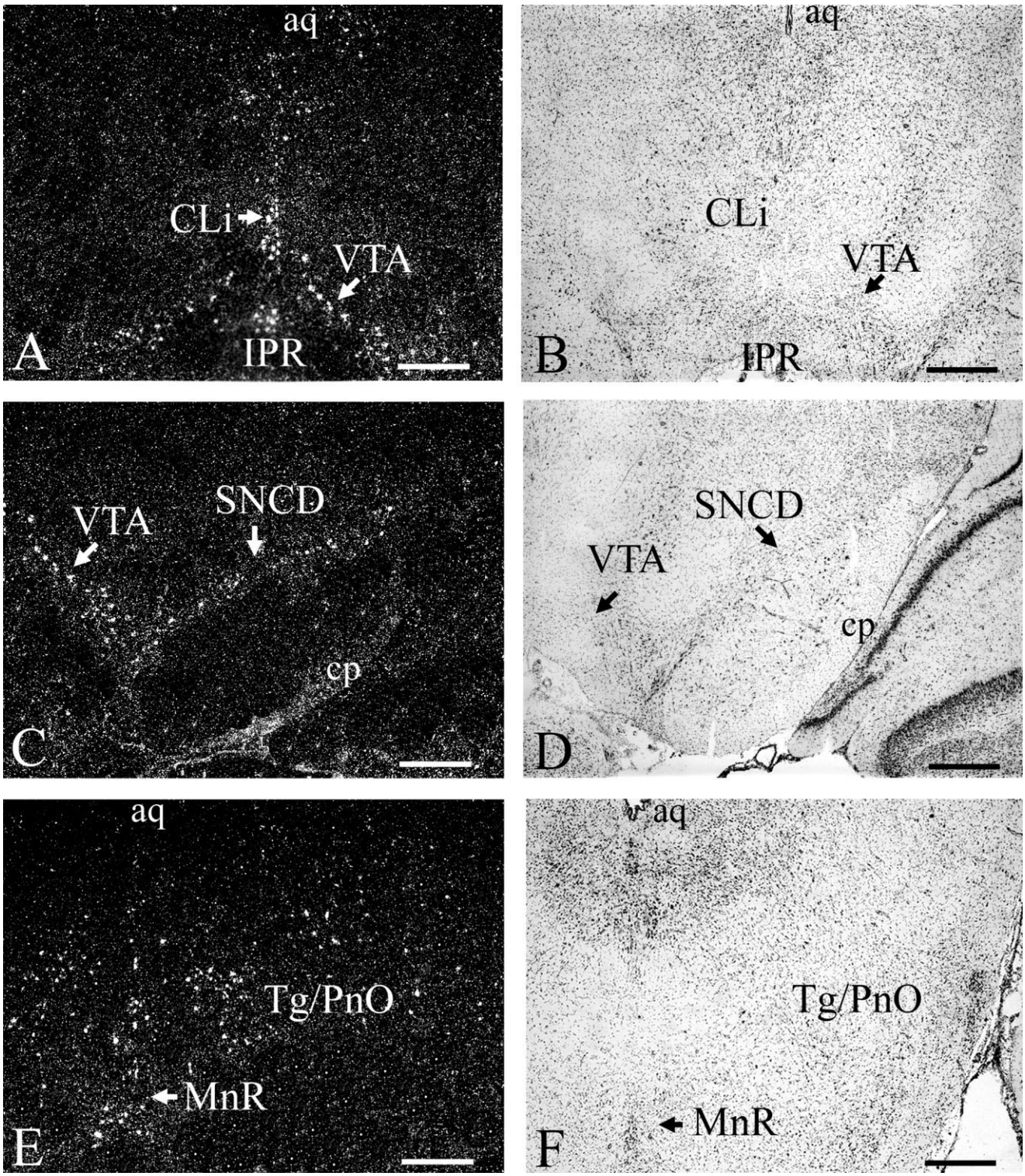

Fig. 10. Distribution of NPSR mRNA in tegmental area of the midbrain and pontine regions of the brainstem. **A,C,E:** Darkfield images of sections displaying NPSR mRNA expression. **B,D,F:** Brightfield images of adjoining sections shown in A,C,E counterstained with cresyl violet. For abbreviations see list. Scale bar = 500 μm.

NPSR mRNA expression. No detectable hybridization was observed in cerebellum.

DISCUSSION

Here we describe the neurochemical profiles of NPS-synthesizing neurons in the rat pontine brainstem and a detailed map of NPSR expression throughout the adult rat brain. Insofar as NPS is a very recently identified neuropeptide system, these data will provide a useful basis for new hypotheses about physiological functions of NPS and its interactions with other neurotransmitter systems in the brain.

Our results show that NPS precursor is coexpressed with excitatory or stimulatory transmitters, such as glutamate, acetylcholine, and CRF. These observations provide a neurochemical link to our initial findings showing that central NPS administration promotes arousal and wakefulness (Xu et al., 2004b). They are also in line with our observation that NPS receptors produce stimulatory intracellular signals, such as mobilization of Ca^{2+} and increase of cAMP (Reinscheid et al., 2005). Most of the NPS-expressing cells in the LC area and the trigeminal principle sensory nucleus are glutamatergic neurons, so it is likely that NPS may be coreleased together with glutamate from these neurons. Therefore, NPS may provide additional excitatory input to the postsynaptic targets of these cells. It is also possible that functional interactions between NPS receptors and glutamate receptors in postsynaptic neurons might lead to enhanced glutamatergic neurotransmission. Interactions between peptide G-protein-coupled receptors and glutamate receptors have been described for dopamine D1 receptors and N-methyl-D-aspartate (NMDA)-type glutamate receptors (Lee et al., 2002) and for prolactin-releasing peptide receptors and GluR2 or GluR3 AMPA receptors (Lin et al., 2001, 2002). Further studies investigating the interaction between NPS receptors and glutamate receptors will be necessary to elucidate the role of the NPS system in excitatory neurotransmission. Similarly, the corelease of NPS and CRF from lateral parabrachial nucleus neurons might have a functional correlate that warrants further studies.

The presence of glutamatergic and cholinergic neurons in the area ventromedial to LC and dorsolateral to Barrington's nucleus is consistent with previously published literature. It has been reported that a few ChAT-positive neurons were found in the area between Barrington's nucleus and LC (Sutin and Jacobowitz, 1988; Rizvi et al., 1994). In situ hybridization studies with VGLUT2 probes have shown that glutamatergic neurons are also localized in this region (Stornetta et al., 2002). So far, the projections of these NPS-containing glutamatergic neurons are unknown. Tracing experiments or immunohistochemical analysis of NPS peptide localization will be needed to map the projections of these neurons. The specific responses that these neurons may regulate are unknown at present. No significant signals of NPSR mRNA were observed in this area. Therefore, it is unlikely that NPS coexpressed in these glutamatergic neurons could modulate glutamate release via a presynaptic mechanism. We have shown that central NPS administration promotes arousal and reduces anxiety. The LC and its major neurotransmitter noradrenaline (NA) are well known to regulate arousal and stress responses. It has been reported that tonic discharges of LC neurons are virtually silent during REM sleep, low during slow wave sleep, and highest during wakefulness (Hobson et al., 1975; Foote et al., 1980). Also, the NA system plays an important role in mediating stress responses (Berridge and Waterhouse, 2003). The absence of NPSR mRNA in LC neurons, however, indicates that NPS may not modulate NA release from these cells via direct action. It is therefore possible that the LC area might contain two neurochemically distinct systems, namely, noradrenaline and NPS containing, that are both involved in regulating arousal and anxiety.

The glutamatergic neurons of the trigeminal principal sensory nucleus are known to transmit sensory information from orofacial fields to thalamocortical relays (Killackey et al., 1995). The expression of NPS mRNA in these neurons suggests that NPS neurotransmission might be involved in the regulation of sensory input into the trigeminothalamic pathway.

The lateral parabrachial nucleus plays an important role in regulating autonomic functions and nociceptive processing, in that it relays visceral afferent input from the nucleus of the solitary tract (Herbert and Saper, 1990) and pain stimuli from the spinal cord (Gauriau and Bernard, 2002) to the forebrain. It has been reported that CRF-positive neurons are identified in the lateral parabrachial nucleus (Swanson et al., 1983; Herbert and Saper, 1990), which is consistent with our findings. Neurons of the lateral parabrachial nucleus have been reported to project to many brain regions, such as ventromedial and paraventricular hypothalamic nuclei, amygdala, and periaqueductal gray matter (Gauriau and Bernard, 2002), where NPSR mRNA are highly expressed. This indicates that the NPS system might also play important roles in integrating and regulating behavioral or emotional responses to autonomic or nociceptive inputs.

Olfaction

Abundant NPSR mRNA was found in many regions that are involved in olfaction. A high level of expression of NPSR mRNA was found in anterior olfactory nucleus, endopiriform nucleus, and piriform cortex. Strong expression is also evident in several nuclei of the amygdala complex that are functionally associated with the olfactory system, including medial, anterior cortical, and posteromedial cortical amygdala (Swanson and Petrovich, 1998). Scattered NPSR mRNA signals were also found in neurons of claustrum and insular cortex, where olfactory afferent pathways terminate (Shipley and Geinisman, 1984). The high expression levels of NPSR mRNA in these regions indicate that NPS may be involved in regulation of perception and/or integration of olfactory or pheromonal information.

Modulation of anxiety

We have shown before that central administration of NPS produces anxiolytic-like behaviors in mice using four different paradigms that measure behavioral responses to stress or fear, including open field, light-dark box, elevated plus maze, and marble burying tests. NPSR mRNA is expressed in various stress-related brain regions, such as the amygdala, hypothalamus, raphe nuclei, and ventral tegmental area (Xu et al., 2004b).

The amygdala plays a major role in regulating stress responses and processing of emotional memory (Swanson and Petrovich, 1998; McGaugh, 2004). NPSR mRNA is densely expressed in the medial, anterior cortical, postero-

medial cortical, and intercalated nuclei of the amygdala. Neurons in these subdivisions project to either the central or the basolateral amygdala, two amygdaloid regions associated with integration of stress responses. The NPSR-expressing amygdala regions also project directly to stress-related regions outside the amygdaloid complex, including, most notably, the hypothalamus (Sah et al., 2003). NPSR mRNA is also expressed at low levels in the bed nucleus of the stria terminals, a region considered as part of the extended amygdala that is strongly implicated in modulating stress responses. Moderate to high levels of NPSR expression were observed in several nuclei of the hypothalamus, including the paraventricular hypothalamic nucleus, which is one of the main centers of CRF synthesis and an integrator of stress responses via the hypothalamus-pituitary-adrenal axis (Koob and Heinrichs, 1999; Heinrichs and Koob, 2004).

Aminergic neurons in the brain have been shown to play important roles in regulation of anxiety. Serotonergic (5-HT) neurons in raphe nuclei project widely to amygdala, frontal cortex, hippocampus, and hypothalamus and are involved in modulating anxious states (Millan, 2003). NPSR mRNA is expressed at moderate levels in raphe nuclei, including the caudal linear nucleus, median raphe, and pontine raphe. NPSR mRNA is also expressed in the ventral tegmental area, which is the origin of mesolimbic and mesocortical dopaminergic pathways. The dopaminergic pathways send input to the amygdala and play important roles in control of stress responses and other emotional processes (Millan, 2003). In summary, the expression of NPSR mRNA in anxiety-associated neurocircuits such as corticolimbic, hypothalamic, and important aminergic centers indicates that the NPS system may interact with other neurotransmitter systems, such as CRF, 5-HT, and dopaminergic systems in the modulation of anxiety and stress responses.

Arousal regulation

Our previous studies have shown that central administration of NPS potently suppressed paradoxical (REM) sleep and slow wave sleep 1 and 2 in rats. Conversely, NPS significantly increased the percentage of time spent in wakefulness during the first hour after injection. In addition, NPS induces hyperlocomotion in mice when injected centrally (Xu et al., 2004b). These data suggest that NPS might be part of the brain arousal systems (Jones, 2003). Indeed, significant expression of NPSR mRNA was found in several activating/arousal pathways. In the thalamus, NPSR mRNA expression was detected in multiple midline thalamic nuclei, such as interanteromedial, rhomboid, and diffuse interlaminar group of thalamic nuclei, which relay extensive input from the brainstem reticular formation to cortical regions and are thus important in regulation of arousal (Van der Werf et al., 2002). In the hypothalamus, significant NPSR expression was found in the perifornical region of the lateral and posterior hypothalamic area, where another important arousal-promoting neuropeptide, hypocretin/orexin, is produced (Sutcliffe and de Lecea, 2002). Significant NPSR mRNA hybridization was localized in the ventral tuberomammillary nucleus, which produces histamine and is well known to regulate vigilance states (Jones, 2003). In the brainstem, NPSR is expressed in the substantia nigra and the VTA. These brain regions are important centers of dopamine synthesis, and activation of dopamine systems has been shown to induce behavioral arousal and hyperlocomotion (Jones, 2003). Moderate NPSR mRNA signals were also found in scattered neurons of the pontine reticular nucleus, a brainstem region known to activate widespread regions of the cortex (Jones, 2003).

In addition to these activating systems, NPSR mRNA is also expressed in brain centers that promote sleep. NPSR mRNA was found in many neurons of the lateral preoptic area and in a cluster of neurons at the interface of the ventrolateral preoptic nucleus (VLPO) and the medial part of the nucleus of the HDB. It has been shown that neurons in these regions use GABA as their neurotransmitter and are active during sleep and relatively inactive during wakefulness (Saper et al., 2001). Lesions of HDB were found to suppress sleep, whereas electrical stimulation of HDB promotes non-REM sleep (Szymusiak and McGinty, 1989). Many HDB neurons are thermosensitive, and local warming of the area facilitates non-REM sleep (Hays et al, 1997). The expression of NPSR mRNA in all these regions suggests possible interaction of the NPS system with various activating as well as sleep-promoting systems in the brain, with the overall result of increasing vigilance. Activation of NPS receptors may stimulate arousal-promoting systems such as glutamatergic, histaminergic, dopaminergic, and hypocretin/orexin systems and/or inhibit GABAergic systems in sleep centers such as the VLPO and HDB. Further double in situ hybridization experiments are necessary to determine whether NPS receptor mRNA in these regions is coexpressed with these neurotransmitters that are involved in sleep/wake regulation.

Learning and memory

NPSR mRNA is expressed at striking levels in major output areas of the hippocampal formation, such as subiculum and posterior parahippocampal regions, including presubiculum and parasubiculum. It is also expressed by major afferents to the hippocampal formation, such as lateral entorhinal and endopiriform cortex. All of these regions are critical in regulating learning and memory, in particular, spatial learning. The subiculum sends out major hippocampal efferents to a wide range of cortical and subcortical targets (Swanson and Cowan, 1977). Neurons in the subiculum are stimulated specifically according to both location and directional information (Sharp and Green, 1994; Laxmi et al., 1999; Sharp et al., 2001). Several studies have shown that selective subiculum lesions can impair spatial learning in rats (Morris et al., 1990; Laxmi et al., 1999). The posterior parahippocampus, including post-, para-, and presubiculum, receives input from various brain regions that are involved in the association of memory with visuospatial information, including CA1, subiculum, thalamus, basolateral amygdala, and retrosplenial cortex (Kohler, 1985; Van Groen and Wyss, 1990, 1992, 1995). Projections of pre- and parasubiculum make up one of the major output pathways for memory information processed in the hippocampus to the entorhinal cortex (Swanson and Cowan, 1977; Van Groen and Wyss, 1990). So-called head direction cells representing the actual position and angle of the head are found in the presubiculum. Selective lesions of parahippocampal regions disrupt object recognition and spatial memory, especially maintenance of spatial information over prolonged periods of time, place memory, and object–place associations (Taube et al., 1990, 1992; Liu et al., 2004). NPSR mRNA is also expressed in deep layers of the lateral

entorhinal cortex, where outputs from the hippocampus primarily reach the entorhinal cortex. Superficial layers of the entorhinal cortex are also known to integrate multimodal sensory information from various cortical fields and to project back to hippocampal structures, such as the dentate gyrus region, via the perforant pathway (Amaral and Witter, 1995). Strong NPSR mRNA expression was also found in several cortical regions such as endopiriform cortex, piriform cortex, and retrosplenial agranular cortex, which project densely to the entorhinal cortex and are presumed to relay multimodal sensory information from other cortical regions (Amaral and Witter, 1995). The strong expression of NPSR in these areas indicates that the NPS system may modulate conveyance of information in and out of the hippocampus and thereby might be a key player in memory formation or consolidation, especially regarding spatial memory.

Energy balance

The hypothalamus is the predominant brain center regulating energy homeostasis. NPS precursor and receptor mRNAs are expressed in various nuclei in the hypothalamus that are involved in feeding. Both NPS precursor and NPSR mRNA are expressed in the dorsomedial hypothalamic nucleus (DMH). The ventromedial hypothalamic nucleus (VMH) also contains strong NPSR mRNA hybridization signals. Lesion studies have shown that destruction of DMH (Bernardis and Bellinger, 1998) or VMH (Shimizu et al., 1987) leads to hyperphagia, demonstrating that these regions may act as "satiety" centers in regulating energy balance. In addition, significant amounts of NPSR mRNA are expressed in the posterior arcuate hypothalamic nucleus, where important orexigenic systems, such as neuropeptide Y (NPY) or agouti gene-related protein (AGRP), and anorexigenic systems, such as proopiomelanocortin (POMC)- or cocaine- and amphetamine-regulated transcript (CART)-expressing neurons, are found (Flier and Maratos-Flier, 1998; Elias et al., 2001). Moderately dense NPSR mRNA labeling was also found in lateral hypothalamic areas and posterior hypothalamus, especially in the perifornical area, where two other neuropeptides, hypocretin/orexin (de Lecea et al., 1998) and melanin-concentrating hormone (MCH; Bittencourt and Elias, 1998), are synthesized. Extensive studies have shown that MCH acts as an orexigenic signal to induce food intake and decrease energy expenditure (Rossi et al., 1997; for review see Xu et al., 2004a). The hypocretin/orexin system can also modulate food intake, and it has been reported that central administration of hypocretin 1 can stimulate food intake (Sakurai, 1999; Yamanaka et al., 2003). The anatomical distribution of NPSR mRNA in the hypothalamus indicates that the NPS system could modulate other orexigenic and anorexigenic signals and play a role in regulating food intake and/or energy expenditure. Recently, it has been reported that acute central administration of NPS (1 μg) decreased food intake in fasted rats during the first hour postinjection (Beck et al., 2005). Cumulative food intake during the first 3 hours was significantly reduced in rats injected with NPS at low doses (1 μg) and during 6 hours in rats injected with a high dose (10 μg) of NPS. NPS injection also significantly inhibited spontaneous intake of high-energy diet in satiated rats during the first hour after injection. This study indicates that NPS could have a potent effect on inhibiting food intake. However, the arousal-enhancing effect of NPS has to be considered when effects of NPS on food intake are evaluated. We have shown that central administration of NPS induces wakefulness in rats and hyperlocomotion in mice during the first hour after injection. NPS-induced inhibition of food intake was also observed, mainly during the first hour postinjection, and was followed by a rebound lasting for up to 3 hours postinjection in fasted rats. It is therefore possible that reduced food intake observed after acute administration of NPS may be partially secondary to the increased exploratory activity or vigilance induced by NPS and not a direct effect on food-seeking behavior.

CONCLUSIONS

In summary, this study is the first report to demonstrate that the majority of NPS-expressing neurons located between LC and Barrington's nucleus are glutamatergic neurons. The present study also provides a comprehensive anatomical description of NPSR mRNA expression in the rat brain. NPSR mRNA is expressed in many brain regions, notably in olfactory regions, amygdala complex, and limbic regions, along with multiple arousal-activating systems and sleep-promoting regions, which supports a role of the NPS system in anxiety and vigilance regulation. NPS receptor mRNA is also expressed significantly in the input and output regions of the hippocampal formation amd multiple hypothalamic regions, such as arcuate nucleus, VMH, and DMH, suggesting possible roles of NPS in modulating learning and memory as well as effects on energy homeostasis. The widespread distribution of NPS receptor mRNA in the brain indicates that the NPS system may be important in regulating a variety of physiological functions.

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