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Abstract

Nogo-A and Nogo-receptor have been considered to play pivotal roles in controlling axonal regeneration and neuronal plasticity. We investigated the total distribution of Nogo-A and Nogo-receptor mRNAs in the adult rat central nervous system using in situ hybridization histochemistry. Nogo-A is abundantly expressed in both neurons and oligodendrocytes throughout the central nervous system. Interestingly, we could not find any neurons which lack Nogo-A mRNA expression, indicating that Nogo-A mRNA is universally expressed in all neurons. In contrast, Nogo-R mRNA expression was very restricted. Nogo-R mRNA was expressed in the olfactory bulb, hippocampus, tentia tecta, some amygdala nuclei, cerebral cortex, some thalamic nuclei, medial habenular, whereas we could not detect it in the other regions. Interestingly, we did not detect Nogo-R mRNA in monoaminergic neurons, which are known to have high regenerative capacity, in the substantia nigra, ventral tegmental area, locus caeruleus, and raphe nuclei. In addition, although neurons in the reticular thalamus and cerebellar nuclei are also known to show high capacity for regeneration, Nogo-R mRNA was not detected there. These data indicate that Nogo-A and Nogo-R mRNAs were differentially expressed in the central nervous system, and suggest that the lack of Nogo-R expression in a given neuron might be necessary to keep its high regenerative capacity.

Theme: development and regeneration
Topic: regeneration

Key words: in situ hybridization, Nogo-A, Nogo-66 Receptor, CNS myelin inhibitor, regeneration.

Introduction

Nogo-A has been discovered as a protein in myelin, which impedes axonal regeneration [3], [4], [7], [18]. This protein is expressed by central nervous system (CNS) myelin-forming oligodendrocytes but not by peripheral Schwann cells [8], [11], [25], and can be observed in immunoelectron micrographs at the innermost adaxonal and outermost myelin membranes [8], [25]. This expression pattern is well coincident with the fact that in the adult CNS axon regeneration is highly restricted [19]. In addition to its localization in oligodendrocytes, Nogo-A is also expressed in a range of central and peripheral neurons [8], [11], [25], strongly suggesting that this protein has additional functions in the CNS. Nogo-A is divided into three regions separated by two hydrophobic segments. Its large N-terminal domain (N-nogo) and small C-terminal domain (C-nogo) reside in the cytoplasmic space, whereas a short stretch of 66 amino acids (Nogo-66) forms an extracellular loop. Interestingly, both N-nogo and Nogo-66 have been reported to inhibit axons in vitro independently [4], [5], [6].

Recently, Nogo-66 receptor (Nogo-R) has been identified [4], [7]. Transfection of Nogo-R gene into retinal ganglion cells at a developmental stage when they otherwise are unresponsive to Nogo-66 promotes growth cone collapse in response to exogenously applied Nogo-66 [4]. As Nogo-R is a glycosyl posphatidylinositol (GPI) anchored protein, it needs the interaction of p75, which is known as the receptor for the neurotrophin family of nerve growth factor [24]. In addition, Mi et al. have reported that a new protein LINGO-1 is a component of the Nogo-R/p75 signaling complex, and plays a role to modulate intracellular Rho activity, which is important in cytoskeletal regulation [15]. Although Nogo-R is not completely dependent on Nogo-A, the interaction of both proteins is considered to play pivotal roles in the inhibition of axon regeneration and other additional phenomena in the CNS.

The distributions of Nogo-A and Nogo-R have been reported using immunohistochemisty and in situ hybridization [4], [8], [9], [11], [12], [13], [21], [25], [26]. Despite the importance of these proteins in the CNS, these reports mainly deal with their expressions in the restricted areas. In addition, they are sometimes very contradictory. For example, in the cerebellum, Fornier et al. and Hunt et al. reported that Nogo-R mRNA was expressed in Purkinje neurons [4], [9], however Josephson et al. did not detect Nogo-R mRNA in Purkinje neurons [12]. In addition, Hunt et al. reported that cerebellar nuclei expressed Nogo-R mRNA [9], whereas Josephson et al. did not [12]. It is, thus, necessary to perform more wide and detailed investigations of the expression patterns of Nogo-A and Nogo-R in the adult rat CNS.

Materials and Methods

In situ hybridization

Six male Wistar rats (SLC, Shizuoka, Japan) weighing approximately 150g were decapitated under diethylether anesthesia. All experiments conformed to the Guidelines for Animal Experimentation at Hamamatsu University School of Medicine on the ethical use of animals and all efforts were made to minimize the number of animals used and their suffering. The fresh brains and nervous tissues were quickly removed and immediately frozen on powdered dry ice. Serial sections (20 μm thick) were cut on a cryostat, thaw-mounted onto silan-coated slides, and stored at –80℃. After being warned to room temperature, slide-mounted sections were fixed in 4% paraformaldehyde in 0.1M phosphatebuffer (pH7.2) for 15 minutes (all steps were performed at room temperature unless otherwise indicated), rinsed three times (5 minutes each) in 4×SSC (pH7.2) (1×SSC contains 0.15M sodium chloride and 0.015M sodium citrate), and dehydrated through a graded ethanol series (70%-100%). The sections were then defatted with chloroform for 5miniutes, and immersed in 100% ethanol (twice for 5 minutes each time) before being subjected to hybridization. Hybridization was performed by incubating the sections with a buffer [4× SSC, 50% deionized formamide, 0.12M phosphate buffer (pH7.2), Denhardt's solution (Nakarai, Kyoto, Japan), 2.5% tRNA (Roche, Tokyo, Japan), 10% dextran sulfate (Sigma, Tokyo, Japan)] containing [^{35}S] dATP (1000-1500 Ci/mmol (37- 555TBq/mmol; New England Nuclear, Boston, MA, USA))-labeled probes (1-2×d. p. m. /ml, 0.2ml/slide) for 24hour at 41℃. After hybridization, the sections were rinsed in 1×SSC (pH7.2) for 10minutes, followed by rinsing three times in 1×SSC at 55℃ for 20minutes each time. The sections were then dehydrated trough a graded ethanol series (70-100%). After film exposure for 7 days at room temperature, the sections were coated with Kodak NBT-2 emulsion (Kodak, Rochester, NY, USA) diluted 1:1 with water. The sections were then exposed at 4℃ for 2 weeks in a tightly sealed dark box. After being developed in

D-19 developer (Kodak), fixed with photographic fixer, and washed with tap water, the sections were counterstained with Thionin solution to allow morphological identification.

Oligonucleotide probes

Two antisense oligo cDNA probes (AS1 and AS2) and a sense oligo cDNA probe (S, complementary to AS1) for each Nogo-A and Nogo-receptor were designed based on the corresponding rat sequences [3], [4] and synthesized commercially (Takara, Tokyo, Japan). The sequences of the probes are as follows:
Nogo-A-AS1,
GCTCTGGAGCTGTCCTTCACAGGTTCTGGGGTACTGGGGAAAGA AGCA;
Nogo-A-AS2,
AGTCTTCTCTGTTATAATTTGGGCCTTCCTTTCTTCTATT;
Nogo-A-S,
TGCTTCTTTCCCCAGTACCCCAGAACCTGTGAAGGACAGCTCCAG AGC;
Nogo-R-AS1,
GTCATGCCGGAATCTCACCATCCTGTGGCTGCACTCAAAT;
Nogo-R-AS2,
GCAAACAGGTAGAGGGTCATGAGTCGGCCAAGGTCCCGGA;
Nogo-R-S,
ATTTGAGTGCAGCCACAGGATGGTGAGATTCCGGCATGAC.

Computer-assisted homology searches (NCBI - BLAST) showed that each probe has less than 50% homology with any sequences contained in the gene banks. The probes were labeled at the 3' end using [^{35}S] dATP (1000 - 1500 Ci/mmol (37-55.5Tbq/mmol); New England Nuclear, Boston, MA, USA) and terminal deoxynucleotidyl transferase (Takara, Tokyo, Japan) to obtain a specific activity of approximately 1.4-2.0 $\times 10^9$ d. p. m. / μ g.

Control studies

We carried out control competition experiments for the six antisense probes using a 100-fold excess of one of the unlabeled probes together with the corresponding labeled probe, and other controls involving RNase A pretreatment just before hybridization. These experiments showed no

positive signals for any of the probes.

Nomenclature

The terminology used in this report follows that of the atlas of Paxinos and Watson [17].

Results

General expression patterns

To investigate the specificity of oligo cDNA probes for detection of Nogo-A and Nogo-receptor (Nogo-R) mRNA, in situ hybridization histochemisty on horizontal sections from the rat brain was performed with two different antisense probes (AS1 and AS2) and a sense probe (S) for each mRNA and the results were visualized using film autoradiography as negative image (Fig. 1). For each mRNA, the two antisense probes showed the same hybridization pattern, and the sense probe exhibited no hybridization signal, indicating that the antisense probes can specifically recognize the corresponding transcripts. Thus, in the following experiments, we used the AS1 probes to detect the corresponding transcripts.

Nogo-A mRNA was expressed intensely throughout the rat central nervous system (Fig. 1 A and B). Very strong Nogo-A mRNA was observed in the olfactory bulb, medial habenular nucleus and hippocampus. Interestingly, the corpus callosum also showed abundant Nogo-A mRNA expression, indicating its glial expression. In contrast, Nogo-R mRNA expression was restricted to the cortex, medial habenular nucleus, hippocampus and cerbellum (Fig. 1 D and E). We could not detect Nogo-R mRNA expression in the other regions (Fig. 1 D and E). Identification of cells expressing Nogo-A and Nogo-R mRNAs was based upon criteria used in our previous study [20]. Positive cells were divided into four categories (very strong, strong, moderate, and low) by visual comparison of the extent of silver grain accumulation. The relative intensity of the in situ signals for Nogo-A and Ngo-R mRNAs in the rat CNS is summarized in Table 1.

Expression of Nogo-A and Nogo-R mRNAs

Telencephalon.

Olfactory bulb

Nogo-A mRNA was abundantly expressed in the olfactory bulb (Fig. 2A). Very strong Nogo-A mRNA expression was observed in the mitral cell layer and internal granular layer (Fig. 2A). The glomerular layer and external plexiform layer expressed Nogo-A mRNA moderately (Fig. 2A). In contrast, we detected Nogo-R mRNA expression only in the mitral cell layer (Fig. 2E).

Septum and nuclei of the diagonal band of Broca

The tenia tecta expressed both Nogo-A and Nogo-R mRNA abundantly

(Fig. 2B and F). In addition, Nogo-A mRNA was strongly expressed in the medial septal nucleus, and moderately in the lateral septal nuclei and diagonal band of Broca (Fig. 2C), while we could not detect Nogo-R mRNA expression in these regions (Fig. 2G).

Piriform cortex and cerebral cortex.

Very strong Nogo-A mRNA expression and strong Nogo-R mRNA expression were observed in the piriform cortex (Fig2. B, C, F and G). Both Nogo-A mRNA expression and Nogo-R mRNA expression were abundantly observed in the cerebral cortex (Fig. 2C and G). Interestingly, Nogo-A mRNA expression was relatively higher in the layer V (Fig. 2C), whereas Nogo-R mRNA expression was relatively weaker in this layer (Fig. 2G).

Hippocampal formation.

Nogo-A mRNA was very strongly expressed in the pyramidal cell layer of CA1-3, while its expression was relatively weaker in the granular cell layer of the dentate gyrus (Fig. 3A). Strong Nogo-R mRNA expression was also seen in the pyramidal cell layer of CA1-3 and the granular cell layer of dentate gyrus (Fig. 3E).

The amygdala and bed nucleus of the stria terminalis.

Nogo-A mRNA was moderately expressed in all nuclei of the amygdala, whereas Nogo-R mRNA expression was detected only in the lateral nucleus and basolateral nucleus (Fig. 3A, B, E and F). The bed nucleus of the stria terminalis expressed Nogo-A mRNA moderately, however we could not detect Nogo-R mRNA there.

Basal ganglia.

Nogo-A mRNA was moderately expressed in the globules pallidus, and the caudate putamen nucleus exhibited weak Nogo-A mRNA expression (Fig. 2C and D). On the other hand, we could not detect Nogo-R mRNA expression in all nuclei of the basal ganglia (Fig. 2G and H).

*Dien*cephalon.

Thalamus.

All thalamic nuclei abundantly expressed Nogo-A mRNA, with relative stronger expression in the anterodorsal, centromedial, reticular, and parafascicular nuclei (Fig. 2D, Fig. 3A and B). On the other hand, Nogo-R mRNA was weakly expressed in most thalamic nuclei (Fig. 2H, Fig. 3E and F), however we could not detect Nogo-R mRNA in the reticular thalamic nucleus (asterisks in Fig. 2H and 3E).

Habenular complex.

Very strong Nogo-A mRNA expression and strong Nogo-R mRNA expression were observed in the medial habenula nucleus. The lateral habenula nucleus also exhibited moderate Nogo-A mRNA expression (Fig. 3A and B), while we could not detect Nogo-R mRNA expression there (Fig. 3E and F).

Hypothalamus.

Strong Nogo-A mRNA expression was observed in the supraoptic nucleus, paraventricular nucleus and ventromedial hypothalamic nucleus (Fig. 3A and B). The other nuclei expressed Nogo-A mRNA moderately (Fig. 2D, 3A and B). In contrast, we could not detect Nogo-R mRNA expression in this area (Fig. 2H, 3E and F).

Others.

Geniculate nuclei express both Nogo-A mRNA and Nogo-R mRNA moderately to weakly (Fig. 3C and G). Nogo-A mRNA was abundantly expressed in the subthalamus and zona incerta, whereas we did not detect Nogo-R mRNA expression there (Fig. 3B and F).

Midbrain.

Strong Nogo-A mRNA expression was observed in the pars compacta of the substantia nigra and red nucleus (Fig. 3C), in addition moderate Nogo-A mRNA expression was seen in the pars reticulata of the substantia nigra, ventral tegmental area and central gray (Fig. 3C). On the other hand we could not detect Nogo-R mRNA expression in these nuclei (Fig. 3G).

Pons and medulla.

Very strong Nogo-A mRNA was observed in the locus caeruleus and trigeminal mesencephalic nucleus (Fig. 4A). Strong Nogo-A mRNA expression was seen in the pontine nucleus and dorsal raphe (Fig. 3D). In addition, we observed strong Nogo-A mRNA expression in the vestibular nuclei and cochlear nuclei (Fig. 4A and B). Furthermore, motor nuclei, such as the facial nucleus (Fig. 4A and B), dorsal motor nucleus of the vagus, hypoglossal nucleus (Fig. 4C), expressed Nogo-A mRNA strongly. Strong Nogo-A mRNA expression was also seen in the external cuneate nucleus and lateral reticular nucleus (Fig. 4C). Moderate to weak Nogo-A mRNA expression was seen in the other nuclei (Fig. 4A-C), whereas we could not detect Nogo-R mRNA expression in these areas (Fig. 4E-G).

Cerebellum.

The granular cell layer of the cerebellum expressed both Nogo-A and Nogo-R mRNA strongly (Fig. 4A-C). Nogo-A mRNA expression was observed in the cerebellar nucleui (Fig. 4A and B), whereas we could not detect Nogo-R mRNA there (Fig. 4E and F).

Spinal cord

The ventral horn of the spinal cord exhibited strong Nogo-A mRNA expression. In addition, moderate Nogo-A mRNA expression was seen in the dorsal horn and white mattar (Fig. 4D). On the other hand, we could not detect Nogo-R mRNA expression in the spinal cord (Fig. 4H).

Comparison in detail

Olfactory bulb.

Nogo-A mRNA was expressed abundantly in the glomerular layer, external plexiform layer, mitral cell layer and internal granular layer (Fig. 5A). In contrast, Nogo-R mRNA was only detected in the mitral cell layer (Fig. 5E). Bright-field observation clearly showed that positive signals for Nogo-A mRNA were detected in mitral neurons and other cells, whereas those of Nogo-R mRNA were only detected in mitral cells (arrowheads in Fig. 5B and F).

Corpus callosum.

As shown in the Fig. 5C, Nogo-A mRNA was abundantly expressed in the corpus callosum, whereas Nogo-R mRNA was not detected there (Fig. 5G). Bright-field observation showed that positive cells were small and formed characteristic chain-like structure (arrowheads in Fig. 5D), indicating that oligodendrocytes express Nogo-A mRNA.

Cerebral cortex.

Both Nogo-A mRNA and Nogo-R mRNA were expressed abundantly in the cerebral cortex (Fig. 6A and B). Interestingly, relative stronger Nogo-A mRNA and relative weaker Nogo-R mRNA were observed in the layer V. Fig. 6B shows that Nogo-A mRNA was detected both in large pyramidal neurons (arrowheads) and small glial-like cells (arrows). In contrast, Nogo-R mRNA was exclusively detected in pyramidal neurons (arrowheads in Fig. 6G), but not in glial-like cells (arrows in the Fig. 6G)

Hippocampal formation.

Nogo-A mRNA was very strongly expressed in the pyramidal cell layer of the Ammon’s horn, while its expression in the granule cell layer of the dentate gyrus was relatively weak (Fig. 6C). Nogo-R mRNA was also

detected in the pyramidal cell layer and granule cell layer (Fig. 6H). Bright-field observation showed pyramidal neurons expressed both mRNAs abundantly (Fig. 6D and I). Interestingly, a significant difference was observed in interneurons. Interneurons expressed Nogo-A mRNA abundantly (Fig. 6E), whereas they were devoid of Nogo-R mRNA expression (Fig. 6J).

Cerebellum

Closer observation revealed that Nogo-A mRNA expression was detected not only in the granule cell layer, but also in the Purkinje cell layer and white matter (Fig. 7A), while Nogo-R mRNA was exclusively expressed in the granule cell layer (Fig. 7E). Bright-field observation showed that Purikinje cells abundantly expressed Nogo-A mRNA (arrowheads in Fig. 7B), but not Nogo-R mRNA (arrowheads in Fig. 7F).

Spinal cord

Cells expressing Nogo-A mRNA were detected in both gray matter and white matter (Fig. 7C), while we could not detect Nogo-R mRNA in the spinal cord (Fig. 7G). Bright-field observation showed that Nogo-A mRNA expression was detected in large motor neurons (arrowheads in Fig. 7D) and also in small glial-like cells (arrows in Fig. 7D).

DISCUSSION

To date, the distributions of Nogo-A and Nogo-R have been reported using immunohistochemisty and in situ hybridization [4], [8], [9], [11], [12], [13], [21], [25], [26]. However, these studies were performed in restricted areas, and sometimes the data are very contradictory. Thus, we investigated the total distributions of Nogo-A and Nogo-R mRNAs in the adult rat CNS. The present results clearly show that Nogo-A is abundantly expressed in both neurons and oligodendrocytes throughout the CNS, whereas Nogo-R mRNA is expressed in very restricted areas. Although the expression level of certain mRNAs generally reflects that of the translated protein, sometimes the presence of mRNA is not a sufficient condition for the expression of the corresponding protein. With this caveat in mind, the present results are discussed.

Nogo-A was first discovered as a protein in CNS myelin, indicating that oligodendrocytes express it. In addition, neuronal Nogo-A expression has been reported in some areas [8], [9], [11]. Consistent with these reports, in the present study, we found that Nogo-A mRNA is expressed in both oligodendrocytes and neurons. Interestingly, we could not find any neuron which lack Nogo-A mRNA expression, indicating that Nogo-A is universally expressed in all neurons. This ubiquitous neuronal expression suggests that Nogo-A is fundamentally necessary to neurons. What does Nogo-A do in neurons? Recently, Hunt et al. showed that in culture neurons Nogo-A presents at branch points, varicosities, and synapses [10]. Thus we speculate that neuronal Nogo-A may be involved in axonal growth, guidance, and interactions with other axons, although additional functions might remain to be elucidated.

In contrast to the ubiquitous Nogo-A mRNA expression, we detected Nogo-R mRNA in very restricted areas. Nogo-R mRNA was expressed in the olfactory bulb, hippocampus, tentia tecta, some amygdala nuclei, cerebral cortex, some thalamic nuclei, medial habenular, whereas we could not detect it in the other regions. These data suggest that Nogo-A/Nogo-R signaling is not the only mechanism to explain the absence of axonal regeneration in the CNS. Since N-nogo is known to function as an inhibitor for axonal regeneration irrespective of Nogo-R [4], [5], [6], N-nogo might function in such regions. Although Nogo-R expression has been reported previously [4], [9], [12], some discrepancies were observed among our data and the previous data. For example, in the cerebellum, Fornier et al. and Hunt et al. reported that Nogo-R mRNA was expressed in Purkinje neurons [9], [11], however we did not detect Nogo-R mRNA in Purkinje neurons. In addition, Hunt et al. reported that cerebellar nuclei expressed Nogo-R mRNA, whereas we could not detect it there[9]. Furthermore, Fornier et al.

and Hunt et al. reported that Nogo-R mRNA was detected in many nuclei in the brain stem and spinal cord [9], [11], in contrast, we could not detect Nogo-R mRNA there. The discrepancy might be explained with a difference of in situ method. Fornier et al. and Hunt et al. used a long digoxigenin-labeled riboprobe [9], [11], whereas we used a short ^{35}S-labeled oligonucleotide probe. The long probe might have hybridized to more mRNA species than a shorter probe would have.

In the olfactory bulb, we detected Nogo-R mRNA in mitral cells, but not in the periglomerular neurons, and granular neurons. Mitral cells are principal output (projecting) neurons, and transmit olfactory information into the primary olfactory cortex using glutamate as a neurotransmitter. In contrast, periglomerular neurons and granular neurons are mainly GABAergic and function as interneurons. In addition, in the hippocampus, pyramidal and granular neurons expressed abundant Nogo-R mRNA, while we could not detect it in interneurons as shown in Fig. 6J. Thus, we speculate that Nogo-R is not expressed in interneurons in these areas. Why do interneurons lack Nogo-R? In the present study, we revealed that Nogo-A is expressed ubiquitously. In addition, Nogo-A and Nogo-R interaction is considered to inhibit not only axonal regeneration but also neuronal plasticity. If a given neuron expresses Nogo-R, the neuron might not show its plasticity well. Thus, we speculate that interneurons might lack Nogo-R expression to keep their ability for plasticity.

The hypothesis that Nogo-A growth inhibition is mediated by Nogo-R is very fascinating. If so, neurons lacking Nogo-R expression should have high regenerative capacity. Monoaminergic neurons are known to have high regenerative capacity in the CNS [14]. Interestingly, we did not detect Nogo-R in related nuclei, such as the ventral tegmental area, locus caeruleus, and raphe nuclei, indicating that the lack of Nogo-R mRNA expression in monoaminergic neurons might have relations with their high regenerative capacity. When grafts have been implanted into the thalamus, many types of CNS neuron are axotomized yet more than 90 % of the CNS axons which regenerate into and through the grafts originate from the reticular thalamic nucleus, the neurons of which project to dorsal thalamic nuclei. Few thalamocortical projection neurons (in the dorsal thalamus) regenerate their axons [1], [16], [22]. Interestingly, we could not detect Nogo-R mRNA in neurons in the reticular thalamic nucleus, indicating again that the absence of Nogo-R expression might explain their high capacity for regeneration. Furthermore, we could not detect Nogo-R mRNA in neurons in the deep cerebellar nuclei, which are good at regenerating axons into nerve grafts [22], [23], [2]. Taken together, the lack of Nogo-R expression in a neuron might be necessary to keep its high regenerative capacity.

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Figure legends

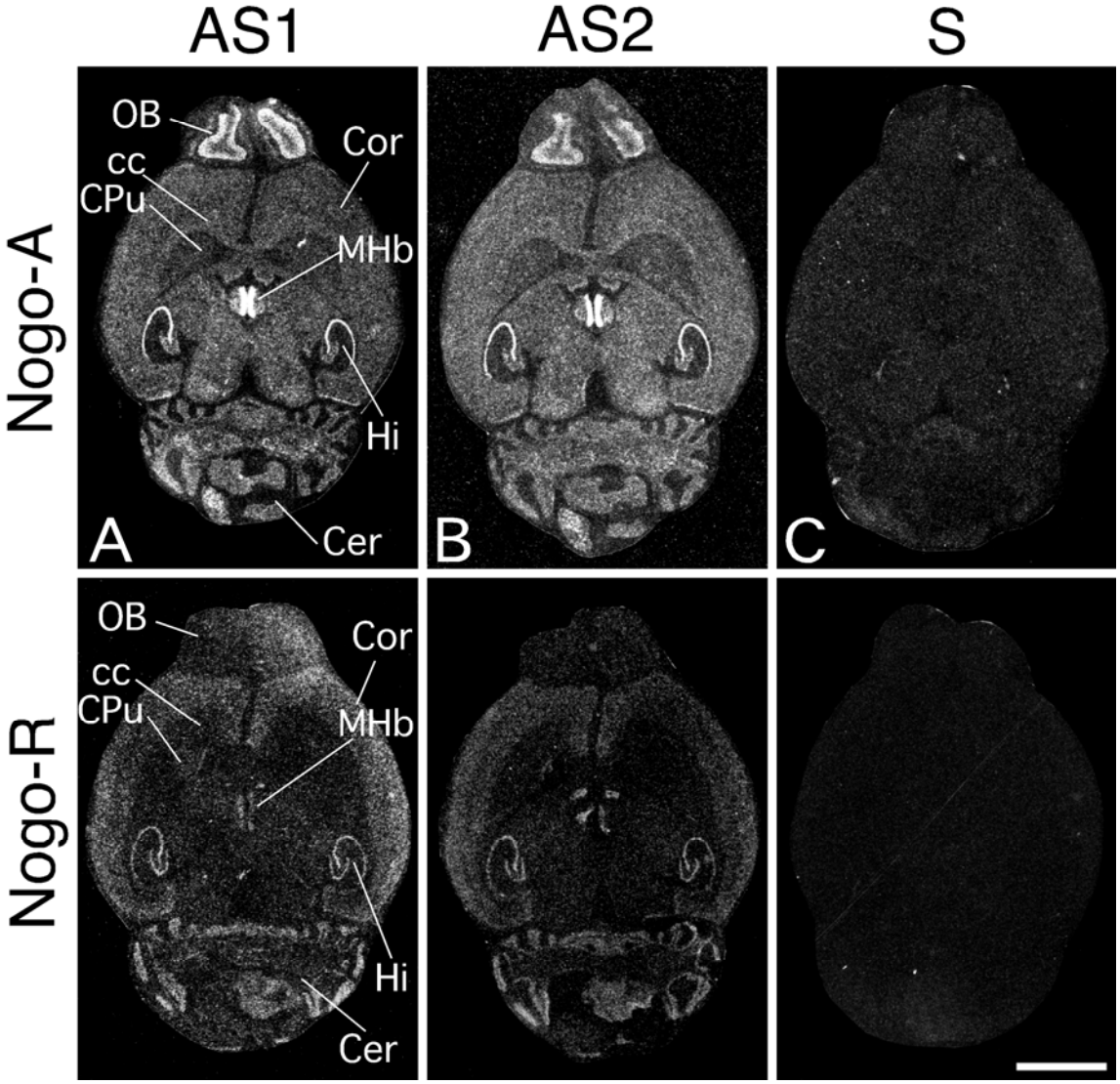

Fig. 1. Expression of Nogo-A mRNA (A, B), Nogo-R mRNA (D, E) in the rat brain. Note that two antisense probes show the same hybridization pattern for each mRNA, and that sense probes show no hybridization signals (C, F). cc; corpus callosum, Cer; cerebellum, Cor; cerebral cortex, CPu; caudate-putamen, Hi; hippocampus, MHb; medial habenular nucleus, OB; olfactory bulb, Th; thalamus, TT; tenia tecta. Scale bar = 5mm.

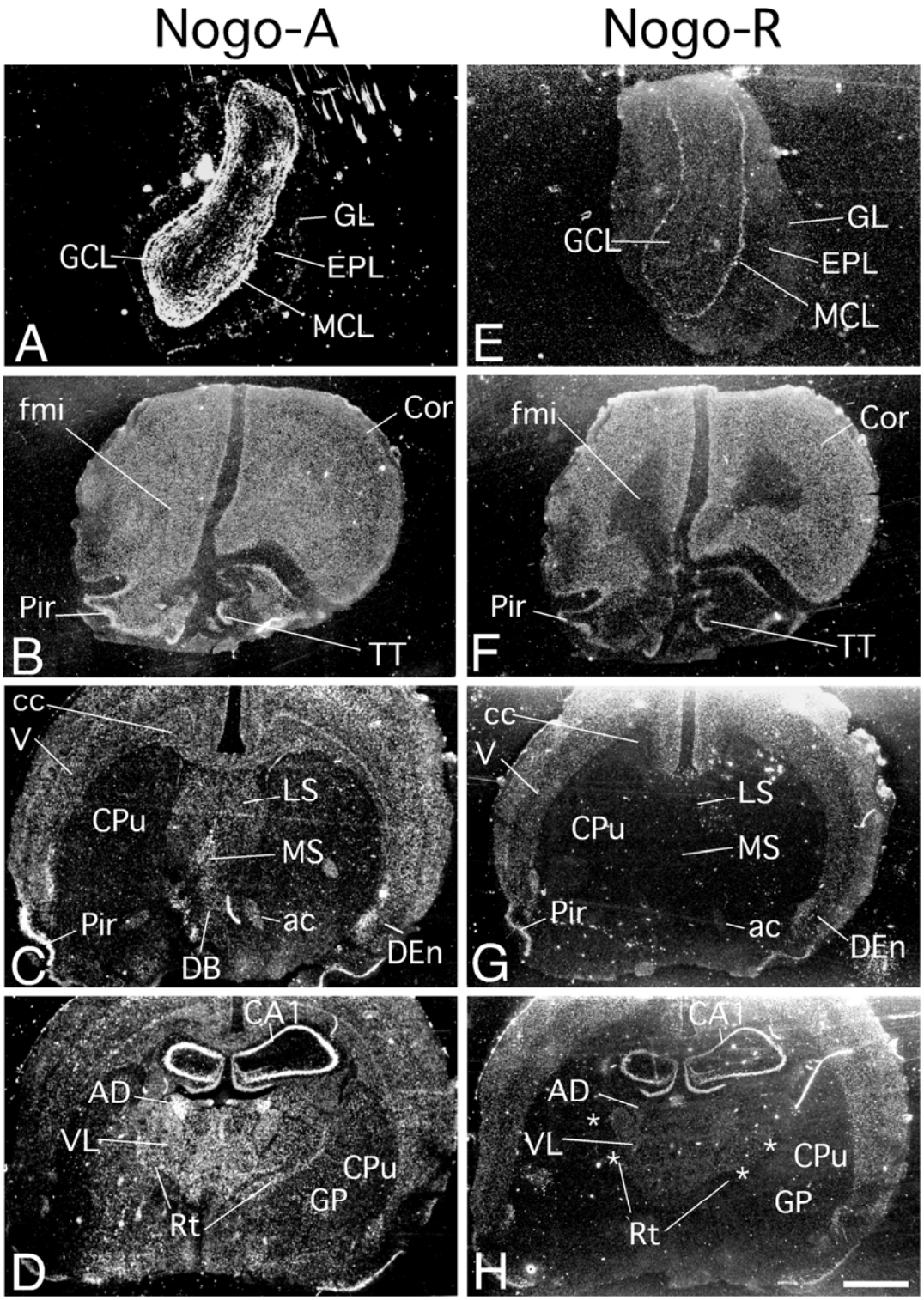

Fig. 2. Dark-field photomicrographs showing Nogo-A mRNA (A-D) and Nogo-R mRNA (E-H) expression in the upper part of the rat brain. Note that the reticular thalamic nucleus is devoid of Nogo-R expression (asterisks in H). ac; anterior commissure, AD; anterodorsal thalamic nucleus, CA1; field CA1of Ammon's horn, Cor; cerebral cortex, CPu; caudate-putamen, DB; diagonal band, DEn; dorsal endopiriform nucleus, fmi; forceps minor of the corpus callousm, GL; glomerular layer, GP; globus pallidus, GCL; granular cell layer of the olfactory bulb, LS; lateral septal nucleus, MCL; mitral cell layer of the olfactory nucleus, MS; medial septal nucleus, Pir; piriform cortex, Rt; reticular thalamic nucleus, TT; tenia tecta, VL; ventrolateral thalamic nucleus. Scale bar =1mm (A, E), 2mm (B-D, F-H).

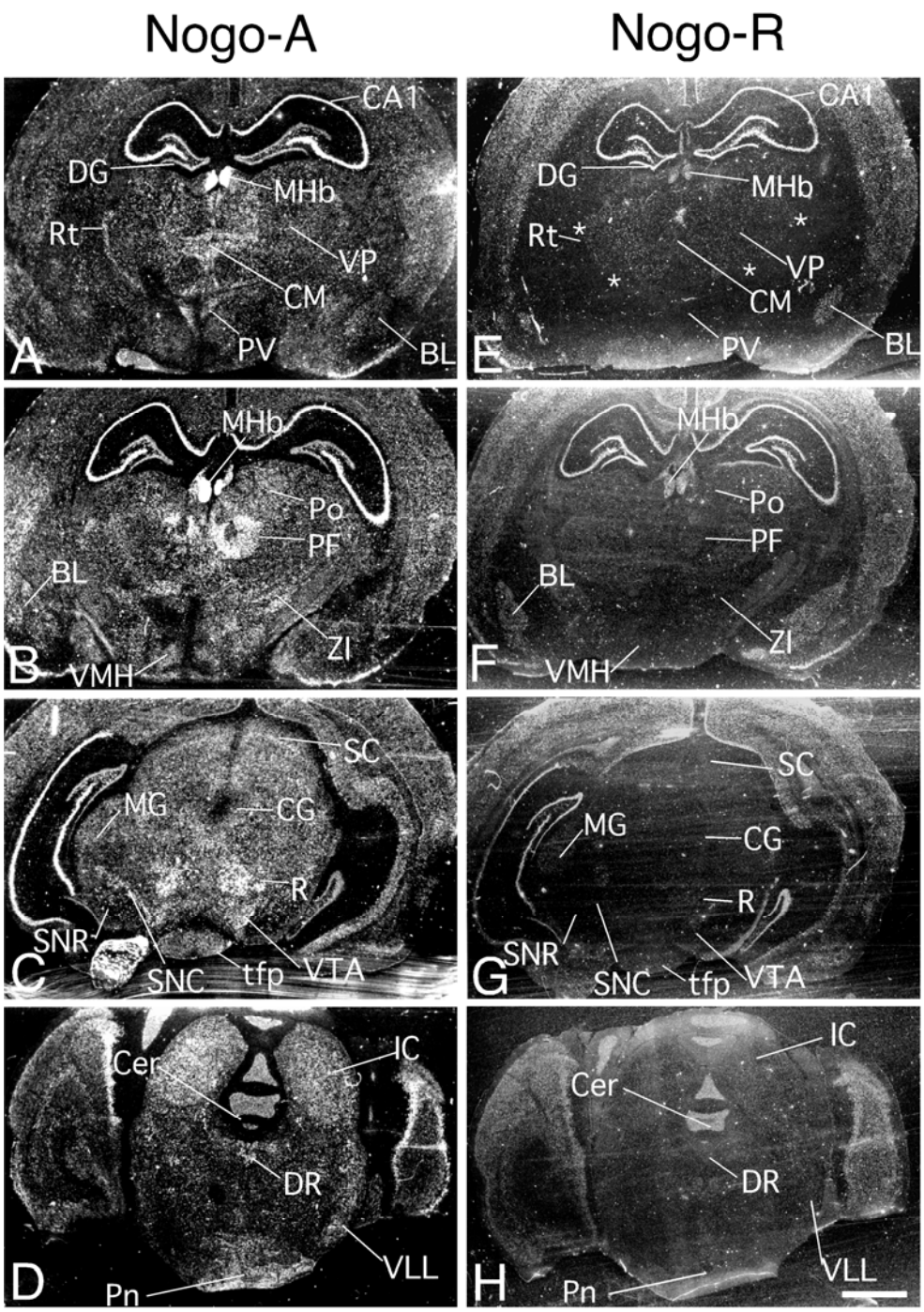

Fig. 3. Dark-field photomicrographs showing Nogo-A mRNA (A-D) and Nogo-R mRNA (E-H) expression in the middle part of the rat brain. Note that the reticular thalamic nucleus is devoid of Nogo-R expression (asterisks in H). BL; basolateral amygdaloid nucleus, CA1; field CA1 of Ammon's horn, Cer; cerebellum, CG; central gray, CM; central medial thalamic nucleus, DG; dentate gyrus, DR; dorsal raphe nucleus, IC; inferior colliculus, MG; medial geniculate nucleus, MHb; medial habenular nucleus, PF; parafascicular nucleus, Pn; pontine nuclei, Po; posterior thalamic nuclear group, PV; paraventricular hypothalamic nucleus, R; red nucleus, Rt; reticular thalamic nucleus, SC; superior colliculus, SNC; substantia nigra, compact part, SNR; substantia nigra, reticular part, tfp; transverse fibers of the pons, VLL; ventral nucleus of the lateral lemniscus, VMH; ventromedial hypothalamic nucleus, VP; ventral posterior thalamic nucleus, VTA; ventral tegmental area, ZI; zona incerta. Scale bar = 2mm.

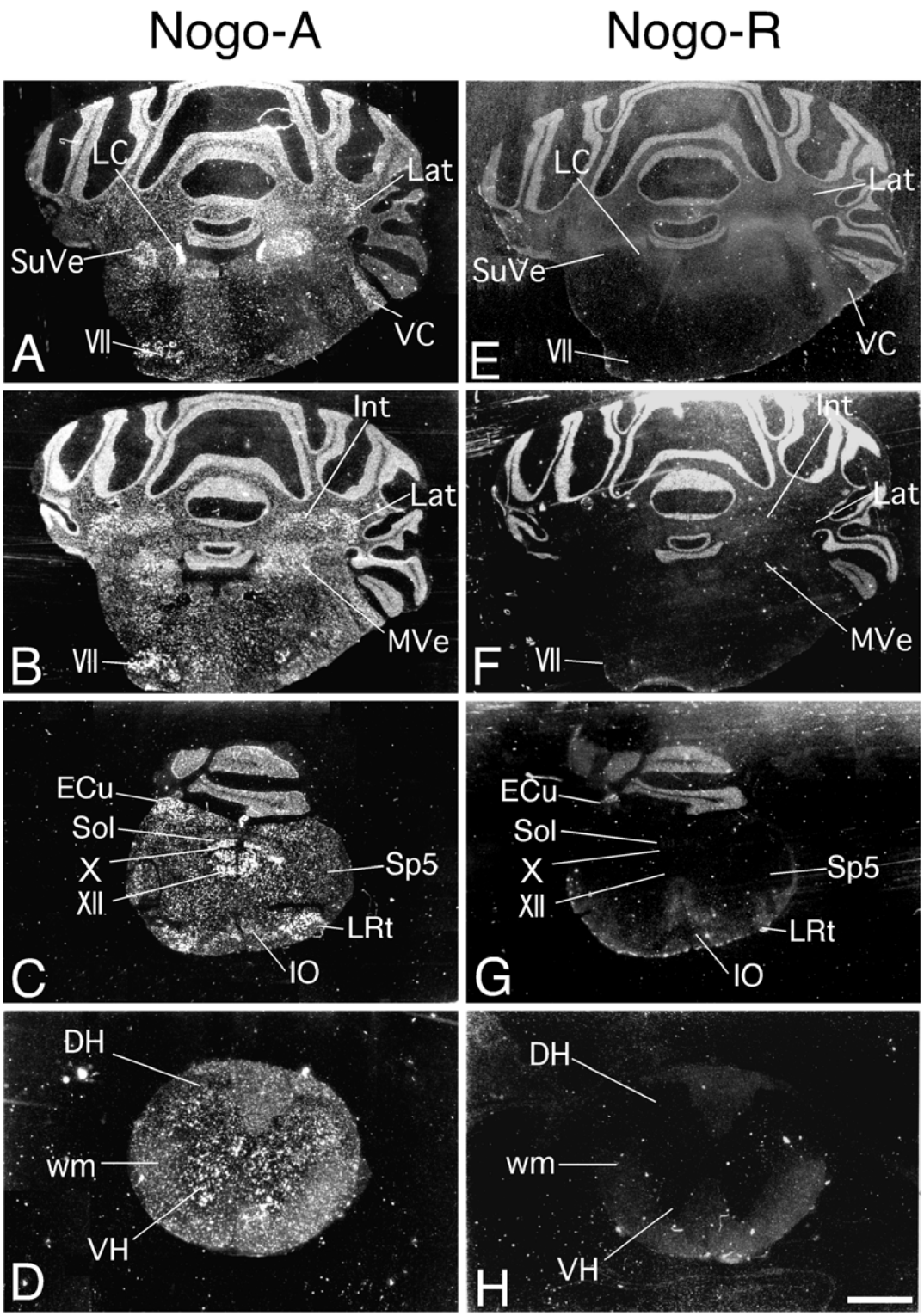

Fig. 4. Dark-field photomicrographs showing Nogo-A mRNA (A-D) and Nogo-R mRNA (E-H) expression in the lower part of the rat brain. Note that Nogo-R mRNA is hardly detected in this region except the cerebellar cortex. VII; facial nucleus, X; dorsal motor nucleus of vagus, XII; hypoglossal nucleus, DH; dorsal horn of the spinal cord, ECu; external cuneate nucleus, IO; inferior olive, Int; interposed cerebellar nucleus, Lat; lateral cerebellar nucleus, LC; locus coeruleus, LRt; lateral reticular nucleus, MVe; medial vestibular nucleus, Sol; nucleus of the solitary tract, Sp5; spinal trigeminal nucleus, SuVe; superior vestibular nucleus, VC; ventral cochlear nucleus, VH; ventral horn of the spinal cord, wm; white matter. Scale bar = 2mm (A, B, E, F), 1mm (C, D, G, H).

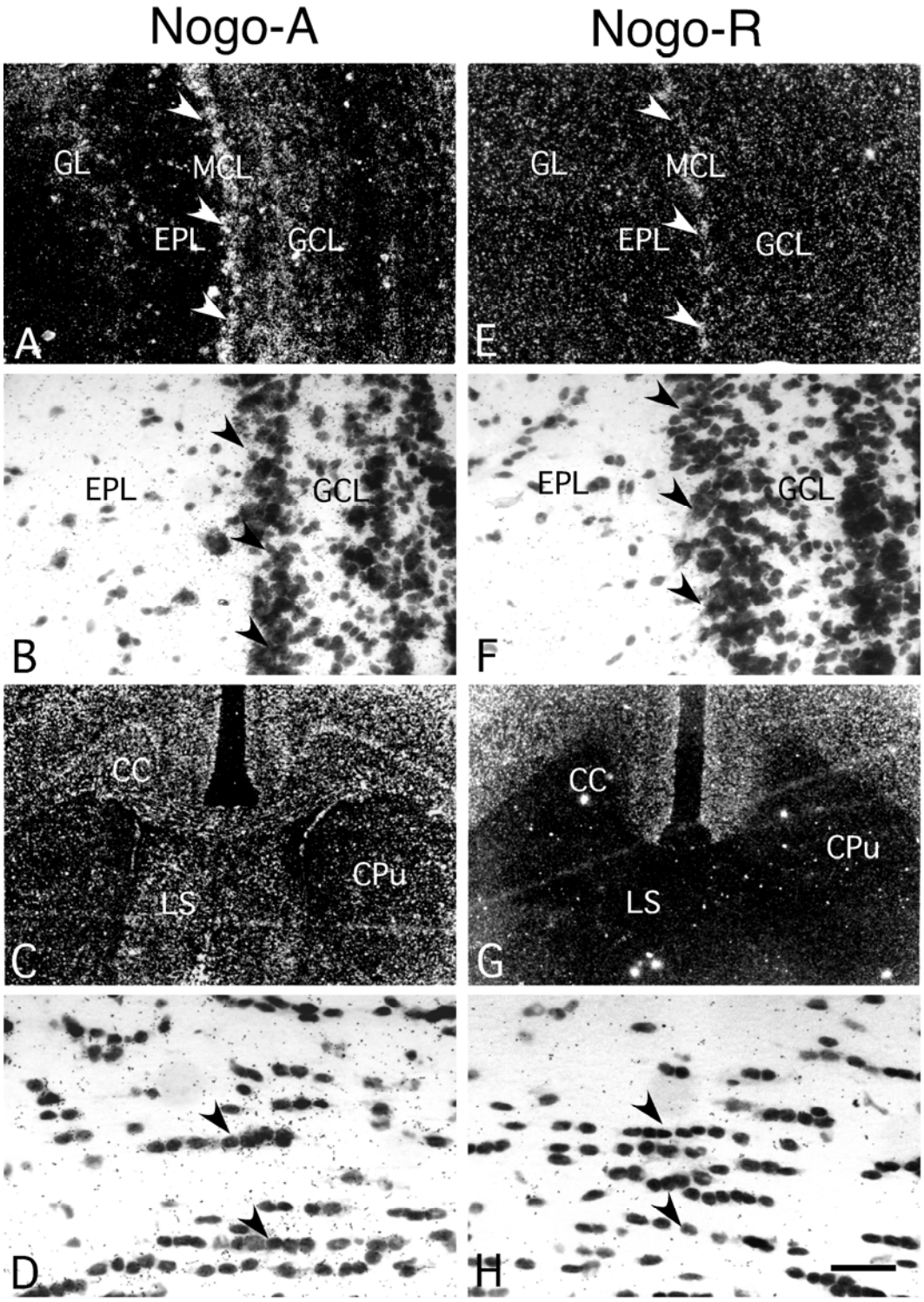

Fig. 5. Dark- and bright-field photomicrographs showing Nogo-A mRNA (A-D) and Nogo-R mRNA (E-H) expression in the olfactory bulb (A, B, E, F) and corpus callosum (C, D, G, H) of the rat brain. Note that Nogo-A and Nogo-R mRNA positive signals were observed on mitral cells of the olfactory bulb (arrowheads in A, B, E, F,), and that oligodendrocytes in the corpus callosum, expressed Nogo-A mRNA, but not Nogo-R mRNA (arrowheads in D, H). cc; corpus callosum, Cpu; caudate-putamen, EPL; external plexiform layer of the olfactory bulb, GCL; granular cell layer of the olfactory bulb, GL; glomerular layer, LS; lateral septal nucleus, MCL; mitral cell layer of the olfactory nucleus. Scale bar = 400 μ m (A, C, E, G), 100 μ m (B, D, F, H).

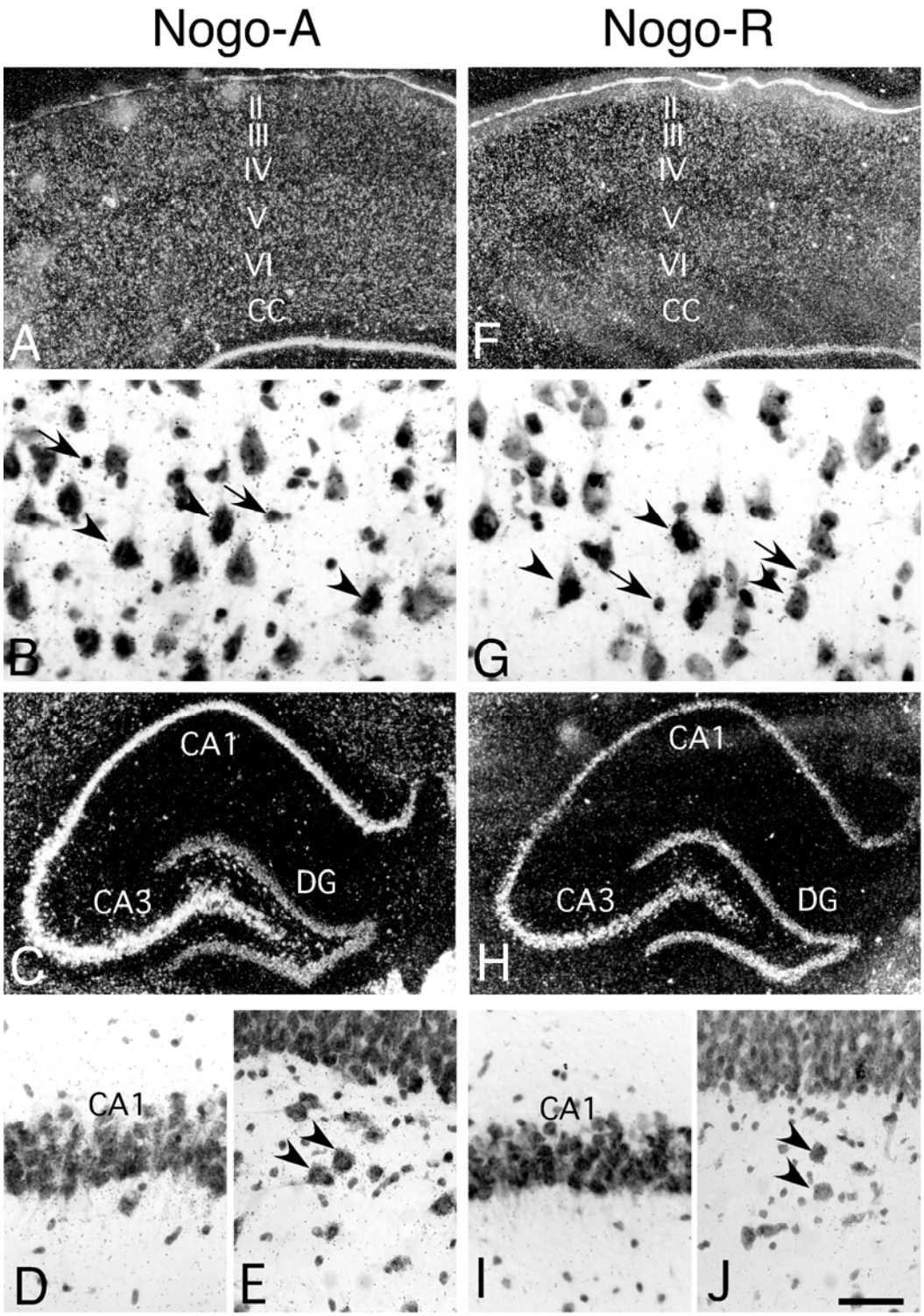

Fig. 6. Dark- and bright-field photomicrographs showing Nogo-A mRNA (A-D) and Nogo-R mRNA (E-H) expression in the cerebral cortex (A, B, E, F), and hippocampus (C, D, G, H) of the rat brain. Note that Nogo-A mRNA signals were observed on pyramidal cells (arrowheads in B) and small glial-like cells (arrows in B), while Nogo-R mRNA signals were observed on pyramidal cells (arrowheads in G) but could not on olygodendrocytes (arrows in G). Nogo-A mRNA signals were observed on internal neurons in the hippocampus (arrowheads in E), but Nogo-R mRNA signals were not observed (arrowheads in J). II-VI; 2-6 cortical layers, CA1-3; field CA1-3 of Ammon's horn, cc; corpus callosum, DG; dentate gyrus. Scale bar = 400 μ m (A, C, E, G), 100 μ m (B, D, F, H).

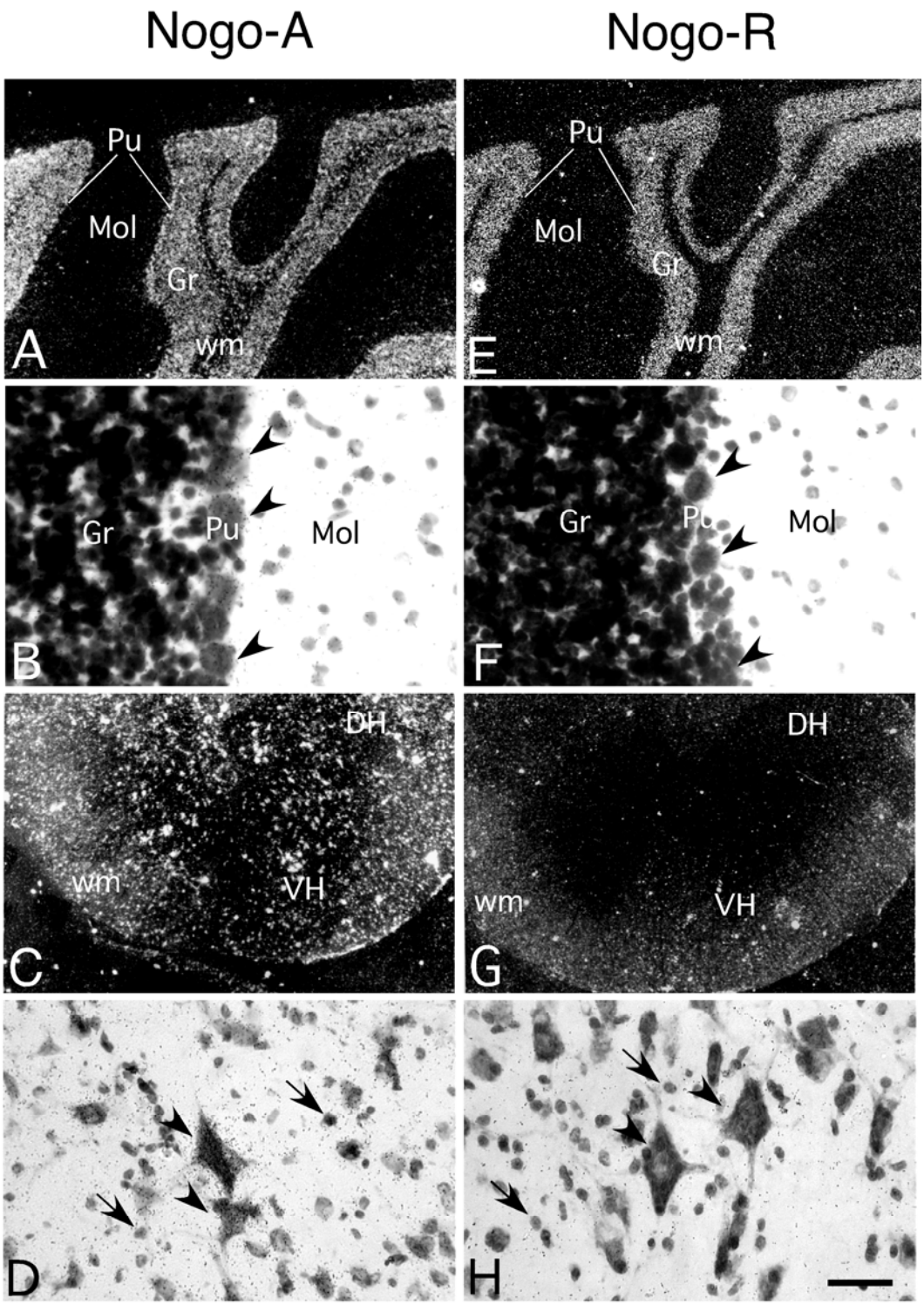

Fig.7. Dark- and bright-field photomicrographs showing Nogo-A mRNA (A-D) and Nogo-R mRNA (E-H) expression in the cerebellar cortex (A, B, E, F) and spinal cord (C, D, G, H) of the rat brain. Note that Nogo-A mRNA signals were observed on Purkinje (arrowheads in B), while Nogo-R mRNA signals were not observed on Purkinje cells (arrowheads in F). Nogo-A mRNA signals were observed on motor neurons (arrowheads in D) and small glial-like cells (arrows in D), while Nogo-R mRNA signals were not observed there. DH; dorsal horn of the spinal cord, Gr; granular layer of the cerebellum, Mol; molecular cell layer, Pu; Purkinje cell, VH; ventral horn of the spinal cord, wm; white matter. Scale bar = 400 μ m (A, C, E, G), 100 μ m (B, D, F, H).

Table 1. Distribution and intensity of the *in situ* hybridization signals for Nogo-A, Nogo-R mRNAs in the rat nervous system

Area and cell type	Nogo-A	Nogo-R
I . Telencephalon		
Olfactory bulb		
Glomerular layer	++	n.d.
Mitral layer	++++	++
Granular layer	++++	n.d.
Piriform cortex	++++	+++
Hippocampal formation		
Ammon's horn	++++	+++
Dentate gyrus	+++	+++
Septal area		
Tenia tecta	++++	+++
Medial septal nucleus	+++	n.d.
Lateral septal nucleus	++	n.d.
Nucleus of the diagonal band of Broca	++	n.d.
Dorsal endopiriform nucleus	+++	++
Amygdala		
Lateral nucleus	++	++
Basolateral nucleus	++	++
Other nuclei	++	n.d.
Bed nucleus of the stria terminalis	++	n.d.
Ventral pallidum	++	n.d.
Globus pallidus	++	n.d.
Caudate putamen	+	n.d.
Nucleus accumbens	+	n.d.
Preoptic nuclei	++	n.d.
Claustrum	++	++
Cerebral cortex	+++	++
II . Diencephalon		
Thalamus		
Anterodorsal nucleus	+++	+
Reticular nucleus	+++	n.d.
Parafascicular nucleus	+++	+
Centromedial nucleus	+++	n.d.
Other nuclei	++	+
Geniculate nuclei	++	+
Subthalamus	+++	n.d.
Zona incerta	++	n.d.
Habenular complex		
Medial nucleus	++++	++
Lateral nucleius	++	n.d.
Hypothalamus		
Supraoptic nucleus	+++	n.d.
Paraventricular nucleus	+++	n.d.
Ventromedial nucleus	+++	n.d.
Mammillary area	+	n.d.
Other nuclei	++	n.d.
III. Midbrain		
Ventral tegmental area	++	n.d.

continued

Subatantia nigra		
Pars compacta	+++	n.d.
Pars retinaculata	++	n.d.
Red nucleus	+++	n.d.
Central gray matter	++	n.d.
Nucleus of Darkschewitsch	+++	n.d.
Interpendicular nucleus	++	n.d.
IV. Pons and Medulla		
Locus caeruleus	++++	n.d.
Visual system		
Pretectal area	++	n.d.
Superior colliculus	++	n.d.
Auditory system		
Inferior colliculus	++	n.d.
Cochlear nuclei	+++	n.d.
Vestibular nuclei	+++	n.d.
Somatosensory system		
Trigeminal mesencephalic nucleus	++++	n.d.
Ttrigeminal principal nucleus	+	n.d.
Gracile nucleus	++	n.d.
Cunate nucleus	++	n.d.
External cunate nucleus	+++	n.d.
Nucleus of the solitary tract	++	n.d.
Ambiguus nucleus	+++	n.d.
Dorsal motor nucleus of the vagus	+++	n.d.
Oculomotor system		
Edinger-Westphal nucleus	+++	n.d.
oculomotor nucleus	+++	n.d.
Motor trigeminal nucleus	++	n.d.
Facial nucleus	++	n.d.
Hypoglossal nucleus	+++	n.d.
Reticular nuclei		
Trigmental reticular nucleus	+++	n.d.
Lateral reticular nucleus	+++	n.d.
Other nuclei	++	n.d.
Raphe nuclei		
Dorsal raphe	+++	n.d
Medial raphe	+	n.d.
Raphe magnus	+	n.d.
Raphe obscurus	+	n.d.
Pontine nuclei	+++	n.d.
Dorsal tegmental nuclei	++	n.d.
Inferior olive	++	n.d.
V . Cerebellum		
Cerebellar nuclei	+++	n.d.
Cortex		
Purkinje cell	+++	n.d.
Granule cell	+++	+++
VI. Spinal cord		
Dorsal horn	++	n.d.
Ventral horn	+++	n.d.

Relative expression levels were estimated by visual comparison of exposed

emulsion-coated slides: n. d., not detected; +, low; ++, moderate; +++, strong; ++++, very strong.