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Distinctive expression pattern of Peg10 in the mouse brain

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SUMMARY

Peg10 (paternally expressed 10) is a retrotransposon-derived gene that is highly conserved across mammalian species. Peg10 is involved in cell proliferation and differentiation, and is essential for placenta formation in mice. Although a number of studies have examined Peg10 expression in the placenta, its cellular localization in the brain is still unclear. The function of Peg10 in the brain is also unknown. Here, we examined Peg10 distribution in the mouse brain. In situ hybridization revealed intense expression of the gene in the core region of the accumbens nucleus, lateral division of the bed nucleus of the stria terminalis, medial preoptic nucleus, paraventricular nucleus, arcuate nucleus, dorsomedial hypothalamic nucleus, premammillary nucleus, central amygdaloid nucleus and lateral parabrachial nucleus.

Moderate to intense expression of Peg10 was also observed in monoaminergic nuclei such as the substantia nigra, dorsal raphe nucleus and locus coeruleus. These results suggest that Peg10 may play a role in motivational processes, emotional regulation, and autonomic functions in the brain. The findings also suggest that Peg10 may have contributed to the evolution of mammals, not only by participating in placenta formation, but also by regulating parental behavior and hormonal secretions necessary for maternal responsiveness.

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INTRODUCTION

Peg10 plays an important role in cell proliferation and differentiation, and is essential for placenta formation in mice (Kaneko-Ishino and Ishino, 2012). Peg10 gene knockout in mice results in embryonic lethality because of defects in the placenta (Ono et al., 2006). Peg10 is a paternally-expressed imprinted gene (Ono et al., 2001) that shares homology with Sushi-ichi, an LTR-type retrotransposon. Peg10 is highly conserved across mammalian species, while birds and reptiles lack the gene (Kaneko-Ishino and Ishino, 2012). Thus, after insertion into the genome, Peg10 conceivably acquired its function in placenta formation over the course of evolution. Although Peg10 seems to have originated from a retrotransposon, it does not possess a long terminal repeat sequence, and thus appears to have lost its ability to retrotranspose.

Peg10 is overexpressed in a number of cancers, including breast cancer, pancreatic cancer, prostate cancer, hepatocellular carcinoma, B cell lymphocytic leukemia, lung cancer, and gallbladder carcinoma (Akamatsu et al., 2015; Bang et al., 2015; Kainz et al., 2007; Xie et al., 2018). A number of studies have reported that Peg10 plays a role in the proliferation of cancer cells. Overexpression of Peg10 contributes to migration and invasion of breast cancer (Xie et al., 2018). Peg10 is also related to poor survival and recurrence in hepatocellular carcinoma (Bang et al., 2015). Inhi-

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bition of apoptosis is one of the characteristics of cancer cells. Peg10 has been reported to have an anti-apoptotic role in cancer cells (Xie et al., 2018).

Although there are a number of studies on Peg10 expression in the placenta and in cancer cells (Kaneko-Ishino and Ishino, 2012; Xie et al., 2018), very few reports have focused on the expression of Peg10 mRNA in the brain. The function of Peg10 in the brain is also unknown. Therefore, in this study, we systematically investigated the expression of Peg10 mRNA throughout the mouse brain.

MATERIALS AND METHODS

Animals

8- to 10-week-old male mice (C57BL/6J) were used. The experimental procedures were approved by the animal ethics committee at Osaka University, and were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

In situ hybridization (ISH)

cDNA fragments of mouse Peg10 (NM_130877.2; 1,583–2,385) were produced using PCR amplification of C57BL/6J mouse brain cDNA, and were used as templates for digoxigenin (DIG)-labeled cRNA probe generation. Under anesthesia, the brains were rapidly removed and frozen on dry ice. Coronal sections (10 μm), from the olfactory bulb to the spinal cord, were prepared on a microtome in a cryostat. Sections were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer at 24°C for 10 min, washed in 0.01M PBS, and then acetylated in a solution of 1.5% triethanolamine and 0.25% acetic anhydride in distilled water. After washing with 2 × saline sodium citrate buffer (SSC), sections were incubated with hybridization buffer (60% formamide, 3 × SSC, 5 × Denhardt's solution, 250 μg/ml yeast tRNA, 20 μg/ml salmon testes DNA, 1 mM DTT and 5% dextran sulfate in distilled water) containing 200 ng/ml DIG-labeled cRNA probe at 55°C overnight. Next, sections were washed with 2 × SSC at 65°C for a few minutes and 0.1 × SSC at 65°C for 20 min. After blocking with 0.1% Tween-20 and 0.5% casein in Tris -buffered saline (100 mM Tris-HCl, pH 7.5, 150 mM NaCl), sections were incubated with alkaline phosphatase-conjugated anti-DIG antibody (sheep; Roche, Mannheim, Germany; 1:2,000) in blocking solution at 4°C overnight, and then incubated with NBT/BCIP solution (100 mM Tris-HCl, pH 9.5, 150 mM NaCl, 50 mM MgCl2, 3.5 μl BCIP (Roche), 3.5 μl NBT (Roche)) overnight for the color reaction, which was stopped by adding TE solution (10 mM Tris-HCl, pH 8.0, 1 mM EDTA). Images were obtained on a BX63 light microscope (Olympus, Tokyo, Japan).

RESULTS

The specificity of the hybridization signal was assessed by hybridizing adjacent sections with antisense or sense RNA probe. No signal was detected in sections hybridized with sense probe.

Forebrain

Septum and bed nucleus of the stria terminalis

Many Peg10 mRNA-containing neurons were found in the ventral part of the lateral septal nucleus (LSV) (Fig. 1a), while a few cells were detected in the dorsal part of the lateral septal nucleus (LSD) (Fig. 1a). A moderate number of Peg10 mRNA-positive cells were observed in the medial and lateral divisions of the bed nucleus of the stria terminalis (BSTM and BSTL) (Figs. 1a and 2b). Intense to moderate expression of Peg10 mRNA was seen in the interstitial nucleus of the posterior limb of the anterior commissure (IPAC) (Fig. 1a).

Amygdaloid complex

Strongly-labeled neurons were seen in the lateral division of the central amygdaloid nucleus (CeL) (Figs. 1c, d and 3b). A moderate number of labeled cells were observed in the medial amygdaloid nucleus (Me) (Figs. 1d and 3b) and basomedial amygdaloid nucleus (BM) (Fig. 1d).

Hippocampal formation

A weak ISH signal was detected in the granule cell layer of the dentate gyrus (GrDG) (Fig. 3c). Extremely strongly-labeled neurons were found in the ventral subiculum (VS) (Figs. 1e and 3d).

Basal ganglia

Intensely-labeled neurons were observed in the core subdivision of the accumbens nucleus (AcbC) (Fig. 2a). Scattered labeled neurons were seen in the ventral pallidum (VP) (Fig. 2a).

Subfornical organ

Weakly to moderately-labeled cells were detected in the subfornical organ (SFO) (Figs. 2c and 4c).

Diencephalon

Thalamus

Moderate hybridization signals were detected in the anterior part of the periventricular thalamic nucleus (PVA) (Fig. 2c), the anteroventral thalamic nucleus (AV) (Fig. 2c), and the reuniens thalamic nucleus (Re) (Fig. 2c). Intensely-labeled cells were found in the lateral habenular nucleus (LHb) (Figs. 1d and 3a).

Hypothalamus

The median preoptic nucleus (MnPO) (Figs. 1a and 2b) contained moderately- to strongly-labeled neurons (Figs. 1a and 2b). A large number of labeled-neurons were observed in the striohypothala-

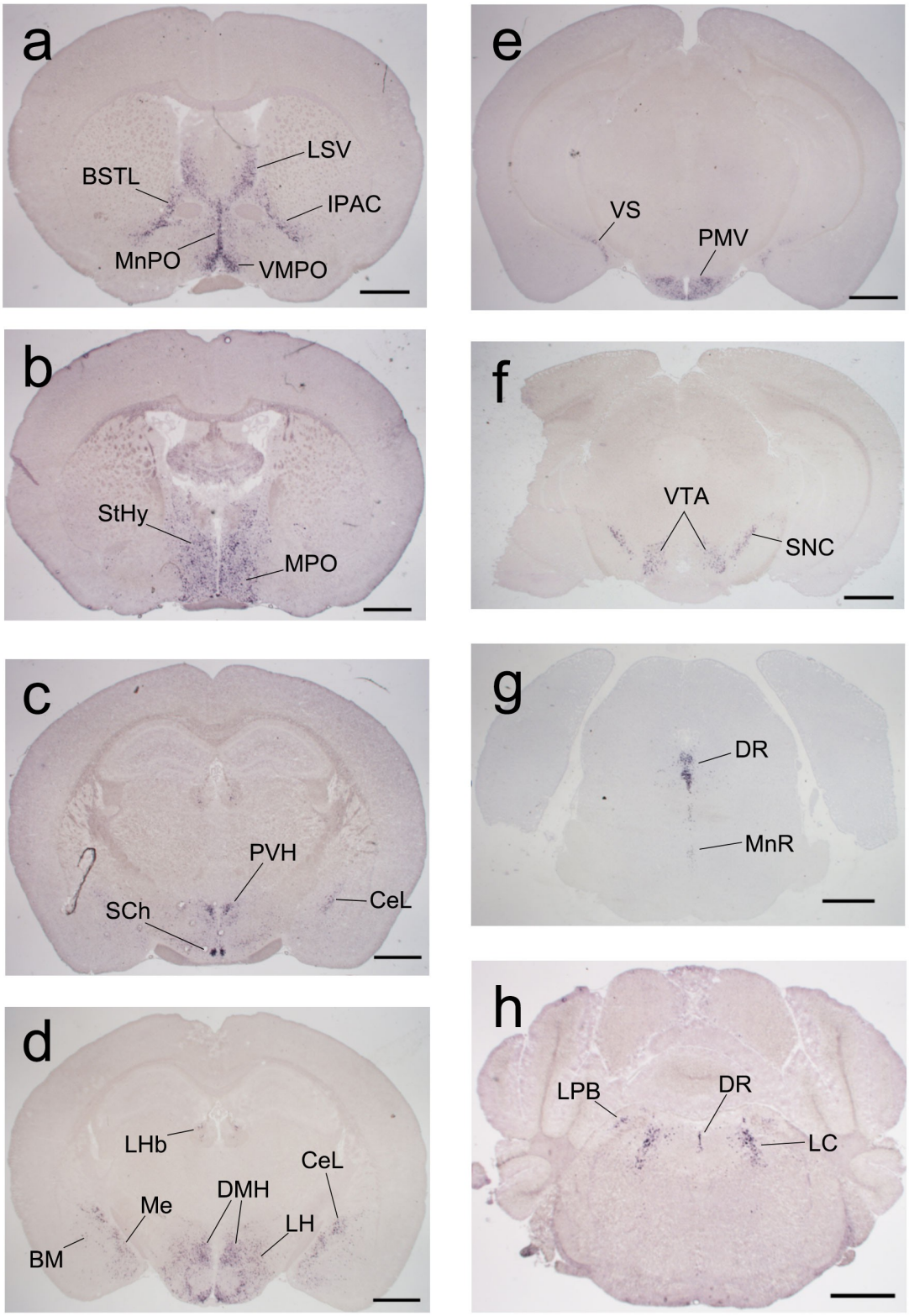

Fig 1. In situ hybridization histochemistry of Peg10 mRNA expression in throughout the mouse brain. Coronal sections from rostral to caudal levels of the brain are shown (**a-h**).

Abbreviations: BM, basomedial amygdaloid nucleus ; BSTL, lateral division of the bed nucleus of the stria terminalis; CeL, central amygdaloid nucleus, lateral division; DMH, dorsomedial hypothalamic nucleus; DR, dorsal raphe nucleus; IPAC, interstitial nucleus of the posterior limb of the anterior commissure; LC, locus coeruleus; LH, lateral hypothalamic area; LHb, lateral habenular nucleus; LPB, lateral parabrachial nucleus; LSV, lateral septal nucleus, ventral part; Me, medial amygdaloid nucleus; MnPO, median preoptic nucleus; MnR, median raphe nucleus; MPO, medial preoptic nucleus; PMV, premammillary nucleus, ventral part; PVH, paraventricular hypothalamic nucleus; SCh, suprachiasmatic nucleus; SNC, substantia nigra, compact part; StHy, striohypothalamic nucleus; VMPO, ventromedial preoptic nucleus; VS, ventral subiculum; VTA, ventral tegmental area. Scale bar: 1 mm.

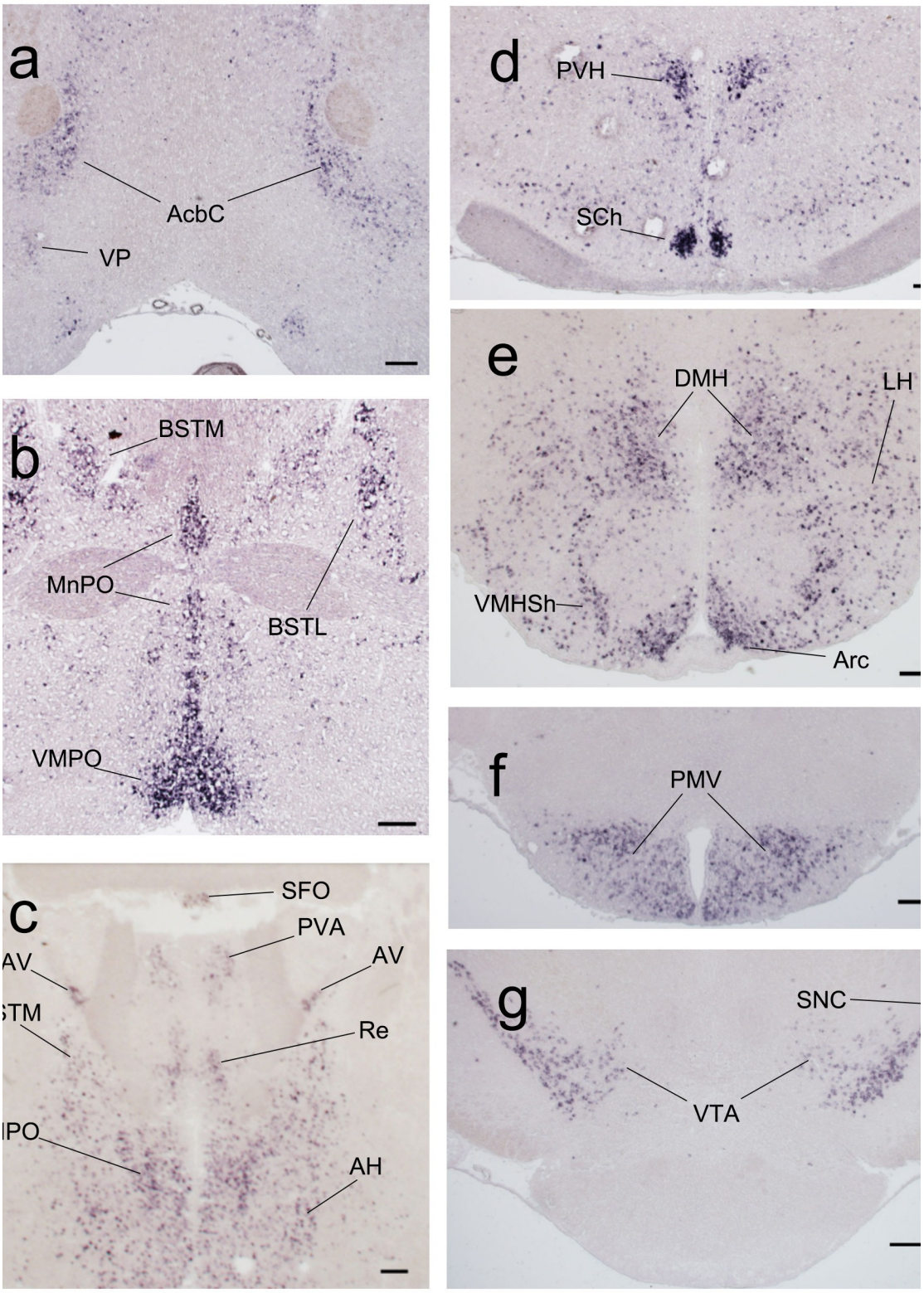

Fig 2. High magnification of the coronal sections of the mouse brain showing Peg10 mRNA expression by in situ hybridization histochemistry.

Abbreviations: AcbC, accumbens nucleus, core subdivision; AH, anterior hypothalamic area; Arc, arcuate hypothalamic nucleus; AV, anteroventral thalamic nucleus; BSTL, lateral division of the bed nucleus of the stria terminalis; BSTM, medial division of the bed nucleus of the stria terminalis; DMH, dorsomedial hypothalamic nucleus; LH, lateral hypothalamus; MnPO, median preoptic nucleus; MPO, medial preoptic nucleus; PMV, premammillary nucleus, ventral part; PVA, periventricular thalamic nucleus; PVH, paraventricular hypothalamic nucleus; Re, reuniens thalamic nucleus; SCh, suprachiasmatic nucleus; SFO, subfornical organ; SNC, substantia nigra, compact part; VMHSh, ventromedial hypothalamic nucleus, shell region; VMPO, ventromedial preoptic nucleus; VP, ventral pallidum; VTA, ventral tegmental area. Scale bar: 200 μm.

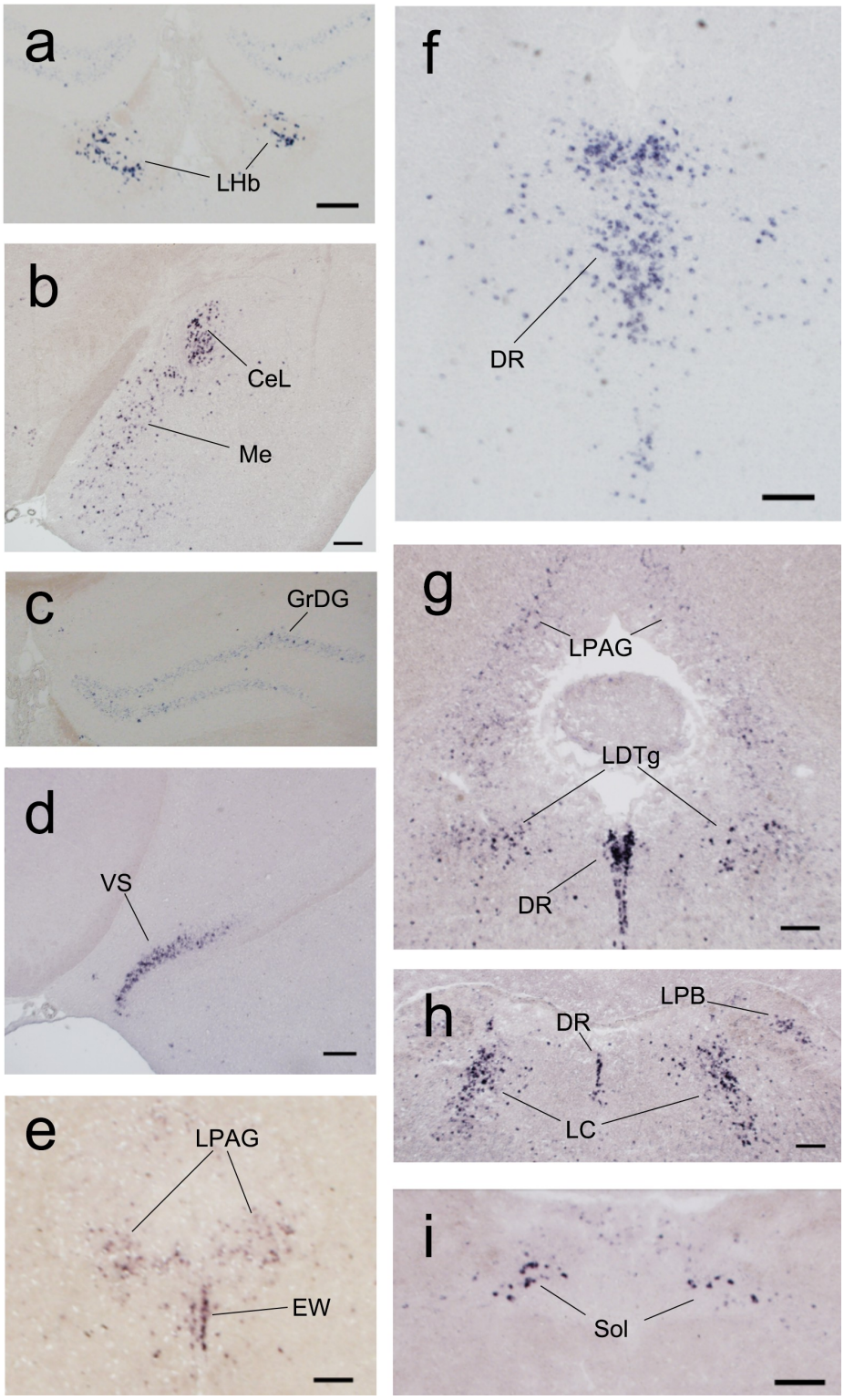

Fig 3. High magnification of the coronal sections of the mouse brain showing Peg10 mRNA expression by in situ hybridization histochemistry.

Abbreviations: CeL, central amygdaloid nucleus, lateral division; DR, dorsal raphe nucleus; EW, Edinger-Westphal nucleus; GrDG, granule cell layer of the dentate gyrus; LC, locus coeruleus; LDTg, laterodorsal tegmental nucleus; LHb, lateral habenular nucleus; LPAG, lateral periaqueductal gray; LPB, lateral parabrachial nucleus; Me, medial amygdaloid nucleus; SolM, solitary nucleus, medial part; VS, ventral subiculum. Scale bar: 200 μm.

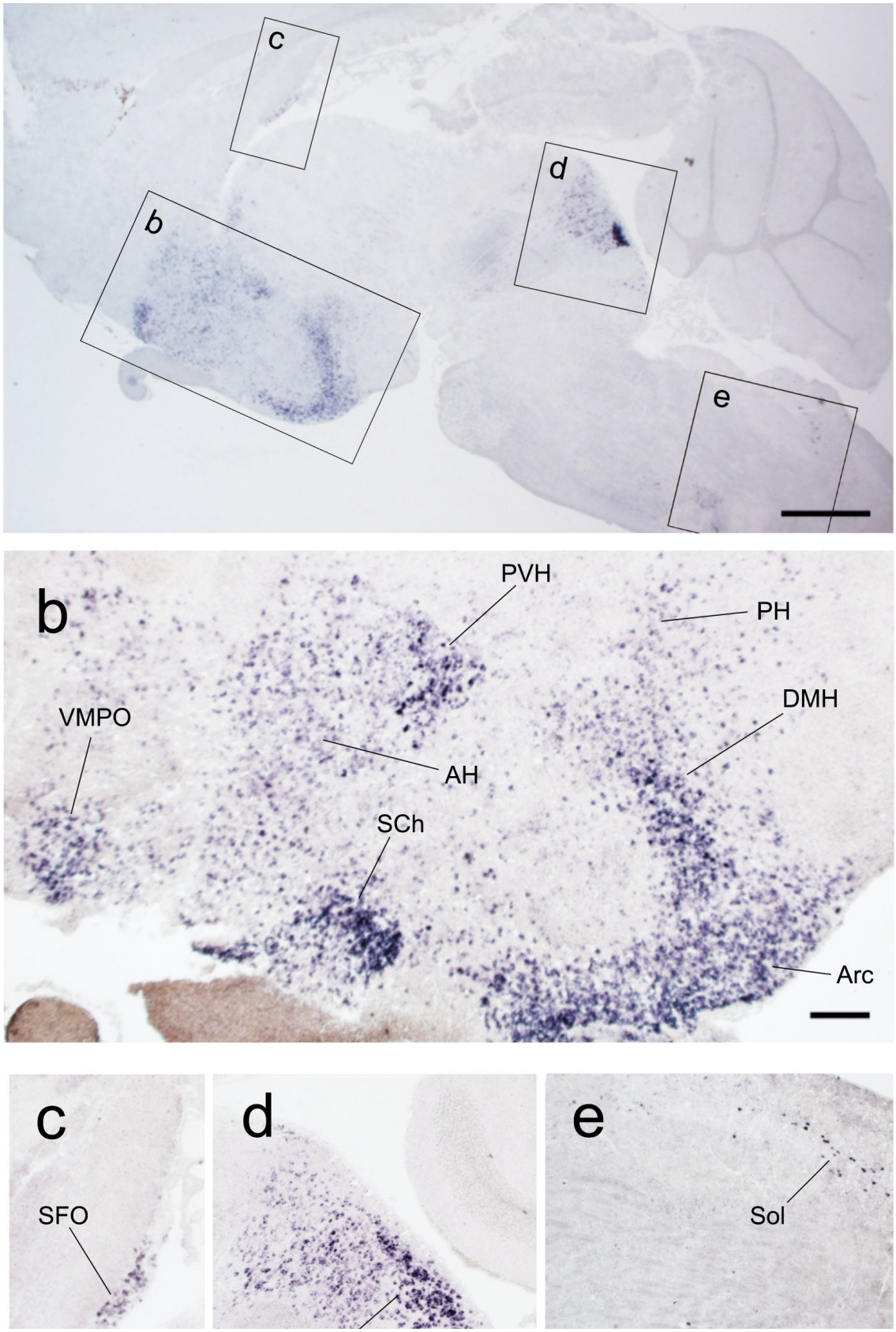

Fig 4. Sagittal sections of the mouse brain showing Peg10 mRNA expression by in situ hybridization histochemistry. (b-e): Higher magnification of squared area in (a).

Abbreviations: AH, anterior hypothalamic area; Arc, arcuate hypothalamic nucleus; DMH, dorsomedial hypothalamic nucleus; DR, dorsal raphe nucleus; PH, posterior hypothalamic nucleus; PVH, paraventricular hypothalamic nucleus; Rt, reticular formation; SCh, suprachiasmatic nucleus; SFO, subfornical organ; SolM, medial part of the solitary nucleus; VMPO, ventromedial preoptic nucleus. Scale bar: 1 mm (a), 200µm (b, c, d, e).

mic nucleus (StHy) (Fig. 1b). Many positive neurons were seen in the medial preoptic nucleus (MPO) as well (Figs. 1b and 2c). A dense accumulation of strongly-labeled neurons was observed in the ventromedial preoptic nucleus (VMPO) (Figs. 1a, 2b and 4b). Intense ISH signals were observed in the paraventricular hypothalamic nucleus (PVH) (Figs. 1c, 2d and 4b). Moderately-labeled neurons were observed in the anterior hypothalamic area (AH) (Figs. 2c and 4b).

A high concentration of Peg10 mRNA ISH signals was found in the suprachiasmatic nucleus (SCh) (Figs. 1c, 2d and 4b). The arcuate hypothalamic nucleus (Arc) (Figs. 2e and 4b) contained densely-labeled neurons. Moderately-labeled scattered cells were seen in the lateral hypothalamus (LH) (Figs. 1d and 2e). Moderate to high levels of Peg10 mRNA were detected in the shell region of the ventromedial hypothalamic nucleus (VMHSh) (Fig. 2e), whereas little hybridization signal was observed in the core region of the ventromedial hypothalamic nucleus. A large number of labeled cells were seen in the dorsomedial hypothalamic nucleus (DMH) (Figs. 1d, 2e and 4b). Moderately-labeled cells were found in the posterior hypothalamic nucleus (PH) (Fig. 4b).

Strongly-labeled cells were densely packed in the ventral part of the premammillary nucleus (PMV) (Figs. 1e and 2f), while labeled cells were barely detected in the dorsal part of the premammillary nucleus (PMD).

Brainstem

Midbrain

Strongly-labeled neurons were found in the ventral tegmental area (VTA) (Figs. 1f and 2g) and the compact part of the substantia nigra (SNC) (Figs. 1f and 2g). Moderately-labeled cells were located in the Edinger-Westphal nucleus (EW) (Fig. 3e). The lateral periaqueductal gray (LPAG) (Fig. 3e, g) contained weakly-labeled scattered cells.

Robustly-labeled neurons were observed in the dorsal raphe nucleus (DR) (Figs. 1g, h, 3f–h and 4d), while moderately-labeled neurons were found in the median raphe nucleus (MnR) (Fig. 1g).

Pons and medulla

Peg10 mRNA-positive neurons were scattered in the laterodorsal tegmental nucleus (LDTg) (Fig. 3g). The lateral parabrachial nucleus (LPB) (Figs. 1h and 3h) contained moderately to intensely-labeled neurons. Very strong ISH signals were observed in the locus coeruleus (LC) (Figs. 1h and 3h). Moderately-labeled scattered cells were observed throughout the reticular formation (Rt) (Fig. 4e). The medial vestibular nucleus displayed moderate hybridization signals. Moderately-labeled cells were found in the solitary nucleus (Sol) (Figs. 3i and 4e).

DISCUSSION

Peg10 appears to have played a key role in the acquisition of the ability to form the placenta in the course of mammalian evolution (Kaneko-Ishino and Ishino, 2012). Peg10 is expressed in the medial preoptic nucleus, the bed nucleus of the stria terminalis, and the paraventricular hypothalamic nucleus, which is known to be associated with parental behaviors (Tsuneoka, 2019). Therefore, Peg10 may have contributed to the evolution of mammals in two major ways: by participating in placenta formation, and by regulating the parental behaviors and hormonal pathways necessary for maternal responsiveness in the brain.

In the present study, we found that Peg10 was expressed throughout the hypothalamus. Robustly-labeled neurons were detected in the suprachiasmatic nucleus, paraventricular hypothalamic nucleus, dorsomedial hypothalamic nucleus, arcuate hypothalamic nucleus and the ventral part of the premammillary nucleus. These hypothalamic areas are associated with the regulation of body temperature, osmolality, sleep, circadian rhythms, feeding, drinking, hormone secretion, ovulation, reproductive behavior, parental behavior, and feeding behavior. Therefore, Peg10-expressing cells could be involved in many critical physiological and behavioral functions. Indeed, Peg10 knockout mice show embryonic lethality, in addition to a defect in placental development.

Peg10-expressing cells were also widely expressed in monoaminergic neuron-rich regions. High levels of Peg10 mRNA were observed in the substantia nigra and ventral tegmental area, which contain the majority of dopaminergic neurons; the dorsal and median raphe nuclei, which are the principal source of serotonergic neurons; and the locus coeruleus, which is a main source of noradrenergic neurons. Furthermore, the distribution of Peg10 mRNA-positive cells partially overlapped that of histaminergic neurons in the tuberomammillary nucleus (Scammell et al., 2019). Thus, Peg10 seems to be expressed in all four monoaminergic systems. These monoaminergic neurons all project diffusely to numerous areas throughout the brain, and play a pivotal role in fundamental brain functions.

Peg10 ISH signals were observed in distinct limbic areas. Strongly-labeled neurons were found in the lateral division of the central amygdaloid nucleus and the lateral division of the bed nucleus of the stria terminalis. Peg10 was also expressed in the parabrachial nucleus. These three areas are tightly connected to each other by neuronal pathways and are associated with anxiety-like behavior (Fetterly et al., 2019), suggesting that Peg10 may be involved in negative affectivity.

The core region of the accumbens nucleus (AcbC) (Fig. 2a) and the ventral tegmental area (VTA) (Figs. 1f and 2g) strongly expressed Peg10.

These areas are the central components of the reward system in the brain (Dickson et al., 2011), and are associated with drug addiction. Intriguingly, Peg10-expressing neurons were found in the laterodorsal tegmental nucleus (LDTg) (Fig. 3g), which is also involved in the reward system. Cholinergic projections from the LDTg to the VTA and dopaminergic projections from the VTA to the AcbC together form the cholinergic–dopaminergic reward pathway. Our finding that Peg10 is expressed in all of these areas suggests that it may play a critical role in reward seeking behavior.

In conclusion, the expression patterns of Peg10 suggest that it is involved in motivational processes, emotional regulation, and autonomic functions in the brain. Peg10 may also have played a key role in the evolution of mammals, not only by participating in placenta formation, but also by regulating parental behaviors and hormones necessary for maternal responsiveness. To prove this hypothesis, further investigation is needed using a brain-specific conditional knockout mice of Peg 10 without affecting placenta formation.

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