Follistatin-like 5is expressed in restricted areas of the adult mouse brain: Implications for its function in the olfactory system

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Short Communication

Follistatin-like 5 **is expressed in restricted areas of the adult mouse brain: Implications for its function in the olfactory system**

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Short title: Fstl5 expression in the mouse brain

ABSTRACT

Follistatin-like 5 (*Fstl5*), a member of the follistatin family genes, encodes a secretory glycoprotein. Previous studies revealed that other members of this family including *Fstl1* and *Fstl3* play an essential role in development, homeostasis, and congenital disorders. However, the *in vivo* function of *Fstl5* is poorly understood. To gain insight into the function of *Fstl5* in the mouse central nervous system, we examined the *Fstl5* expression pattern in the adult mouse brain. The results of *in situ* hybridization analysis showed a highly restricted pattern of *Fstl5*, namely, with localization in the olfactory system, hippocampal CA3 area and granular cell layer of the cerebellum. Restricted expression in the olfactory system suggests a possible role for *Fstl5* in maintaining odor perception.

Key words: central nervous system, coronal section, follistatin, KIAA clones, mouse

INTRODUCTION

The Kazusa cDNA project by Kazusa DNA Research Institute pioneered an extensive sequencing project of human cDNAs in their entirety and focused particularly on large cDNAs encoding large proteins (Nagase et al. 2006). The novel genes identified by this project were systematically designated as 'KIAA' plus a four-digit number known as KIAA genes (Nomura et al. 1994). KIAA genes are characterized by their long cDNA sequences encoding large proteins and by isolation from the brain. Large human proteins are expected to play an important role in cellular structure, gene expression, cell signaling, and so on. Previously, we used the Kazusa cDNA array system and obtained several genes based on their strong expression in the embryonic spinal cord (Masuda et al. 2009). These genes include mKIAA0655 (mouse *Huntingtin-interacting protein 1-related*; *mHip1r*) and mKIAA1263 (mouse *follistatin-like 5*; *Fstl5*). The *mHip1r* gene may be correlated with Huntington's disease, which is one of the representative congenital disorders displaying choreic movement, personality changes, and dementia (Walker 2007).

Fstl5, which is also called *follistatin-related protein 5*, encodes an 847-amino acid secretory glycoprotein. *Fstl5* belongs to the follistatin family of genes based on the presence of a conserved follistatin domain. *Fstl1*, another follistatin gene, is known to be involved in early mesoderm patterning and neural development (Amthor et al. 1996; Patel et al. 1996; Towers et al. 1999); whereas *Fstl3* plays a crucial role in regulating metabolic homeostasis and testicular aging (Mukherjee et al. 2007; Oldknow et al. 2013). In addition, *Fstl3* is one of the genes responsible for congenital heart malformation (Archer et al. 2005). As in the case of these genes, it is highly possible that *Fstl5* might also play an essential role in development or be a gene responsible for some congenital disorder. Although the transcriptional profiling of human normal tissues in the Expression Atlas (http://www.ebi.ac.uk/gxa/) showed that levels of human *Fstl5* (*FSTL5*) gene expression in the brain and spinal cord are higher compared with those in other organs, the *in vivo* function of the *Fstl5* gene is yet unknown.

To begin to assess the role of *Fstl5* in the mature brain, a precise knowledge of the expression pattern of the *Fstl5* gene is required. Thus, we describe here the expression patterns of *Fstl5* in the mouse brain. Our data will be beneficial for a better understanding of the role of *Fstl5* in the mouse central nervous system.

MATERIALS AND METHODS

Tissue preparation

ICR male mice (CLEA Japan, Tokyo, Japan), 12 weeks old, were used for experiments. These mice were killed by CO_2 narcosis. Thereafter, they were decapitated rapidly, and their brains were removed and frozen in Tissue-Tek OCT compound (Bayer AG, Leverkusen, Germany). Coronal serial sections (25-μm thickness) were cut with a cryostat for *in situ* hybridization processing. All procedures in the animal experiments in this study were approved by the Animal Care Committee of the National Institute of Neuroscience.

In situ hybridization

In situ hybridization was performed by using the digoxygenin (DIG)-labeled RNA probe transcribed from the full-length cDNA of *Fstl5*, as described earlier (Masuda et al. 2009). This probe was synthesized by using a DIG RNA-labeling kit (Roche Diagnostics, Mannheim, Germany). Hybridization and detection procedures were performed as described previously (Masuda et al. 2009). No labeling was detectable in the control sections, which were hybridized with the sense cRNA probe (data not shown).

Figure illustrations

The drawings in Figures 1 and 2 were made with Adobe Illustrator CS5 (Adobe Systems, CA, USA) and Bamboo Tablet (Wacom, Saitama, Japan) adapted from the corresponding figures from Paxinos and Franklin (2013).

RESULTS AND DISCUSSION

To examine the expression pattern of the *Fstl5* gene in the adult mouse brain, we performed *in situ* hybridization analysis on a series of coronal sections of the 12-week-old murine brain. The drawings were selected from Paxinos and Franklin (2013) at specific distances from Bregma to give a schematic representation of the anteroposterior spread of labeling in the adult mouse brain, as shown in Figures 1 and 2. In the olfactory bulb seen in coronal sections of the anterior part of the brain, the *Fstl5* mRNA was detected in the internal plexiform layer and in the border cells between the external plexiform layer or in the glomerular layer (Fig. 1A–C). No signals were seen within the external plexiform layer and the glomerular layer (Fig. 1C). In coronal sections made slightly more posterior, *Fstl5* was highly expressed in the olfactory pathway, that is, in the piriform cortex, olfactory tubercle, and the islands of Calleja (Fig. 1D–K). In addition, some nuclei of the thalamus and the hypothalamus including the anterior part of the paraventricular thalamic nuclei and the periventricular hypothalamic nuclei were *Fstl5*-positive (Fig. 1I,K).

Next, we examined the expression of the *Fstl5* gene in the posterior part of the adult mouse brain. In the coronal section made through the hippocampus and the third ventricle at the level of Bregma –2.30 mm, the *Fstl5* mRNA was strongly detected in the various regions including the olfactory pathway, that is, in the hippocampal CA3 area, posterolateral cortical amygdala nuclei, piriform cortex, posterior part of the basolateral amygdala nuclei, posteromedial cortical amygdala nuclei, ventral premammillary nuclei, and the mammillothalamic tract (Fig. 2A,B). In contrast to the high expression level in the CA3 area, no hybridization signals for *Fstl5* were seen in the hippocampal CA1 and CA2 areas or in the dentate gyrus (Fig. 2C). In the coronal section through the cerebellum, the *Fstl5* transcripts were detected in the granular cell layer of the cerebellar cortex, but not in the molecular layer (Fig. 2D,E). Furthermore, the *Fstl5* mRNA was detected in

posterior and lateral parts of the dorsal tegmental nuclei and in some unknown cell population (Fig. 2D,F).

Regarding the data on *Fstl5* expression in the mouse brain, some histological information can be found in the Allen Brain Atlas (http://www.brain-map.org/). However, detailed spatial expression analysis of *Fstl5* in the adult mouse brain had not been examined. Our study revealed for the first time that the *Fstl5* gene showed a highly restricted expression in the olfactory system. Based on this fact, it is highly likely that *Fstl5* may be involved in maintaining or assisting in odor perception.

Previous DNA analysis revealed that human *FSTL5* is a promising biomarker for medulloblastoma (Remke et al. 2011). This is the only report about the relationship between the *Fstl5* gene and a human disorder so far. Interestingly, a recent study revealed that Sonic hedgehog (Shh) signaling is impaired in *Fstl1*-deficient mouse embryos (Xu et al. 2012). Shh signaling plays a pivotal role in the cell-fate specification and in the development of the central nervous system (Komada 2012). Furthermore, many congenital disorders in humans are known to involve mutations of the Shh signaling pathway (Nieuwenhuis and Hui 2004; Rathod et al. 2012; Vaze et al. 2012). Taken together, the available data suggest that it is highly possible that *Fstl* genes including *Fstl5* might be involved in pathologies of congenital disorders via regulation of Shh signaling.

Our study represents a first step toward the characterization of the Fstl5 protein. Detailed analysis of *Fstl5*-deficient mice or individuals who carry a mutation in his/her *FSTL5* gene should lead to a better understanding of the function of the *Fstl5* gene.

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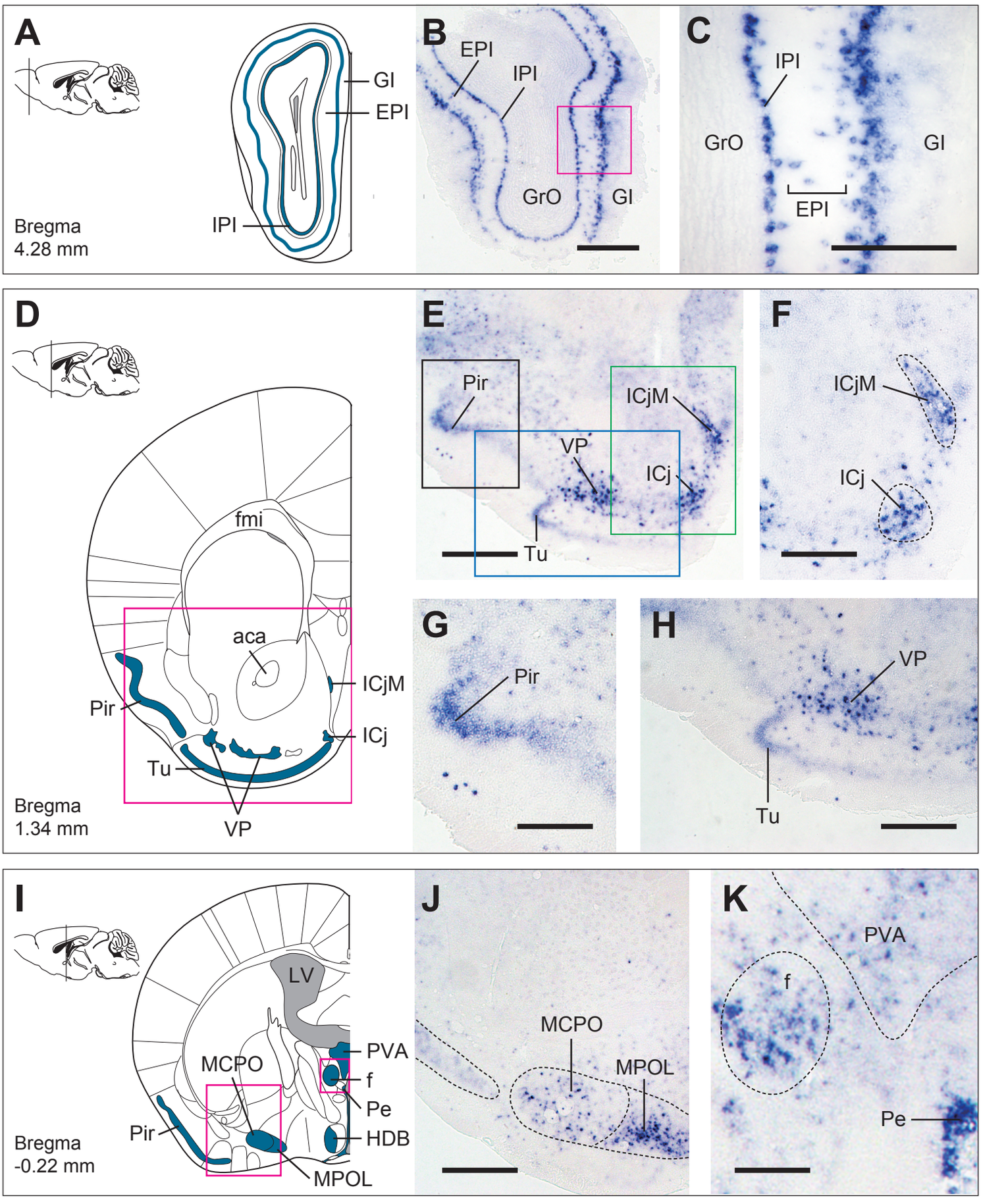
Figure Legends

Fig. 1

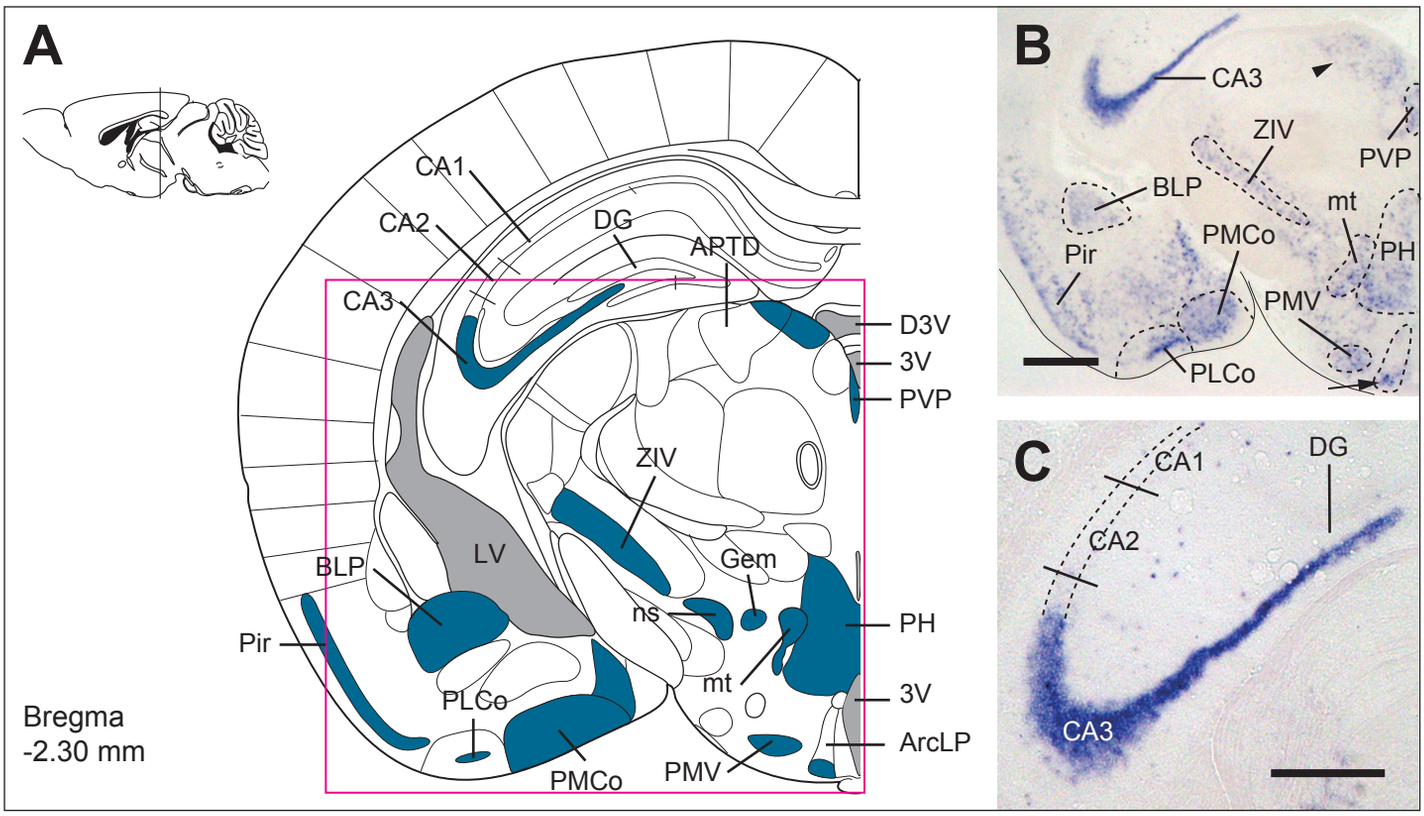
Expression of the *Fstl5* gene in the anterior part of the adult mouse brain. (A) The drawing of a coronal section at 4.28 mm distance from Bregma represents the *Fstl5* mRNA distribution (dark blue). (B) Coronal section through the adult mouse brain at the level of 4.28 mm from Bregma. Dark blue color represents positive signals. The *Fstl5* mRNA was detected in the internal plexiform layer (IPI) of the olfactory bulb and the border cells between the external plexiform layer (EPI) and the glomerular layer (GI). (C) High magnification view of the magenta-boxed region in B. (D) The expression of *Fstl5* mRNA was shown in the drawing of a coronal section at 1.34 mm distance from Bregma. (E) The photograph of the magenta-boxed region in D. The *Fstl5* transcripts were prominently detected in the piriform cortex (Pir), olfactory tubercle (Tu), ventral pallidum (VP), island of Calleja (ICj), and in the major island of Calleja (ICjM). (F, G, H) High magnification views of the respective green-, black- and blue-boxed regions in E. (I) The drawing of a coronal section at –0.22 mm distance from Bregma represents the expression patterns of *Fstl5*. (J) Photograph of the large magenta-boxed region in I. The *Fstl5* mRNA was highly expressed in the lateral part of the medial preoptic nuclei (MPOL). Moderate hybridization signals of *Fstl5* expression were detected in the magnocellular preoptic nuclei (MCPO). (K) Photograph of the small magenta-boxed region in I. The strong expression of the *Fstl5* gene was seen in the periventricular hypothalamic nuclei (Pe). *Fstl5* gene expression was also detected in the fornix (f) and in the anterior part of the paraventricular thalamic nuclei (PVA). aca, anterior part of the anterior commissure; fmi, forceps minor of the corpus callosum; GrO, granular cell layer of the olfactory bulb; HDB, nuclei of the horizontal limb of the diagonal band; LV, lateral ventricle. Scale bars = 0.5 mm in B, E, 0.25 mm in C, 0.25 mm in F, 1 mm in G, 0.8 mm in H, 0.6 mm in J, 0.2 mm in K.

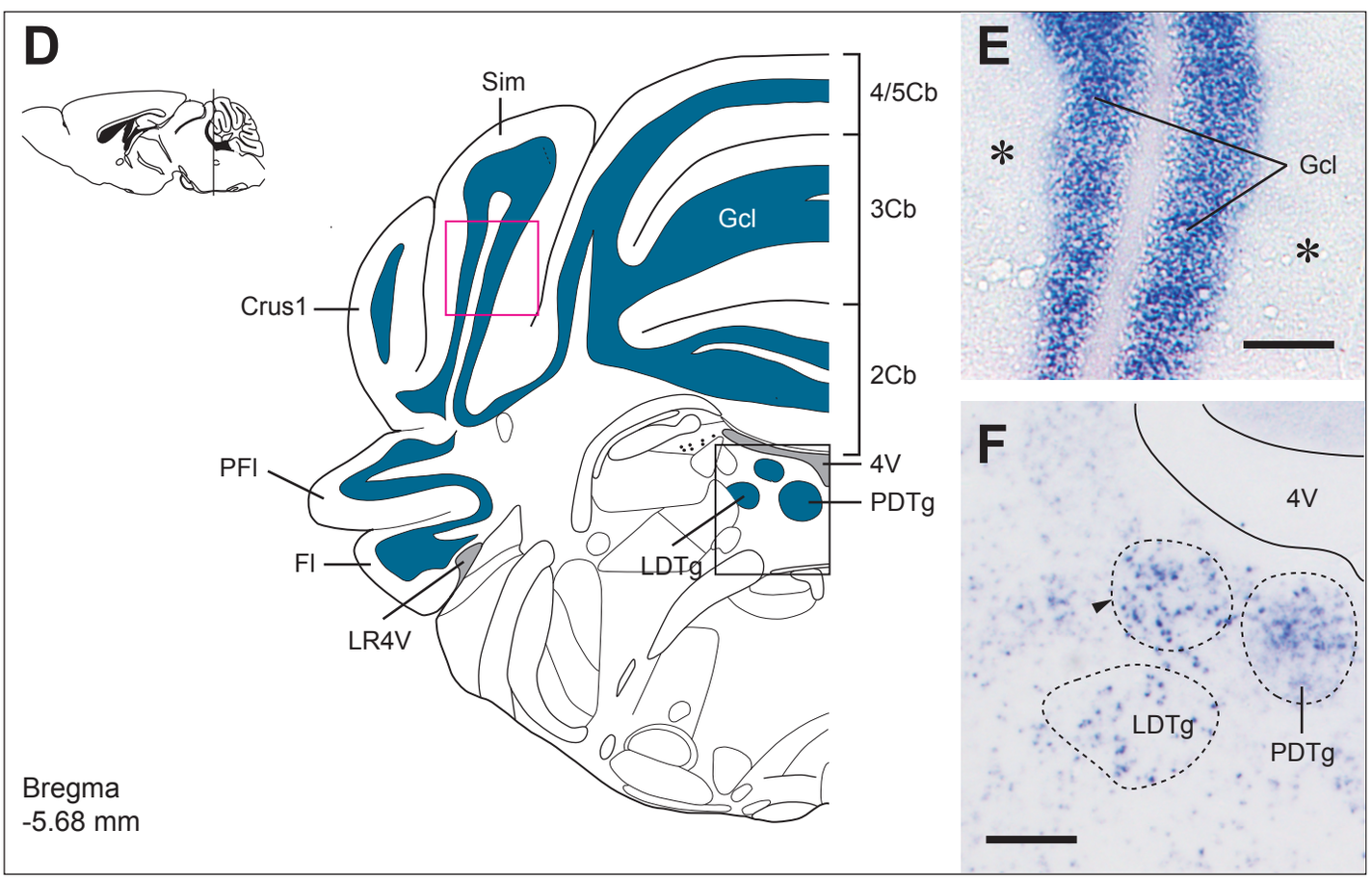
Fig. 2

Expression of the *Fstl5* gene in the posterior part of the adult mouse brain. (A) The drawing of a coronal section at –2.30 mm distance from Bregma represents the *Fstl5* mRNA distribution (dark blue). (B) Photograph of the magenta-boxed region in A. Dark blue color represents positive signals. The *Fstl5* transcripts were strongly detected in the hippocampal CA3 area (CA3) and in the posterolateral cortical amygdala nuclei (PLCO). Moderate signals of *Fstl5* were seen in the piriform cortex (Pir), posterior part of the basolateral amygdala nuclei (BLP), posteromedial cortical amygdala nuclei (PMCO), ventral zona incerta (ZIV), ventral premammillary nuclei (PMV), ventral part of the lateral posterior hypothalamic arcuate nuclei (ArcLP; arrow), mammillothalamic tract (mt), posterior hypothalamic nuclei (PH), posterior part of the paraventricular thalamic nuclei (PVP), and the dorsal part of the anterior pretectal nuclei (APTD; arrowhead). (C) High-magnification view of the expression pattern of the hippocampal CA areas. No hybridization signals of *Fstl5* were seen in the hippocampal CA1 and CA2 areas (CA1, CA2) or dentate gyrus. (D) Expression pattern of *Fstl5* was shown in the drawing of a coronal section made at –5.68 mm distance from Bregma. (E) Photograph of the magenta-boxed region in D. The *Fstl5* gene was expressed in the granular cell layer of the cerebellar cortex (Gcl), but not in the molecular layer (asterisks). (F) Photograph of the black-boxed region in D. The *Fstl5* mRNA was detected in both posterior and lateral parts of the dorsal tegmental nuclei (PDTg, LDTg). The hybridization signals were also seen in an unknown cell population (arrowhead). 2Cb, lobule 2 of the cerebellar vermis; 3Cb, lobule 3 of the cerebellar vermis; 3V, 3rd ventricle; 4V, 4th ventricle; 4/5Cb, lobules 4 and 5 of the cerebellar vermis; Crus1, crus 1 of the ansiform lobule; D3V, dorsal 3rd ventricle; Fl, flocculus; Gem, Gemini hypothalamic nuclei; LR4V, lateral recess of the 4th ventricle; LV, lateral ventricle; ns, nigrostriatal bundle; PFl, paraflocculus; Sim, simple lobule. Scale bars = 1 mm in B, 0.6 mm in C, 0.2 mm in E, 0.25 mm in F.



Masuda et al. Fig. 1





Masuda et al. Fig. 2