

Interactive report

Murine *Lix1*, a novel marker for substantia nigra, cortical layer 5, and hindbrain structures

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Abstract

We describe the expression of *Lix1* in the mouse. Starting at E8, transcripts are present in a regionalized fashion and persist throughout development. *mLix1* is expressed in the cortical plate, subventricular zone, layer 5 of the postnatal cortex, the substantia nigra, dorsal root ganglia, specific nuclei of the brain stem and in spinal cord. Limb buds and facial primordia show transient expression. The prominent expression of *mLix1* in the developing cerebral cortex and in the substantia nigra pars compacta makes this novel gene a candidate marker for both of these tissues.

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Theme: Development

Topic: Cell migration, neuronal differentiation

Keywords: *Lix1*; Mouse; Substantia nigra; Cerebral cortex; Cortical layer 5; Dorsal root ganglia; Limb; Craniofacial primordia

1. Methods

In situ hybridization on whole mounts was performed as described [3,4]. Sections ISH data were generated using robotic devices [2]. Accession number for *mLix1*: AF351204.

2. Results

mLix1 expression was first detected in the ectodermal component of the primitive streak (E8.0, Fig. 1A,D) in which it persisted until E9.5 (Figs. 1A–C). At E8.5 transcripts appear in the headfold (Fig. 1B,F,G) that develops—by E9.5—into prosencephalon, mesencephalon and metencephalon, all of which strongly expressed *Lix1* in their dorsal portion. After E10, expression in the neuroepithelium was markedly reduced but still detectable

(not shown). Following a reduction in level of expression between stages E10 and E12 in the CNS, *Lix1* expression resumed. At E12.5 it was expressed throughout the neuroepithelium of the brain (Fig. 2A) and spinal cord (not shown). By E13.5, transcripts in the telencephalon became restricted to the cortical plate. At E15.5 cortical expression exhibited a rostrocaudal gradient, with the younger, more caudal tissue (i.e., hippocampal region) showing much stronger expression than the developmentally more advanced rostral tissue (Fig. 2B). *mLix1* was expressed also in the subventricular and intermediate layers and very intensely in the hippocampal region (Fig. 2A,B). In addition *mLix1* transcripts were found in the ventricular layer of the developing striatum (Fig. 2B), the neuroepithelium of the third ventricle that gives rise to thalamus and hypothalamus, the mantle layer of epithalamus and ventral thalamus (not shown). The cortical expression at P7 was limited to layer 5 (the bottom inset shows a magnification of layer 5 neurons, which show expression, whereas the inset at the top shows some layer 2–3 neurons, in which the signal is not distinguishable from background), and by P56 it was barely detectable (Fig. 2A,C).

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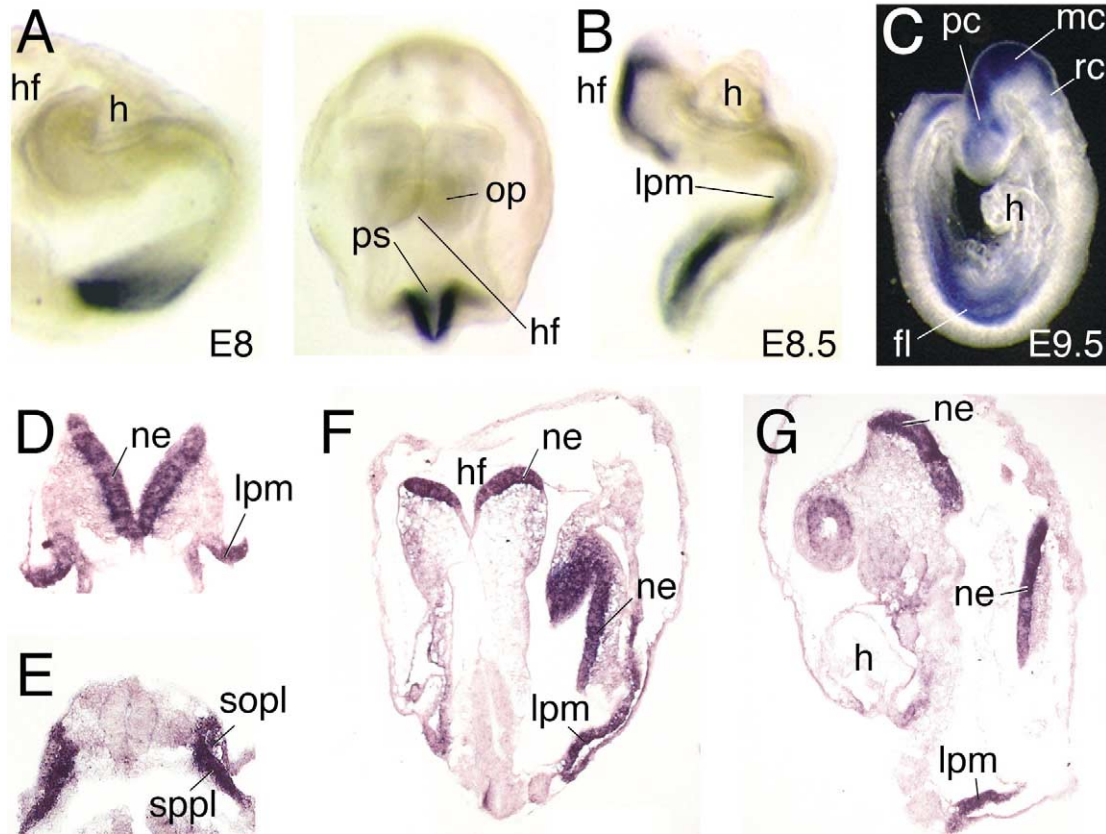
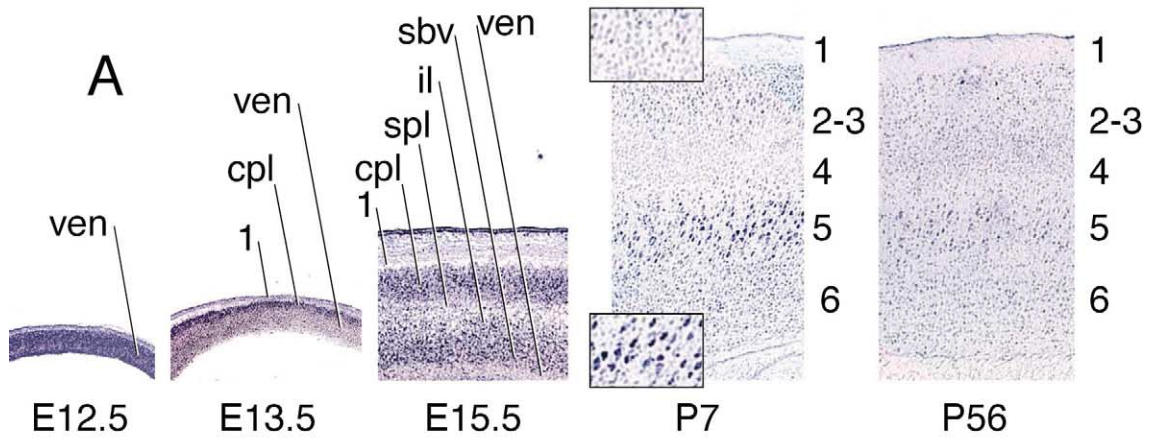


Fig. 1. Expression of *mLiX1* in early stages of mouse development from E8 until E9.5. (A) Expression in the primitive streak region in an 8-day-old embryo, lateral view. (A') presenting the same embryo, frontal view. (B) *mLiX1* expression in an E8.5 embryo, transcripts are visible in the headfold as well as in the primitive streak region (note that the medial break in the lateral plate mesoderm expression is an effect of the perspective, *mLiX1* is expressed along the entire future forelimb region). (C) Expression in an E9.5 embryo. (D,E) Transverse section of an E8.5 old embryo at tail and trunk level, respectively. (D) *mLiX1* is expressed in the neuroepithelium of the primitive streak region and in the lateral plate mesoderm. (E) After the lateral plate mesoderm has divided into somatopleure and splanchnopleure, *mLiX1* transcripts are detectable in both compartments. (F,G) horizontal and sagittal sections of an E8.5 old embryo. Abbreviations: fl, forelimb bud; h, heart; hf, headfold; lpm, lateral plate mesoderm; mc, mesencephalon; op, optic placode; ne, neuroepithelium; pc, prosencephalon; rc, rhombencephalon; sopl, somatopleure; sppl, splanchnopleure.

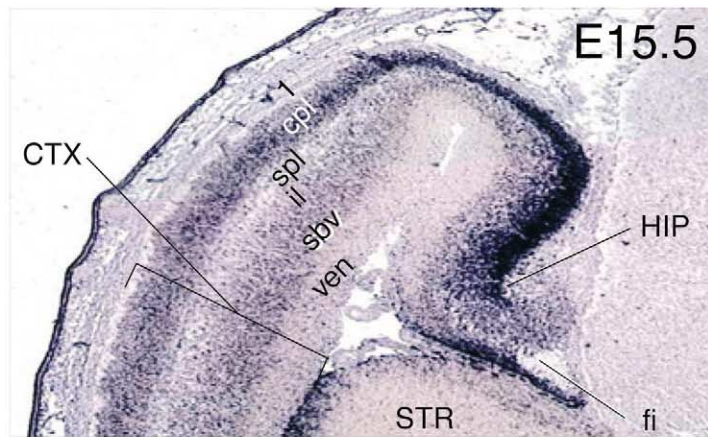
Expression in the embryonic midbrain is complex. By E14.5, the midline neuroepithelium showed expression in the tegmentum and in the presumptive inferior colliculus (not shown). Expression of *mLiX1* in the future substantia nigra was first evident at E14.5 in the neuroepithelium of the tegmentum at the border between diencephalon and mesencephalon (not shown). From E15.5 on *mLiX1* expression became prominent in the substantia nigra pars compacta (SNc) and in the ventral tegmental area (VTA) (Fig. 3A). Expression in the SNc was very similar to that of tyrosine hydroxylase [1] and *Nurr1* [5] (Fig. 3A), although the *mLiX1* expression domain was narrow. *mLiX1* expression in VTA and SNc persisted through postnatal stages (Fig. 2C) and into adulthood (not shown).

In the rhombencephalon *mLiX1* transcripts were observed in a cell population rostradorsal to the cerebellum (Fig. 3B, asterisk), from E14.5 to adulthood; in the motor nuclei of the fifth and the seventh cranial nerves (Fig. 3B) as well as in the trigeminal and the glossopharyngeal ganglia (Fig. 3C). At E14.5, there was strong expression in the presumptive differentiating cranial nerve nuclei (probably hypoglossal and vagus, not shown) at the border between medulla oblongata and spinal cord. By the same age, the floor of the fourth ventricle was labeled along the midline; in lateral sections, the neuroepithelium corresponding to the rhombic lip showed expression, continuous with the mesencephalic neuroepithelial domain (see above). *Lix1* was expressed in the spinal cord and in the

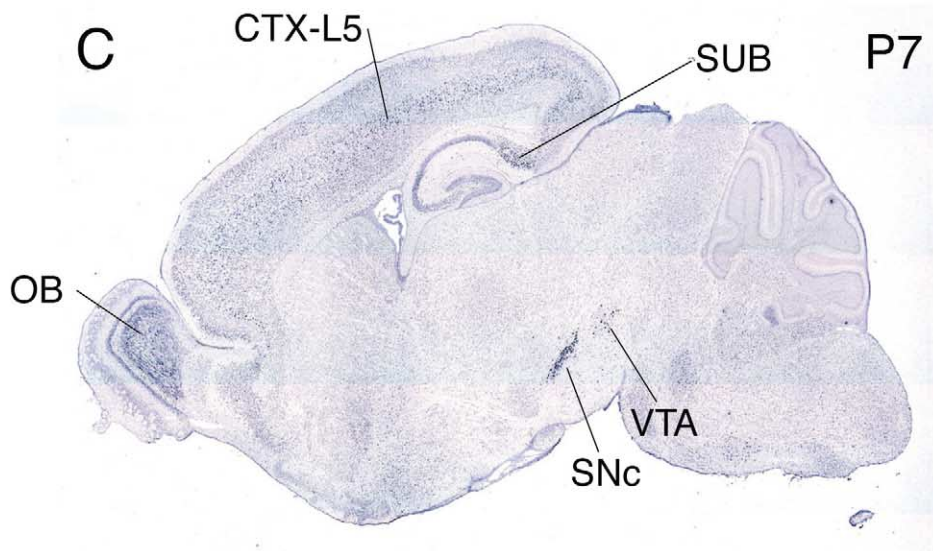
Fig. 2. Expression of *mLiX1* in the developing cerebral cortex. (A) Successive stages of cortical development from E12.5 on. (B) Enlarged view of the caudal cortex and the hippocampus of an E15.5 brain. *mLiX1* is detected in the cortical plate and the subventricular zone of the cortex as well as in the hippocampus. (C) Sagittal section of a P7 brain. *mLiX1* is expressed in layer 5 of the cortex, the subiculum, the olfactory bulb and the pars compacta of the substantia nigra. Abbreviations: cpl, cortical plate; CTX, cortex; CTX-L5, layer 5 of the cortex; fi, fimbria; HIP, hippocampus; il, intermediate layer; OB, olfactory bulb; ppl, preplate; sbv, subventricular layer; SNc, substantia nigra pars compacta; spl, subplate; ven, ventricular layer; STR, striatum; numbers resemble the according cortical layers.

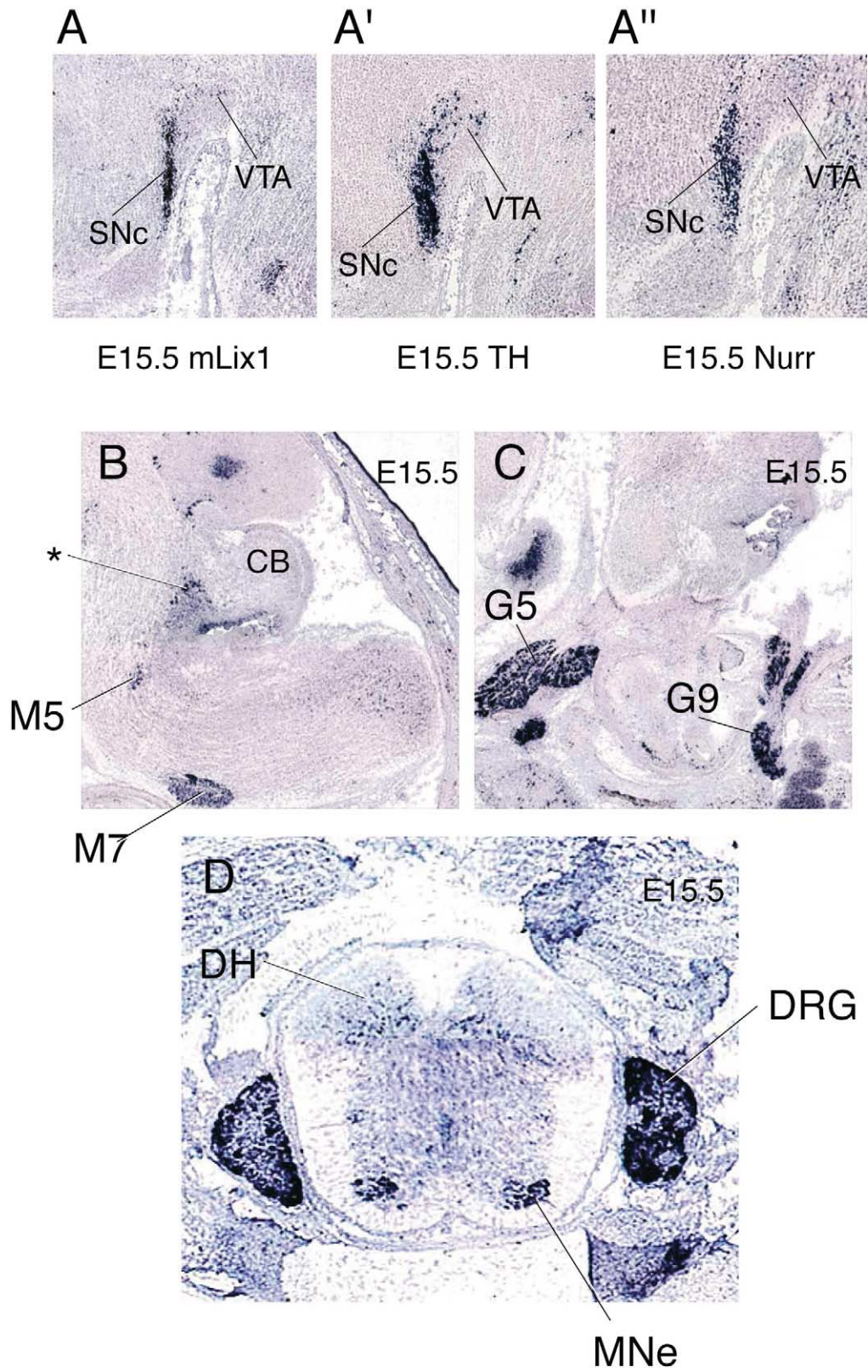


B



C





dorsal root ganglia, initially only weakly but expression increased from E12.5 onward and persisted through development (Fig. 3D and data not shown). In the spinal cord expression was restricted to a subset of motoneurons and dorsal horn interneurons (Fig. 3D).

Outside the CNS and PNS *mLix1* was expressed in the lateral plate mesoderm from E8.5 on (Fig. 1B,D–G), in fore- and hindlimb buds (E9.5–E11) (Fig. 1C and not shown), and in condensing cartilage of limbs in older stages (not shown). In the developing face *mLix1* was restricted to the maxillary component of the first brachial arch, the medial and the lateral nasal processes in later stages. Starting at E12.5, *mLix1* is also expressed prominently by the lens and the genital tubercle (not shown).

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Fig. 3. Expression of *mLix1* in the substantia nigra, the hindbrain, and the peripheral nervous system at E15.5. (A) Comparison of *mLix1* expression (left panel) with the known nigrostriatally expressed genes tyrosine hydroxylase (TH) (A') and Nurr1 (Nurr) (A"). (B) Expression in the E15.5 in the motornuclei of the fifth and seventh cranial ganglia and in an unknown cell population rostral to the developing cerebellum (see asterisk and text). (C) *mLix1* is transcribed in the trigeminal ganglion and in the glossopharyngeal ganglion. (D) Expression of *mLix1* in the spinal cord. At stage E15.5 spinal cord expression is detectable in some motor neurons in the ventral horn as well as in some interneurons in the dorsal horn of the spinal cord. This expression starts at E12.5 (see text). Abbreviations: CB, cerebellum; DH, dorsal horn; DRG, dorsal root ganglion; G5, ganglion of the fifth cranial nerve/trigeminal ganglion; G9, ganglion of the ninth cranial nerve/glossopharyngeal ganglion; M5, motor nucleus of the fifth cranial ganglion; M7, motor nucleus of the seventh cranial ganglion; MNe, motor neurons; SNc, substantia nigra pars compacta; VTA, ventral tegmental area.