

Short communication

Reelin is expressed in the accessory olfactory system, but is not a guidance cue for vomeronasal axons

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Abstract

Reelin is an extracellular matrix protein that regulates neuronal migration in the developing cerebral cortex, and axon outgrowth in the hippocampus. In the developing vomeronasal system, Reelin mRNA is expressed in perineural cells near the vomeronasal nerve, as well as in the vomeronasal organ, olfactory epithelium and olfactory and accessory olfactory bulbs, suggesting that it might regulate axon guidance or fasciculation. We tested that hypothesis by crossing *reeler* mice with VN12-IRES-tau-lacZ mice to investigate the role of reelin. The vomeronasal nerves are indistinguishable in normal and *reeler* mutant mice, strongly suggesting that Reelin does not provide a guidance cue for vomeronasal axons.

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The autosomal recessive mouse mutant, *reeler*, has been used as a model for neuronal development for over 50 years. Characteristics of the mutant include abnormal lamination of the cerebral, cerebellar, and hippocampal cortices and neuronal ectopia in several brainstem nuclei [12]. The mice display motor dysfunctions including tremor, dystonia, and ataxia [12]. The gene that is mutated in *reeler* mice, Reelin, encodes an extracellular matrix protein that is expressed in a number of regions in the developing mouse central and peripheral nervous systems. Reelin has been most widely studied for its role in neuronal migration. However, other work indicates that Reelin may function in guiding elongating axons in the hippocampus [3,6–8]. On the other hand, *in vitro* studies have not revealed a direct axonal guidance role for recombinant Reelin protein [11], so the issue is still somewhat unclear. Ikeda and Terashima [10] observed

prominent Reelin expression along the optic nerve and olfactory nerve fibers. Since Reelin expression has been observed along the olfactory nerve and there is strong expression in the olfactory bulb it seems likely that Reelin could function as an axon guidance molecule in the olfactory system.

There are two distinct chemosensory systems that comprise the olfactory system of most vertebrates. Primary sensory neurons within the main olfactory epithelium relay odor information to secondary neurons in the main olfactory bulb (MOB). Neurons in four distinct zones of the main olfactory epithelium project axons to four specific zones in the MOB. Pheromone-responsive sensory neurons from the vomeronasal organ (VNO) relay information to secondary neurons in the accessory olfactory bulb (AOB). Neurons from the VNO project axons to distinct regions in the AOB. Recent work describes the role of neuropilin-2 and class 3 secreted semaphorins in the patterning of vomeronasal sensory neuron projections to their targets in the AOB [4]. There are likely other guidance molecules responsible for the patterning in the vomeronasal sensory

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pathway. Reelin seems to be a likely candidate based on its expression pattern and its known role as a guidance cue. By breeding *reeler* mice with VN12-IRES-tau-lacZ mice [1] we were able to visualize a subpopulation of the projections of VNO neurons in the brain of *reeler* mutant mice. The projections and targeting of these neurons in the mutants were indistinguishable from normal, suggesting that even though Reelin is expressed in the olfactory system it may not be involved in axon path finding.

Mice were treated and housed according to protocols approved by the Harvard Medical School Institutional Animal Care and Use Committees. VN12-IRES-tau-lacZ mice [1] were bred with heterozygous B6 mice carrying the *reeler* mutation (The Jackson Laboratory). Initially homozygous VN12-IRES-tau-lacZ mice were bred with heterozygous *reeler* mice to produce double heterozygous mice. Then, double heterozygous mice were mated so that mice carrying both the *reeler* mutation and the VN12-IRES-tau-lacZ locus were generated for analysis. The genotype of these mice was verified by PCR. Genotyping for the VN12-IRES-tau-lacZ locus was completed using

two sets of primers. One set amplifies a 1.2-kb band if the VN12 WT is present (VN12F 5': GCCATCATCCTCAGTCCC) and (VN12R 5': CAGAGAAGTCATACATTTTAGGTTG). The other set amplifies a 1.0 kb band if lacZ is present (LacZF 5': TGTTCCGTCATAGCGATAACGAG) and (LacZR 5': AGATGGCGATGGCTGGTTTC). Genotyping of the *reeler* locus was determined as described [5].

Five *reeler*/VN12-IRES-tau-lacZ mice and three wild-type/VN12-IRES-tau-lacZ mice were analyzed. The mice were sacrificed at 4–8 weeks of age by perfusion with 4% paraformaldehyde–PBS. Whole-mount staining was performed as described [1,13].

In situ hybridization was performed by the In situ hybridization core at Beth Israel Deaconess Medical Center. Non-radioactive in situ hybridization was performed as described [2] using digoxigenin (DIG)-labeled cRNA probes. Probes were generated from the following: *Reln* 0.7 kb from the 3' end of *Reln* cDNA sequence.

Whole-mount images were acquired using a Zeiss dissecting microscope. In situ hybridization images were

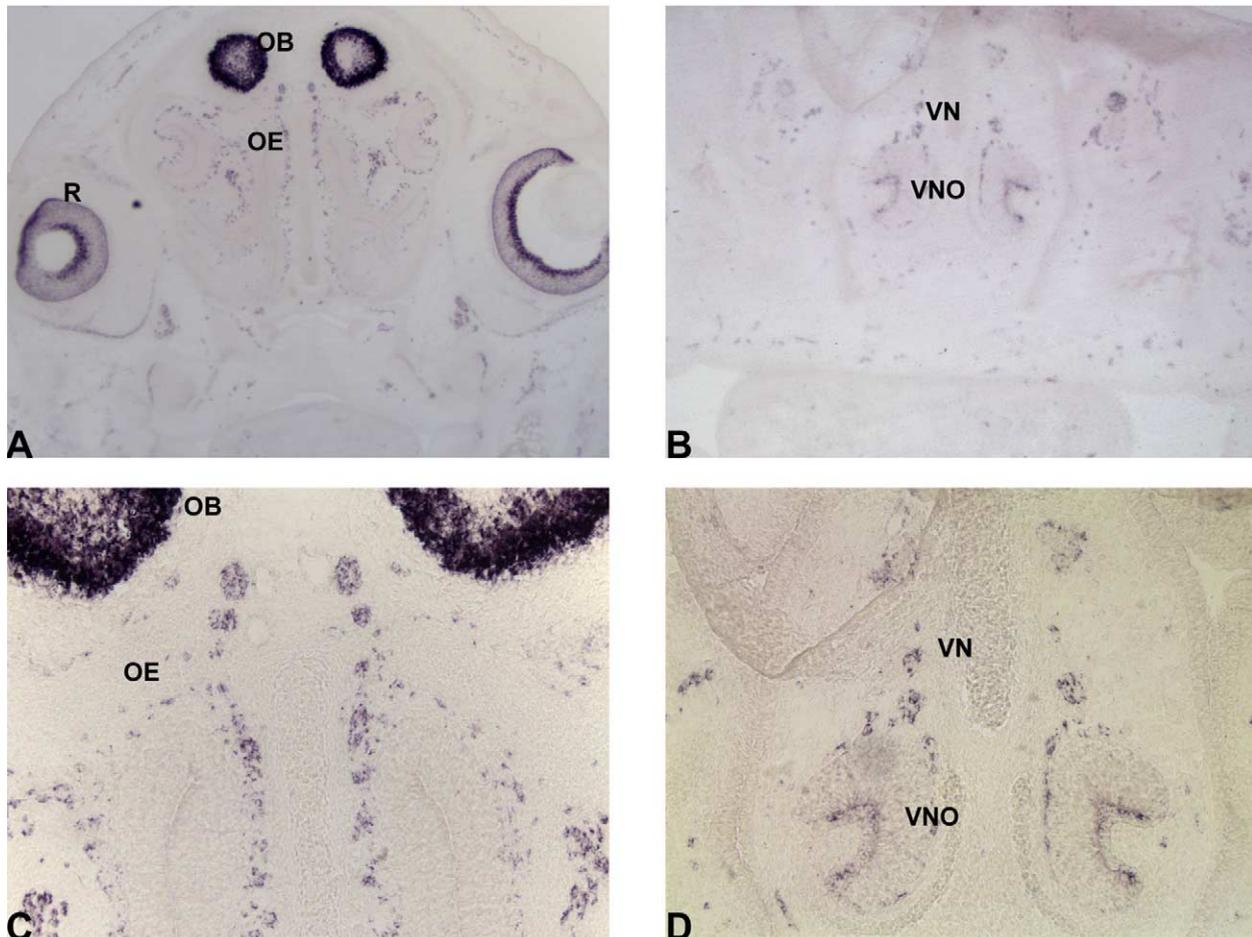


Fig. 1. *Reln* mRNA expression at E16. Coronal sections of an E16.5 mouse head probed with *Reln* mRNA where (A) and (C) show *Reln* expressed in the olfactory bulbs and olfactory epithelium and (B) and (D) show *Reln* expressed around the vomeronasal organ and the vomeronasal nerve. (C) is a higher magnification of the boxed area in (A) and (D) is a higher magnification of the boxed area in (B). OB, olfactory bulb; OE, olfactory epithelium; VNO, vomeronasal organ; VN, vomeronasal nerve; and R, retina.

acquired using a Zeiss light microscope. Figures were prepared using Adobe Photoshop.

Reln mRNA is expressed in the mouse olfactory system at E16.5 (Fig. 1). *Reln* is strongly expressed in the olfactory bulbs and olfactory epithelium (Fig. 1A,C). As described previously [10], high levels of *Reln* mRNA are present in the olfactory nerve layer of the olfactory bulb and accessory olfactory bulb. In addition, a number of

connective tissue cells presumably corresponding to perineural cells, express *Reln* mRNA. These cells lie near the olfactory epithelium and along the entire length of the olfactory nerve. Similar patterns of expression of *Reln* are seen in relation to the vomeronasal organ, and along the route of the vomeronasal nerve (Fig. 1B,D).

Despite the widespread expression of *Reln* mRNA along the length of the olfactory and vomeronasal systems, *reeler*

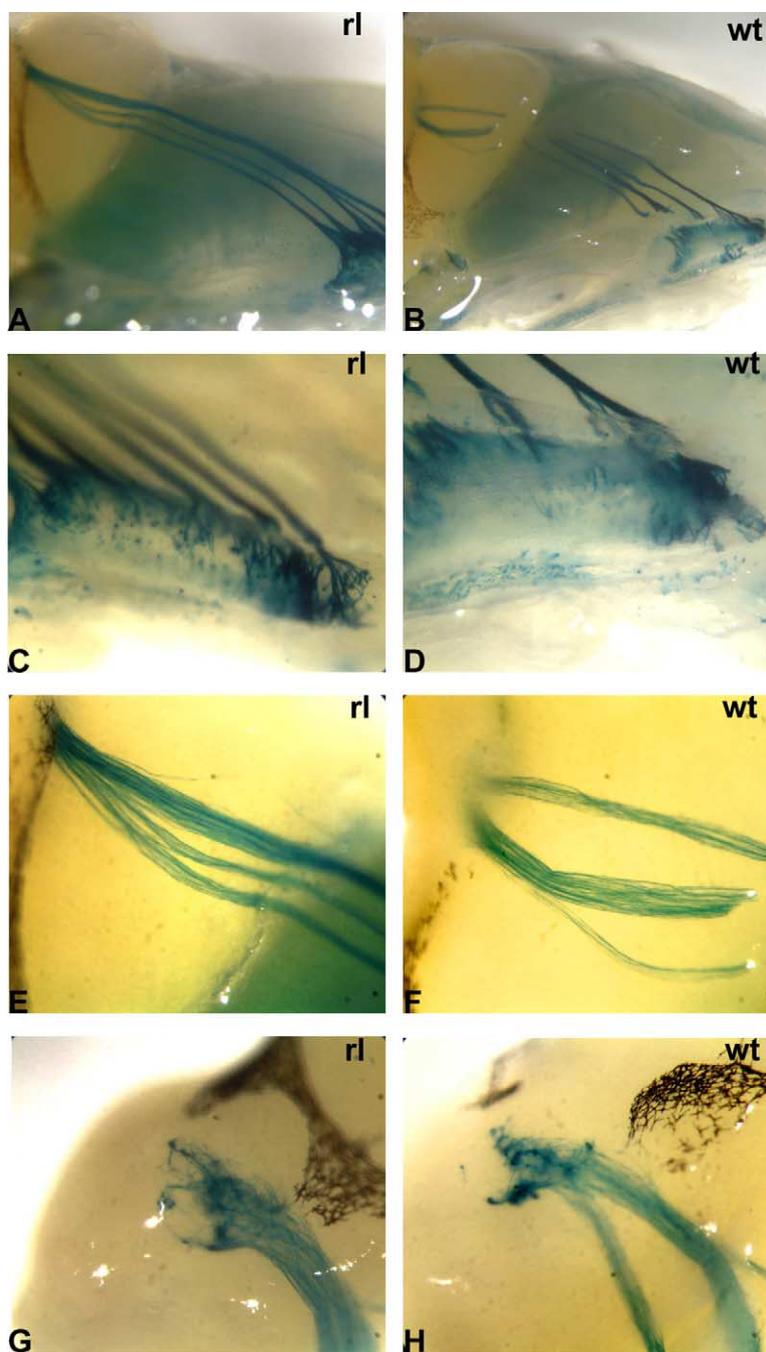


Fig. 2. Axonal projections from VN12-IRES-tau-lacZ expressing neurons in *reeler* and normal animals. Whole mount views of *reeler* (rl) (A,C,E,G) and wild-type (wt) (B,D,F,H) neurons stained with X-Gal. (A,B) Projections of neurons from the VNO, along the nasal septum, and across the MOB. (C,D) Neurons originating in the VNO. (E,F) Axons projecting across the MOB and into the AOB. (G,H) Axons converging in the AOB. There is no difference between the *reeler* and the wild-type animals. VNO, vomeronasal organ; MOB, main olfactory bulb; and AOB, accessory olfactory bulb.

mutants showed patterns of vomeronasal nerve labeling that were not obviously different from controls (Fig. 2). In *reeler* mutants and wild-type mice, vomeronasal sensory neurons labeled by the VN12-IRES-lacZ transgene originate in the VNO and soon after leaving the VNO form bundles of axons. Initially there are between 16 and 25 bundles that converge and form between three and five bundles as the neurons project across the nasal septum towards the olfactory bulb. When the axons cross the main olfactory bulb they converge into two or three bundles that terminate in specific regions of the accessory olfactory bulb. In the *reeler* mice the vomeronasal sensory neuron projections are indistinguishable from the wild-type animals (Fig. 2A,C,E,G versus B,D,F,H). The pathfinding, axonal fasciculation, and axonal convergence of the VN12 neurons appear to be normal in the *reeler* mice.

Reln mRNA is expressed at varying levels in the mouse olfactory system. Our findings, as well as previous findings, indicate that *Reln* is strongly expressed in the olfactory bulb and olfactory epithelium. *Reln* is also expressed along the VN and surrounding the VNO. After examining a subunit of neurons in the vomeronasal sensory pathway of *reeler* mice it appears that Reelin is not involved in axon pathfinding, axonal fasciculation, or axonal convergence. The data suggest that there is no significant difference between *reeler* and wild-type mice and that even though Reelin is present in the olfactory system it does not act as a guidance cue.

Reelin is most widely studied for its role in neuronal migration and organization of the cerebral cortex. However, recent data indicates that Reelin may act as a guidance cue in other parts of the central nervous system. Del Rio et al. [6] suggest that Reelin is essential for the formation of layer-specific hippocampal connections. Reelin acts as a guidance cue for the targeting of various fibers in the hippocampus [3,6–8]. Yip et al. [14] suggest that Reelin affects neuronal migration outside of the brain. Their data show that during development migration of sympathetic preganglionic neurons is dependent on Reelin signaling. In both of these cases Reelin protein is recognized adjacent to the migrating neurons or fibers, yet they do not overlap. Our data, as well as Ikeda and Terashima's [10] data, show that Reelin is expressed along the olfactory nerve without overlapping the nerve. However, Reelin does not appear to be a cue for the targeting of vomeronasal neurons [11].

Neuropilin-2 has been indicated to play an important role in the wiring and patterning of sensory neurons in the accessory olfactory system [4]. Neuropilin-2 binds to a subunit of class 3 semaphorin holoreceptors and the family of semaphorins has been implicated in the guidance of growing axons [4]. Thus, Npn-2 was a likely candidate for mediating axon wiring and pathfinding in the olfactory system. Reelin is not as well established as a guidance molecule and it appears that it is not essential for the wiring and targeting of the sensory neurons in the vomeronasal olfactory system.

What is the role of Reelin in the olfactory system? It is present at varying levels in the olfactory bulb, olfactory epithelium, vomeronasal organ, and along the vomeronasal nerve. It is known that Reelin is necessary for the development of the cerebral cortex that includes the cortex of the olfactory bulbs. However, the question remains as to why Reelin is expressed outside of the brain and within the olfactory system. It is possible that Reelin could play a role in fasciculation of the neurons or that Reelin's expression is not related to axon guidance. For example, humans with *RELN* gene mutations have evidence for defects in lymphatic development [9], so it is possible that expression of Reelin outside of the nervous system may relate to vascular or lymphatic development. Further investigation is necessary in order to understand the significance of Reelin within the accessory olfactory system.

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