EXTENDED PDF FORMAT
SPONSORED BY

BIO-RAD

http://discover.bio-rad.com

Anterior-Posterior Guidance of Commissural Axons by Wnt-Frizzled Signaling

Anna I. Lyuksyutova, *et al.*

Science **302**, 1984 (2003);
DOI: 10.1126/science.1089610

The following resources related to this article are available online at www.sciencemag.org (this information is current as of March 30, 2007):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:
http://www.sciencemag.org/cgi/content/full/302/5652/1984

Supporting Online Material can be found at:
http://www.sciencemag.org/cgi/content/full/302/5652/1984/DC1

A list of selected additional articles on the Science Web sites **related to this article** can be found at:
http://www.sciencemag.org/cgi/content/full/302/5652/1984#related-content

This article **cites 15 articles**, 5 of which can be accessed for free:
http://www.sciencemag.org/cgi/content/full/302/5652/1984#otherarticles

This article has been **cited by** 99 article(s) on the ISI Web of Science.

This article has been **cited by** 29 articles hosted by HighWire Press; see:
http://www.sciencemag.org/cgi/content/full/302/5652/1984#otherarticles

This article appears in the following **subject collections**:
Neuroscience
http://www.sciencemag.org/cgi/collection/neuroscience

Information about obtaining **reprints** of this article or about obtaining **permission to reproduce this article** in whole or in part can be found at:
http://www.sciencemag.org/about/permissions.dtl

Downloaded from www.sciencemag.org on March 30, 2007

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005. Copyright c 2003 by the American Association for the Advancement of Science; all rights reserved. The title SCIENCE is a registered trademark of AAAS.

using kynurenic acid, a different rapidly equilibrating antagonist. As for γ-DGG, the fractional block of the EPSP by kynurenic acid was not significantly different in 2 mM Ca^{2+} and 1.25 mM Ca^{2+} (55 ± 3% and 53 ± 6%, respectively; $P = 0.61$; $n = 4$; Fig. 4D). Our results contrast with the substantial increase in the level of block of excitatory postsynaptic currents (EPSCs) by γ-DGG and kynurenic acid at the climbing fiber, as the release probability was lowered (*7*) from a similar initial value (*11*). The fact that rapidly equilibrating competitive antagonists are equally effective at blocking the EPSP when P_R was reduced by a factor of 4 suggests that the concentration of transmitter in the synaptic cleft, following release, is independent of P_R at L4 to L2/3 synapses. These results indicate that each excitatory synaptic contact releases a single quantum of glutamate (probably one vesicle but could be a group of vesicles whose number is independent of P_R) and operates independently, in an all-or-none manner.

The high P_R at L4 to L2/3 synapses is well suited to promote reliable transmission of the one (76%) or two spikes that whisker stimulation usually evokes in L4 cells in vivo (*25*), because short-term depression becomes significant during longer bursts of L4 activity [Fig. 4A and (*9*)]. The depression of high P_R synapses, together with the precise synaptic latency between L4 and L2/3 (1 to 3 ms) and rapid EPSP kinetics (*9*), may also be important for maintaining and strengthening L4 to L2/3 synapses, because this connection exhibits a spike-timing–based plasticity rule with a narrow coincidence window (*26*). Curiously, the small numbers of release sites, all-or-none uniquantal signaling and small quantal size are likely to endow an individual L4 to L2/3 connection with a relatively low capacity for transmitting information. These properties contrast with synapses in the retina, where large numbers of release sites (~200) and graded transmission allow high information transmission rates (*27*). Moreover, transmission is finely tuned because only 10% of the 300 to 400 spiny stellate cells that innervate an individual L2/3 pyramidal cell fire synchronously, within 15 ms of single whisker stimulation (*25*), close to the minimum number required for a pyramidal cell to reach voltage threshold (*9*). Our results suggest that the functional and anatomical properties of L4 to L2/3 synaptic connections are well adapted for efficient, timing-based distributed signaling.

References and Notes

1. B. Katz, *The Release of Neural Transmitter Substances* (Liverpool Univ. Press, Liverpool, 1969).
2. D. Vere-Jones, *Aust. J. Stat.* **8**, 53 (1966).
3. A. Zador, *J. Neurophysiol.* **79**, 1219 (1998).
4. M. London, A. Schreibman, M. Häusser, M. E. Larkum, I. Segev, *Nature Neurosci.* **5**, 332 (2002).
5. H. Korn, A. Triller, A. Mallet, D. S. Faber, *Science* **213**, 898 (1981).
6. A. I. Gulyas *et al.*, *Nature* **366**, 683 (1993).
7. J. I. Wadiche, C. E. Jahr, *Neuron* **32**, 301 (2001).
8. T. G. Oertner, B. L. Sabatini, E. A. Nimchinsky, K. Svoboda, *Nature Neurosci* **5**, 657 (2002).
9. D. Feldmeyer, J. Lübke, R. A. Silver, B. Sakmann, *J. Physiol. (London)* **538**, 803 (2002).
10. Methods, table S1, and references are available as supporting online material on *Science* Online.
11. R. A. Silver, A. Momiyama, S. G. Cull-Candy, *J. Physiol. (London)* **510**, 881 (1998).
12. J. D. Clements, R. A. Silver, *Trends Neurosci* **23**, 105 (2000).
13. C. A. Reid, J. D. Clements, *J. Physiol. (London)* **518**, 121 (1999).
14. J. M. Bekkers, J. D. Clements, *J. Physiol. (London)* **516**, 227 (1999).
15. R. A. Silver, S. G. Cull-Candy, T. Takahashi, *J. Physiol. (London)* **494**, 231 (1996).
16. M. A. Castro-Alamancos, B. W. Connors, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 4161 (1997).
17. V. N. Murthy, T. J. Sejnowski, C. F. Stevens, *Neuron* **18**, 599 (1997).
18. H. Markram, J. Lübke, M. Frotscher, A. Roth, B. Sakmann, *J. Physiol. (London)* **500**, 409 (1997).
19. J. Lübke, A. Roth, D. Feldmeyer, B. Sakmann, *Cereb. Cortex* **13**, 1051 (2003).
20. J. J. Jack, S. J. Redman, K. Wong, *J. Physiol. (London)* **321**, 65 (1981).
21. P. Jonas, G. Major, B. Sakmann, *J. Physiol. (London)* **472**, 615 (1993).
22. R. A. Silver, in *Central Synapses: Quantal Mechanisms and Plasticity*, D. S. Faber, H. Korn, S. J. Redman, S. M. Thompson, and J. S. Altman, Eds., 4th Human Frontier Science Workshop, Strasbourg, France, 22 to 24 April 1997 (Human Frontier Science Program, Strasbourg, France, 1998), pp. 130–139.
23. K. A. Foster, A. C. Kreitzer, W. G. Regehr, *Neuron* **35**, 1115 (2002).
24. J. S. Diamond, C. E. Jahr, *J. Neurosci.* **17**, 4672 (1997).
25. M. Brecht, B. Sakmann, *J. Physiol. (London)* **543**, 49 (2002).
26. D. E. Feldman, *Neuron* **27**, 45 (2000).
27. S. B. Laughlin, R. R. de Ruyter van Steveninck, J. C. Anderson, *Nature Neurosci.* **1**, 36 (1998).

28. Supported by the Max-Planck Society and the Wellcome Trust. R.A.S. is in receipt of a Wellcome Trust Senior Fellowship. We thank A. Roth and C. Saviane for help with simulations; D. Attwell, D. DiGregorio, M. Farrant, S. Mitchell, T. Nielsen, and I. Vida for comments on the manuscript; and N. Nestel and B. Joch for technical assistance.

Supporting Online Material

www.sciencemag.org/cgi/content/full/302/5652/1981/DC1
Materials and Methods
Table S1
References

23 May 2003; accepted 15 October 2003

Anterior-Posterior Guidance of Commissural Axons by Wnt-Frizzled Signaling

Anna I. Lyuksyutova,[2] Chin-Chun Lu,[1] Nancy Milanesio,[1] Leslie A. King,[2] Nini Guo,[4] Yanshu Wang,[4] Jeremy Nathans,[4] Marc Tessier-Lavigne,[5]* Yimin Zou[1,2,3]†

Commissural neurons in the mammalian dorsal spinal cord send axons ventrally toward the floor plate, where they cross the midline and turn anteriorly toward the brain; a gradient of chemoattractant(s) inside the spinal cord controls this turning. In rodents, several Wnt proteins stimulate the extension of commissural axons after midline crossing (postcrossing). We found that *Wnt4* messenger RNA is expressed in a decreasing anterior-to-posterior gradient in the floor plate, and that a directed source of Wnt4 protein attracted postcrossing commissural axons. Commissural axons in mice lacking the Wnt receptor Frizzled3 displayed anterior-posterior guidance defects after midline crossing. Thus, Wnt-Frizzled signaling guides commissural axons along the anterior-posterior axis of the spinal cord.

Axonal connections are patterned along the anterior-posterior (A-P) and dorsal-ventral (D-V) neuraxes. Guidance molecules that play essential roles in the D-V guidance of axons have been identified, whereas the nature of the A-P guidance cues has remained an enigma (*1, 2*). The dorsal spinal cord commissural neurons form several ascending somatosensory pathways. During embryonic development, they project axons to the ventral midline (Fig. 1A). At the floor plate, commissural axons cross the midline, enter the contralateral side of the spinal cord, and make a sharp anterior turn toward the brain (Fig. 1B) (*3*). The initial ventral growth of the commissural axons is directed by a collaboration of two chemoattractants, netrin-1 (*4–6*) and Sonic hedgehog (Shh) (*7*), and chemorepellents of the bone morphogenetic protein (BMP) family (*8*). As the axons cross the midline, they lose responsiveness to these chemoattractants (*9*) but gain responsiveness to several chemorepellents, including Slit and semaphorin proteins (*10*), which guide axons from the D-V axis into the A-P axis (*10*).

To determine why axons turn in an anterior direction, we studied the turning of commissural axons after midline crossing in "open-book" spi-

[1]Department of Neurobiology, Pharmacology and Physiology, [2]Committee on Developmental Biology, [3]Committee on Neurobiology, University of Chicago, Chicago, IL 60637, USA. [4]Departments of Molecular Biology and Ophthalmology, Howard Hughes Medical Institute, Johns Hopkins Medical School, Baltimore, MD 21205, USA. [5]Department of Biological Sciences, Howard Hughes Medical Institute, Stanford University, Stanford, CA 94305, USA.

*Present address: Genentech Inc., South San Francisco, CA 94080, USA.
†To whom correspondence should be addressed. E-mail: yzou@bsd.uchicago.edu

Downloaded from www.sciencemag.org on March 30, 2007

nal cord explants in culture. We observed that when a segment of E13 (embryonic day 13) rat spinal cord was cultured in collagen gel, commissural axons projected ventrally cross the midline and turned anteriorly within the explant (Fig. 1E). We reasoned that if A-P guidance is controlled by a diffusible gradient of either an attractant(s) or a repellent(s), then cutting these open-book explants shorter might eliminate the gradient and lead to abnormal pathfinding along the A-P axis (Fig. 1C); if A-P guidance is controlled by a nondiffusible cue(s), commissural axons will still have the normal anterior turn in shorter explants, because the gradient will be maintained (Fig. 1D).

We systematically cultured open-book explants of different A-P lengths (3 mm to 0.5 mm) and found that at 0.5 mm, abnormal pathfinding of postcrossing commissural axons was observed, including knotting and stalling (Fig. 1F) and randomized turning along the A-P axis (Fig. 1G). In contrast, all axons turned anteriorly in 3-mm explants (Fig. 1E). In both short and long explants, commissural axon pathfinding from the dorsal spinal cord to the floor plate was normal. Only 18% of the injection sites in the short explants showed normal anterior turning, presumably due to the loss of guidance information; this finding suggests that the guidance cue(s) are diffusible.

To address whether the A-P guidance cue is attractive or repulsive, we injected 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI) focally into the dorsal spinal cord close to the anterior end, in the middle, and close to the posterior end (Fig. 1I) of long explants (3 mm or longer). Axons in the middle and close to the posterior end of the explants always projected anteriorly, whereas axons close to the anterior end almost always made mistakes, either stalling or projecting randomly along the A-P axis after midline crossing, similar to those in the short explants (0.5 mm). These results indicate that a gradient of an attractive cue(s) plays a role in the anterior turn of the postcrossing commissural axons (fig. S1). We further determined that a cut in the open-book explants did not prevent axons from projecting rostrally (fig. S2), indicating that the A-P gradient of the guidance cue(s) is preserved in such a preparation and a cut (damage) to the spinal cord itself does not produce a cue(s) to repel postcrossing commissural axons.

To identify the anterior turning signal, we tested candidate molecules by expressing them in COS cell aggregates positioned next to rat spinal cord explants of postcrossing commissural axons in collagen gels (*10*). Candidate molecules found in the limb bud were considered, because embryonic limb bud tissue stimulates extension of commissural axons only after midline crossing (*11*): hepatocyte growth factor (HGF), fibroblast growth factors 4 and 8 (FGF4 and FGF8), BMP4, BMP7, Shh, and Wnt1, Wnt4, Wnt5a, Wnt 6, and Wnt7b. Of these factors, only Wnt1 (*11*), Wnt4, Wnt5a, Wnt6 (*11*), and Wnt7b stimulated the extension of the postcrossing commissural axons (Fig. 2, B to D). HGF, the FGFs, the BMPs, and Shh did not show stimulation of postcrossing commissural axon growth in our assay (*11*). None of these Wnts affected the outgrowth of precrossing commissural axons (fig. S3).

In the presence of Wnt4, most growth cones of the postcrossing commissural axons (79%) became enlarged and complex (large arrow-

Downloaded from www.sciencemag.org on March 30, 2007

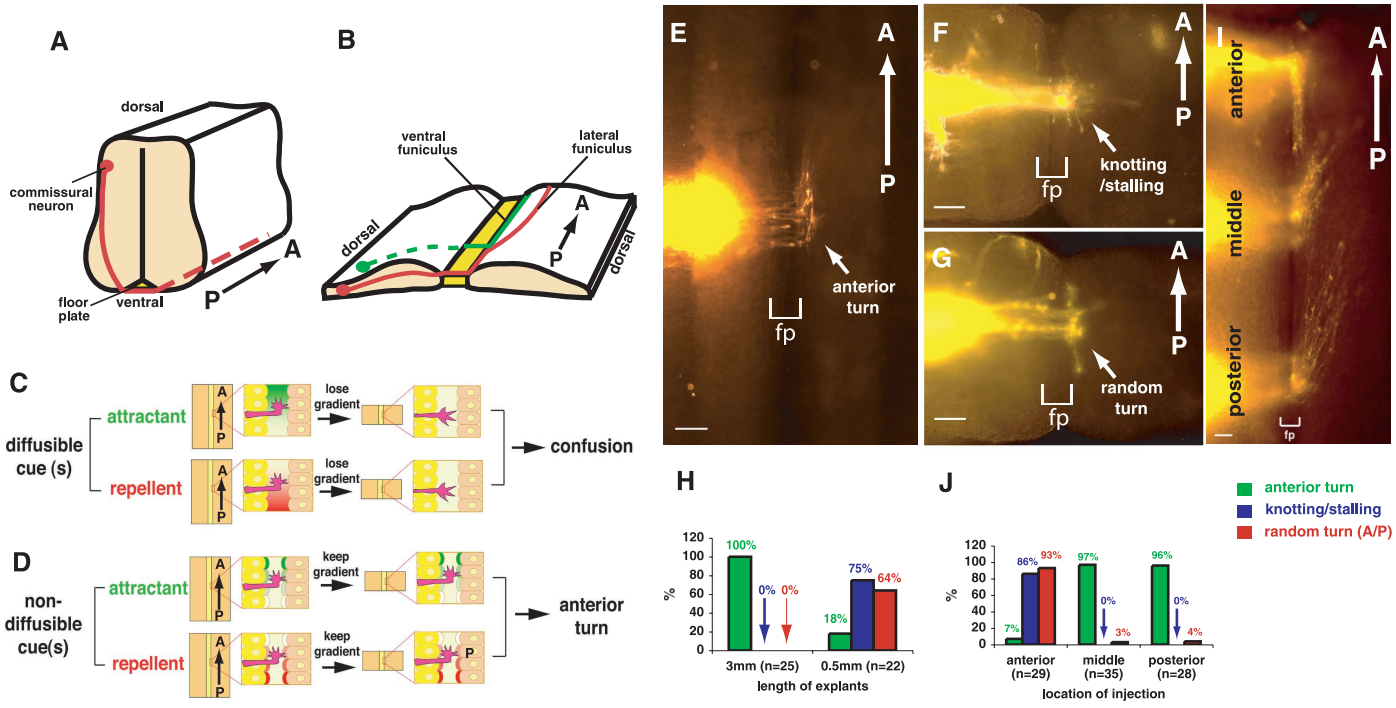

Fig. 1. Chemoattractant(s) guides commissural axons along the A-P axis. (**A**) Transverse section of E13 rat spinal cord showing the D-V trajectory (solid red line) and A-P trajectory (dashed red line) of commissural axons. (**B**) "Open-book" view of E13 rat spinal cord showing midline crossing and anterior turning. The green subpopulation of commissural axons projects anteriorly along a medial pathway, close to the floor plate (ventral funiculus). The red subpopulation projects along the floor plate initially but gradually fans out to occupy more lateral positions (lateral funiculus). Both populations project anteriorly immediately after midline crossing. (**C**) A gradient of diffusible guidance cue(s) might be disrupted when explants are cut shorter, causing misrouting of commissural axons along the A-P axis. (**D**) A gradient of nondiffusible guidance cue(s) will not be affected when the explants are cut shorter, and the axons should still project anteriorly. (**E**) In longer explants (3 mm), all postcrossing commissural axons projected anteriorly after midline crossing (fp, floor plate) after 16 to 20 hours of culture. Commissural axons were labeled by lipophilic DiI injection into the dorsal side of the explants by iontophoresis. (**F**) In shorter explants (0.5 mm), axons frequently stalled after midline crossing. (**G**) In shorter explants (0.5 mm), axons often projected randomly along the A-P axis. (**H**) Quantification of data. Anterior turn indicates normal projection. Knotting/stalling and random A-P turns are abnormal behaviors observed in shorter explants. *n* = number of explants. (**I**) A long explant (3 mm) was injected with DiI close to the anterior end, in the middle, and at the posterior end. The axons at anterior ends are almost always misrouted (~90%). (**J**) Quantification of the open-book assays with anterior, middle, and posterior injections. *n* = number of injection sites. Scale bars, 100 μm.

heads, Fig. 2, F and G). Recombinant secreted Frizzled-related proteins (sFRPs) are soluble proteins that can block the interaction of Wnts with their receptors, the Frizzleds (*12*); addition of sFRP2 reduced these effects within 1 hour (Fig. 2F). The percentage of large and complex growth cones was reduced to 50% after 1 hour, 37% after 2 hours, and 30% after 3 hours. In control cultures, 78% growth cones were small, regardless of whether sFRP2 protein was added (Fig. 2G). Although diffusion of molecules in collagen gel matrix is likely reduced compared

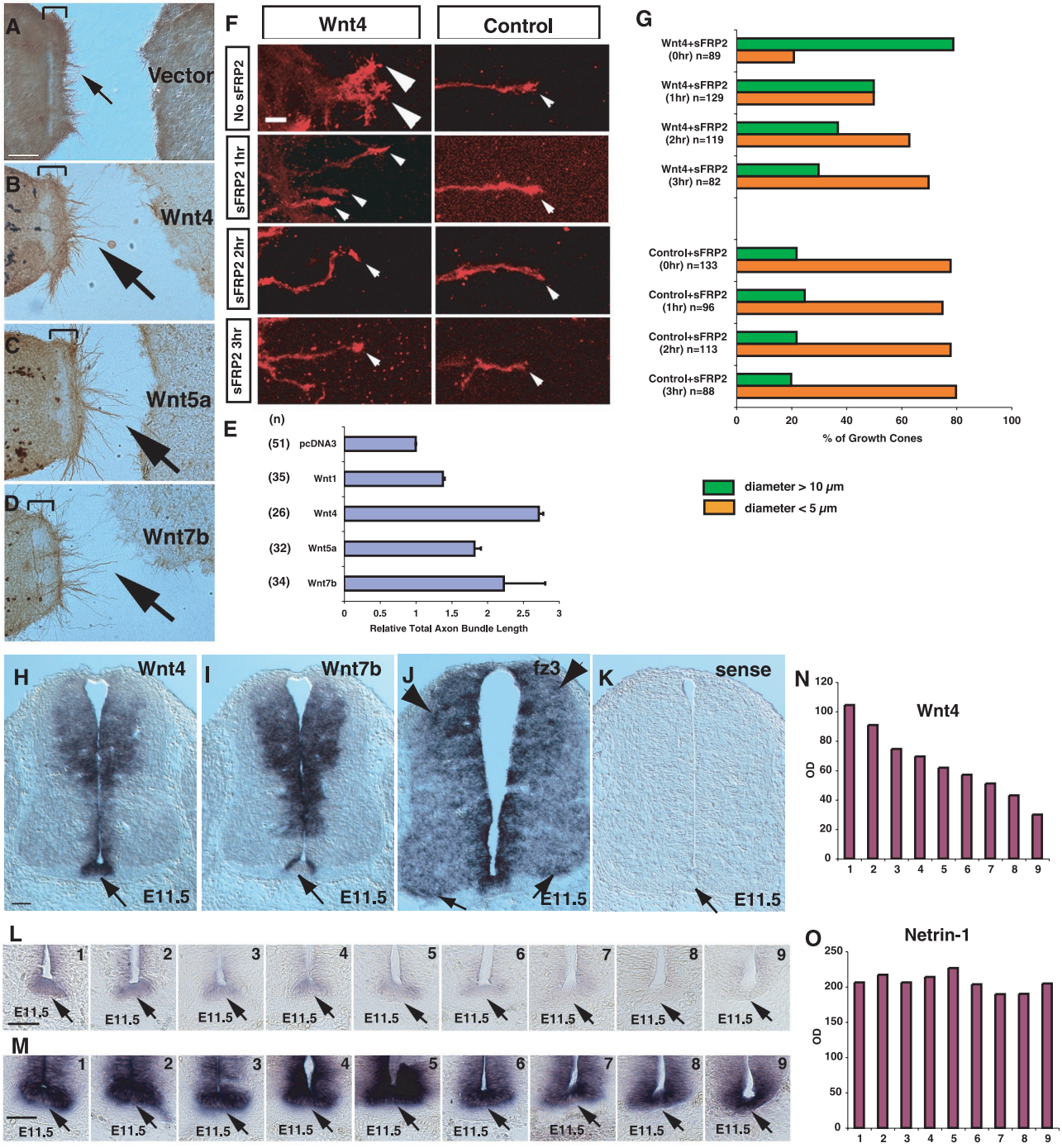

Fig. 2. Wnt proteins stimulate the extension of postcrossing commissural axons and expression of Wnts in the developing spinal cord. (**A** to **D**) Wnt proteins stimulated the extension of postcrossing commissural axons. (**E**) Quantification of postcrossing commissural axon extension stimulated by Wnts as described (*10*). (**F**) The growth cones of postcrossing commissural axons were enlarged and more complex in the presence of Wnt4. Large arrowheads indicate large and complex growth cones; small arrowheads indicate small and simple growth cones. Growth cones were stained with a rabbit antibody to GAP-43 and visualized in three dimensions by confocal microscopy. Addition of purified sFRP2 protein (0.2 μg/ml) reversed Wnt4-induced increase of growth cone size and complexity within 1 hour. Scale bar, 10 μm. (**G**) Quantification of growth cone morphology. Green bars indicate the percentage of large and complex growth cones (diameter ~10 μm). Orange bars indicate small and simple growth cones (diameter ≤5 μm). (**H** to **K**) In situ hybridization of E11.5 mouse transverse sections (scale bar, 100 μm). (**L**) Nine serial sections along the A-P axis at 400-μm intervals probed by *Wnt4*. Arrows indicate floor plate. Scale bar, 100 μm. (**M**) Nine serial sections along the A-P axis at 400-μm intervals probed by *netrin-1*. These sections are the corresponding adjacent sections to those shown in (L). Arrows indicate floor plate. Scale bar, 100 μm. (**N**) Quantification of signal intensity for Wnt4. (**O**) Quantification of signal intensity for netrin-1. OD was determined on the basis of signal density using NIH ImageJ (http://rsb.info.nih.gov/ij/index.html).

Downloaded from www.sciencemag.org on March 30, 2007

to that in culture medium, the blocking effect of sFRP2 was still observed within 1 hour, suggesting that Wnt proteins likely stimulate postcrossing commissural axon growth by directly and acutely acting on the growth cones.

We examined the expression pattern of Wnts by in situ hybridization in developing mouse embryos during the stages when commissural axons are turning anteriorly. At E11.5 (equivalent to E13 in the rat), *Wnt4*, *Wnt7b*, and *Wnt5a* (*11*) were found expressed in the areas where the postcrossing axons turn anteriorly. *Wnt4* was found specifically enriched in the floor plate and the ventricular zone (Fig. 2H), exhibiting a decreasing A-P gradient along the entire length of the floor plate from E10.5 (*11*) to E11.5 (Fig. 2L) and E13.5 (fig. S4). Netrin-1 did not display a decreasing A-P gradient (Fig. 2M). *Wnt7b* was found expressed on the two lateral margins of the floor plate, where anterior turning of postcrossing commissural axons occurs (Fig. 2I). *Wnt5a* was widely expressed in the spinal cord and was particularly abundant in the floor plate and the ventral areas of the spinal cord next to the lateral funiculus (*11*). Therefore, several Wnts are expressed in the correct place during development, with *Wnt4* (from E10.5 to E13.5) displaying an A-P decreasing gradient in the floor plate. A similar Wnt4b gradient in the floor plate was found in zebrafish embryos at similar stages (*13*).

The Wnt receptor *frizzled* genes *fz3*, *fz8*, and *fz9* were found expressed in the spinal cord from E9.5 to E13.5 during the time when commissural axons are making the anterior turn. *fz3* is expressed in the area where commissural neuron cell bodies are located (arrowheads, Fig. 2J) and in the ventral and lateral margins of the spinal cord where postcrossing commissural axons project longitudinally (arrows, Fig. 2J).

To test whether Wnts are required for the proper anterior turning of the postcrossing commissural axons, we used sFRPs to block Wnt function in the open-book explants. sFRP-expressing COS cells were embedded in the bottom layer of collagen gel, and long open-book spinal cord explants were placed on top and embedded in the top collagen gel. The efficacy of this experimental system was first tested using netrin-1–expressing COS cells included in the bottom collagen gel, which stimulated outgrowth of precrossing commissural axons from explants placed in top collagen gel (fig. S5). In the presence of any of the three sFRPs (sFRP1, sFRP2, and sFRP3) or a mixture of all three sFRPs, anterior turning of commissural axons after midline crossing was severely impaired (Fig. 3, B to D). Axons either stalled or turned randomly along the A-P axis, displaying behaviors similar to those observed in the short explants and the anterior injection sites in the long explants (Fig. 1, F, G, and I). In contrast, in the presence of the control COS cells, all commissural axons turned anteriorly after midline crossing (Fig. 3A). No abnormal pathfinding behavior was observed in the precrossing segment of the commissural axons, which suggests that the Wnt signaling pathway is not required for the D-V projection of the precrossing commissural axons.

To further test the role of Wnts in A-P guidance, we examined whether applying an ectopic anterior source of Wnt protein(s) could rescue the anterior turn of commissural axons after midline crossing. Wnt4-expressing COS cell aggregates that were placed anterior to the short explants attracted postcrossing commissural axons and rescued A-P guidance defects (Fig. 3G). COS cells transfected with vector only had no effect (Fig. 3F). Furthermore, Wnt4-expressing COS cells placed posteriorly to the short explants redirected axons to turn

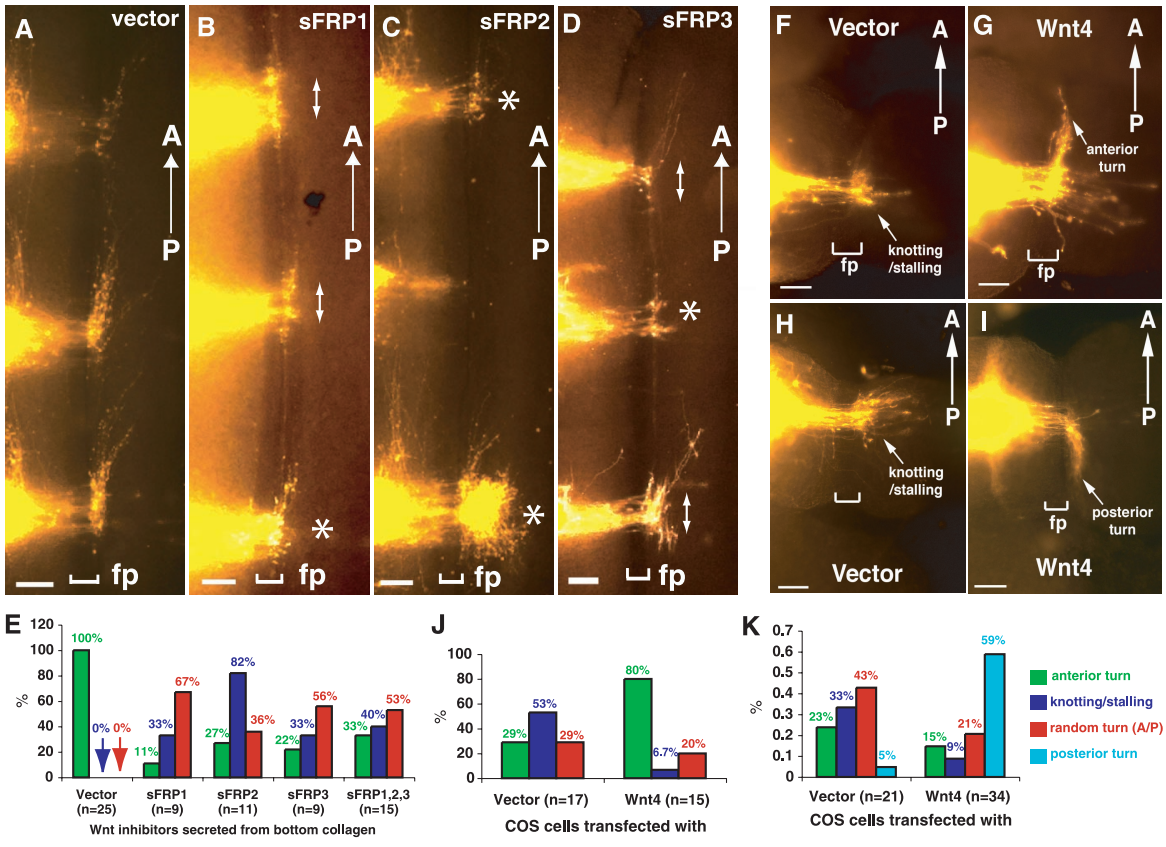

Fig. 3. Wnts are required for proper anterior turning of postcrossing commissural axons in open-book explants, and Wnt4 can act as an instructive axon attractant. (**A**) When COS cells transfected with vector only were resuspended and embedded in the bottom collagen gel, postcrossing commissural axons displayed normal anterior turning in the open-book explants. (**B** to **D**) When sFRP-expressing COS cells were embedded in collagen gel, commissural axons displayed severe defects in the A-P axis, including knotting/stalling (asterisks) and A-P random turns (double-headed arrows). (**E**) Quantification (as in Fig. 1) of effects of sFRP1, 2, and 3, alone or combined. *n* = number of injection sites. (**F**) Vector-only COS cells did not rescue guidance defects. (**G**) Wnt4-transfected COS cells attracted postcrossing commissural axons toward them when positioned on the anterior end of the explants, rescuing the A-P pathfinding defects. (**H**) Vector-only COS cells did not reorient commissural axons after midline crossing. (**I**) COS cells expressing Wnt4 reoriented the postcrossing commissural axons to project posteriorly when positioned on the posterior side of the explants. (**J**) Quantification (as in Fig. 1) of Wnt4 rescue experiments. (**K**) Quantification of the Wnt4 reorientation experiments. *n* = number of injection sites. Light blue bars indicate the percentage of the injection sites whereby all axons turned posteriorly. Scale bars, 100 μm.

Downloaded from www.sciencemag.org on March 30, 2007

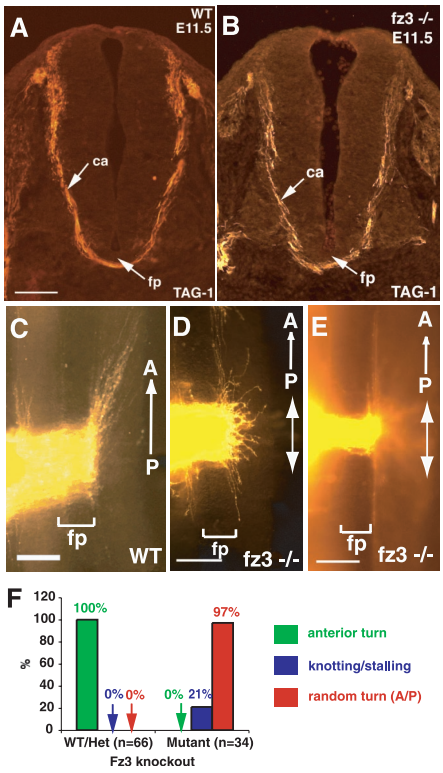

Fig. 4. Frizzled3 is specifically required for A-P guidance of postcrossing commissural axons. (**A**) D-V trajectory of precrossing commissural axons in a wild-type embryo, as revealed by TAG-1 mAb. (**B**) Normal D-V projection of precrossing commissural axons in *fz3* knockout embryos. (**C**) Commissural axons crossed the midline and turned anteriorly after midline crossing in an E11.5 wild-type littermate embryo. (**D**) Commissural axons reached the midline and crossed to the contralateral spinal cord but projected randomly along the A-P axis in an E11.5 *fz3* mutant embryo. These axons are oriented randomly immediately after midline crossing. (**E**) A-P guidance defects of postcrossing commissural axons in an E11.5 *fz3* mutant embryo slightly older than the one shown in (D). These axons extended further than those in (D). (**F**) Quantification of the postcrossing A-P guidance defects in *fz3* knockout mice. Four litters of *fz3* knockout mice were analyzed (three litters were analyzed in blinded experiments). A total of seven mutant embryos were analyzed. *n* = number of injection sites. Scale bars, 100 μm.

posteriorly (Fig. 3I), which suggests that Wnt4 is an instructive cue rather than a permissive cue. In both experimental conditions, postcrossing commissural axons projected along a restricted trajectory between the floor plate and the ventral spinal cord, even though the Wnt4 source was presented in a wider area because of the size of the COS cell aggregates. This suggests that other guidance cues shape the trajectory and keep commissural axons within the narrow corridor immediately after midline crossing. For instance, the Slit proteins and a subset of secreted semaphorins play a role in "squeezing" postcrossing commissural axons between the floor plate and the ventral spinal cord as they exit the midline (*10*).

Midline pathfinding behavior of commissural axons in *fz3* knockout embryos (*14*) was examined by DiI labeling and immunohistochemistry with a monoclonal antibody (mAb) to TAG-1, a commissural axonal marker present only in the precrossing and the midline-crossing segments of commissural axons. D-V projection of precrossing commissural axons was normal (Fig. 4B) compared to wild-type control (Fig. 4A), but postcrossing commissural axons projected randomly along the A-P axis after midline crossing with 100% penetrance along the length of the spinal cord (Fig. 4, D and E); this finding suggests that the Wnt-Frizzled pathway is specifically required for A-P axon guidance after midline crossing in vivo. No spinal cord patterning defects were observed in the *fz3* knockout mice at this stage of development, as assessed by markers such as Nkx2.2, HNF-3β, Lim2, and Isl1 (*14*). LRP6 is a coreceptor of Frizzleds for the canonical Wnt–β-catenin signaling pathway, required in patterning and cell fate determination. Both D-V and A-P pathfinding of commissural axons were normal in *LRP6*$^{-/-}$ embryos, although patterning defects such as spina bifida, truncation of axial skeleton, and loss of distal limb structures were observed in these mice (*15*), which suggests that LRP6 is not involved in the differentiation, D-V pathfinding, or A-P guidance decision of commissural axons at the midline (*11*).

The nature of the molecular guidance cues controlling A-P pathfinding has long remained a mystery. Our results show that a Wnt-Frizzled pathway controls the anterior turning of spinal cord commissural axons after midline crossing via an attractive mechanism. A recent study suggested that a Wnt5-Derailed pathway mediates axonal repulsion in regulating the pathway selection of a subset of axons before entering the fly midline (*16*). We found that the mouse Derailed homolog Ryk (a receptor tyrosine kinase–like molecule) is not expressed in the spinal cord commissural neurons (*11*), providing evidence that Wnt proteins can act as axonal attractants via a signaling pathway involving Frizzled3 but independent of Ryk/Derailed.

References and Notes

1. M. Tessier-Lavigne, C. S. Goodman, *Science* **274**, 1123 (1996).
2. B. J. Dickson, *Science* **298**, 1959 (2002).
3. P. Bovolenta, J. Dodd, *Development* **109**, 435 (1990).
4. T. Serafini *et al*., *Cell* **78**, 409 (1994).
5. T. E. Kennedy, T. Serafini, J. R. de la Torre, M. Tessier-Lavigne, *Cell* **78**, 425 (1994).
6. T. Serafini *et al*., *Cell* **87**, 1001 (1996).
7. F. Charron, E. Stein, J. Jeong, A. P. McMahon, M. Tessier-Lavigne, *Cell* **113**, 11 (2003).
8. A. Augsburger, A. Schuchardt, S. Hoskins, J. Dodd, S. Butler, *Neuron* **24**, 127 (1999).
9. R. Shirasaki, R. Katsumata, F. Murakami, *Science* **279**, 105 (1998).
10. Y. Zou, E. Stoeckli, H. Chen, M. Tessier-Lavigne, *Cell* **102**, 363 (2000).
11. A. I. Lyuksyutova, L. A. King, Y. Zou, data not shown.
12. A. Wodarz, R. Nusse, *Annu. Rev. Cell Dev. Biol.* **14**, 59 (1998).
13. A. Liu, A. Majumdar, H. E. Schauerte, P. Haffter, I. A. Drummond, *Mech. Dev.* **91**, 409 (2000).
14. Y. Wang, N. Thekdi, P. M. Smallwood, J. P. Macke, J. Nathans, *J. Neurosci.* **22**, 8563 (2002).
15. K. I. Pinson, J. Brennan, S. Monkley, B. J. Avery, W. C. Skarnes, *Nature* **407**, 535 (2000).
16. S. Yoshikawa, R. D. McKinnon, M. Kokel, J. B. Thomas, *Nature* **422**, 583 (2003).
17. We thank X. He for the Wnt5a and sFRP1 cDNA constructs, G. Martin for FGF4 and FGF8 cDNAs, W.-b. Gan for help in establishing the iontophoresis labeling method, and V. Bindokas for help with confocal microscopy and quantification with NIH ImageJ software. Supported by a Basil O'Connor Starting Scholar Award from the March of Dimes Foundation, a Schweppe Foundation Career Development Award, and a University of Chicago Brain Research Foundation Seed Grant (Y.Z.). Y.Z. is an Alfred P. Sloan Research Fellow.

Supporting Online Material
www.sciencemag.org/cgi/content/full/302/5652/1984/DC1
Materials and Methods
Figs. S1 to S5

24 July 2003; accepted 14 October 2003

Downloaded from www.sciencemag.org on March 30, 2007