The latter neurons required both modalities to describe the action event, which reflects what normally occurs in nature, where, within a social environment, vision and sound of hand actions are typically coupled. Finally, in the remaining three neurons the response to sound alone was the strongest.

A population analysis (Fig. 2B, rightmost column) based on all 33 neurons analyzed confirmed the data observed in individual neurons (*4*). The population of neurons responded to the sound of actions and discriminated between the sounds of different actions. The actions whose sounds were preferred were also the actions that produced the strongest vision-only and motor responses.

In conclusion, area F5 contains a population of neurons—audio-visual mirror neurons—that discharge not just to the execution or observation of a specific action but also when this action can only be heard. Multimodal neurons have been described in several cortical areas and subcortical centers, including the superior temporal sulcus region (*6–8*), the ventral premotor cortex (*9–14*), and the superior colliculus (*15*). These neurons, however, responded to specific stimulus locations or directions of movement. The difference with the neurons described here is that they do not code space, or some spatial characteristics of stimuli, but actions when they are only heard.

A further difference is that audiovisual mirror neurons also discharge during execution of specific motor actions. Therefore, they are part of the vocabulary of action previously described in area F5. This vocabulary contains not only schemas on how an action should be executed (for example, grip selection) but also the action ideas—that is, actions expressed in terms of their goals (for example, grasp, hold, or break) (*16*). Audiovisual mirror neurons could be used, therefore, to plan/execute actions (as in our motor conditions) and to recognize the actions of others (as in our sensory conditions), even if only heard, by evoking motor ideas.

Mirror neurons may be a key to gestural communication (*17*). The activity of ripping neurons in my brain leads me (if the circumstances are appropriate) to rip a sheet of paper. This overt action will activate your F5 ripping mirror neurons. The action becomes information. This information can be decoded in your brain thanks to the matching properties of your mirror neurons. What is intriguing about the discovery of audiovisual mirror neurons is that they are observed in an area that appears to be the homolog of human Broca's area (area 44) (*18*). The recent demonstration of a left-right asymmetry in the ventral premotor cortex of great apes (*19*) indicates that the human motor speech area is the result of a long evolutionary process, already started in nonhuman primates. The discovery of audiovisual mirror neurons in this location may shed light on the evolution of spoken language for two main reasons: First, these neurons have the capacity to represent action contents; second, they have auditory access to these contents so characteristic of human language.

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Supporting Online Material

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Materials and Methods

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Ectodermal Wnt Function as a Neural Crest Inducer

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Neural crest cells, which generate peripheral nervous system and facial skeleton, arise at the neural plate/ectodermal border via an inductive interaction between these tissues. Wnts and bone morphogenetic proteins (BMPs) play roles in neural crest induction in amphibians and zebrafish. Here, we show that, in avians, Wnt6 is localized in ectoderm and in vivo inhibition of Wnt signaling perturbs neural crest formation. Furthermore, Wnts induce neural crest from naïve neural plates in vitro in a defined medium without added factors, whereas BMPs require additives. Our data suggest that Wnt molecules are necessary and sufficient to induce neural crest cells in avian embryos.

The vertebrate neural crest arises at the border of the neural plate and epidermis early in development. After neurulation, neural crest cells emigrate from the dorsal neural tube, migrate extensively along defined pathways, and subsequently differentiate into diverse cell types, including most of the peripheral nervous system, cranial cartilage, and melanocytes (*1*). In vivo grafts and in vitro coculture experiments have shown that induction of the neural crest in both amphibian and avian embryos can occur by interaction between the neural plate and ectoderm (*2–6*). The classical view is that a signal emanates from the ectoderm and is received by the neuroepithelium, although some bidirectional signaling occurs (*4*).

Adding growth factors to naïve neural plate tissue in vitro has identified candidate molecules with neural crest–inducing ability, including several members of the transforming growth factor–β (TGF-β) family [e.g., dorsalin-1, bone morphogenetic protein (BMP)–4, BMP-7, and activin (*6–8*)]. BMP-4 and -7 were proposed to be the ectodermal-inducing signal, on the basis of their expression pattern and function in vitro (*6*, *8*, *9*). Neural crest induction is ongoing at the border region between the open neural plate and ectoderm of the stage 10 chick embryo (*10*, *11*). BMPs are expressed transiently in the caudal-most ectoderm. However, most prevalent BMP expression at this stage is in the primitive

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streak, neural folds, and dorsal neural tube, with weaker expression in the ectoderm abutting the neural folds (Fig. 1, B and C) (*8*, *12*–*14*). Higher expression of BMP in the responding than in the inducing tissue complicates interpretation of its function. Therefore, the nature of the "ectodermal inducer" of neural crest remains unclear.

Wnt glycoproteins play important roles in both development and carcinogenesis [reviewed in (*15*, *16*)]. In the developing nervous system, Wnt1 and Wnt3a are expressed on the dorsal neural tube shortly after its closure. Combined mutation of these genes results in a diminution of some neural crest derivatives (*17*) but does not affect neural crest induction, which precedes expression of Wnt1 and Wnt3a (*5*). In amphibian embryos, Wnts in combination with fibroblast growth factors or inhibition of BMP signaling are required for neural crest induction (*18*–*22*). The role of Wnts as neural crest inducers in aves was previously unexplored.

Here, we examine the role of Wnt molecules on induction of chick neural crest cells both in vivo and in vitro. We first examined the expression pattern by in situ hybridization of a number of Wnt family members to identify candidates with proper spatiotemporal distribution during neural crest induction. Wnt5a, Wnt5b, and Wnt8c were not expressed in the ectoderm but were observed in the caudal-most region of the open neural plate, with highest expression occurring caudal to Hensen's node in the prospective neuroepithelium (see supporting online material). Wnt6 was expressed in the ectoderm adjacent to the neural folds, but was absent from the neural folds and neural plate, which is a pattern consistent with a candidate ectodermal neural crest inducer (Fig. 1A and 1B, i and ii) (*23*). In contrast, BMP-4 is expressed strongly in the primitive streak, neural folds, and dorsal neural tube but weakly in the ectoderm immediately adjacent to the open neural plate (Fig. 1B, iii and iv, and C) (*8*, *12*–*14*).

We tested the role of Wnt signaling in the induction process in vivo by using a broad-spectrum Wnt inhibitor to challenge neural crest formation. Cells expressing a dominant-negative Wnt1 construct (DnWnt1) (*23*–*25*) were injected either adjacent to the open neural plate or into the closing neural tube. DnWnt1 has a 71–amino acid carboxy-terminal deletion, which was shown previously to block Wnt signaling nonautonomously, possibly by binding to receptors, coreceptors, and/or Wnts themselves. Embryos incubated for 12 to 18 hours after injection were assayed for premigratory neural crest formation by Slug expression (*26*). After injection of DnWnt1 cells, marked inhibition of Slug expression was noted adjacent to the injection sites (n = 28 out of 33 embryos). Control cells rarely produced Slug inhibition (n = 2 out of 38 embryos) (Fig. 1, D and E). DnWnt1 injection did not alter expression of other Wnt genes (Wnt1, Wnt3a, and Wnt4) in the neural tube (n = 8 to 12 embryos each) (*27*). Specificity was confirmed by showing that exogenous Wnt was sufficient to rescue the inhibitor's phenotype. DnWnt1-expressing cells alone (n = 9 out of 12 embyros) or mixed 1:1 with the parental cell line (n = 7 out of 8 embryos) inhibited Slug expression. In contrast, only limited inhibition was observed (n = 2 out of 9 embryos) when DnWnt1 cells were mixed with Wnt1 expressing cells (Fig. 1, F and G).

The effects of Wnt perturbations on migratory neural crest cells were examined 36 hours postinjection using an antibody (HNK-1) that recognizes the carbohydrate antigen HNK-1 expressed by migratory neural crest cells (*28*). Embryos injected with DnWnt1 cells adjacent to the open neural folds had altered neural crest migration (n = 19 out of 25 embryos), as compared with controls (n = 3 out of 18 embryos) (Fig. 1, H and I). A truncated form of Frizzled 7 (extracellular domain only) also caused some inhibition of Slug (*27*), although not as robustly as DnWnt1. 4′,6′-diamidino-2-phenylindole staining provided no evidence of pyknotic nuclei adjacent to the injections, suggesting that the DnWnt1 inhibition of neural crest formation was not due to cell death (*27*).

Activation of the canonical Wnt pathway stabilizes and translocates β-catenin to the nucleus where it functions as a transcription factor in cooperation with members of the TCF/LEF (T cell factor/lymphocyte enhancer binding factor) family. Nuclear localization of β-catenin was found in the neural folds of the open neural plate, anterior to Hensen's node (Fig. 1, J and K). This is consistent with a role of the canonical Wnt signaling in neural crest induction, as suggested by previous

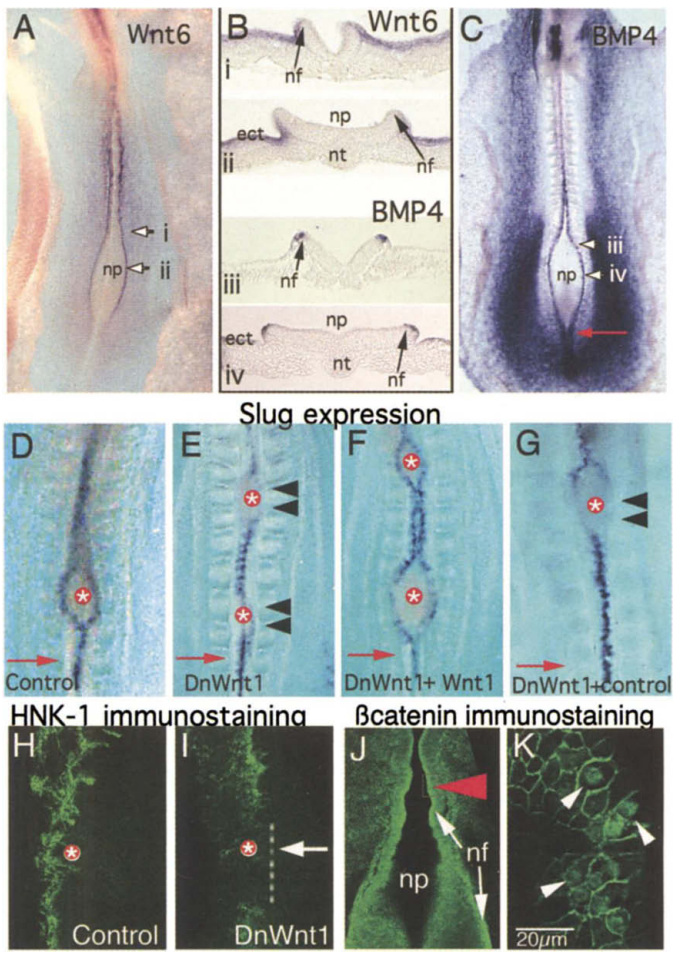

Fig. 1. (**A** to **C**) Wnt6 has an expected distribution for an ectodermal inducer of neural crest. Whole mount in situ hybridization (*44*) of Wnt6 and BMP4 [(A) to (C)] on stage 10 chick embryos (*45*). [(A) and (B, i and ii)] Wnt6 is expressed on the ectoderm (ect) adjacent to the neural plate, abutting but absent from the neural folds [(nf) designated by the arrows]. [(C) and (B, iii and iv)] BMP-4 is expressed in the primitive streak (red arrow) and neural folds; note the gap in expression lateral to the neural folds. [(B), i to iv] Sections corresponding to the white arrows in (A) and (C). (**D** to **I**) Neural crest formation in vivo requires Wnt signaling. [(D) to (G)] Embryos 12 to 18 hours after injection assayed by Slug expression; the red arrows indicate last formed somite, and asterisk marks injection site. Normal Slug expression adjacent to control cells (D), while DnWnt1 cells (E) (*48*) inhibit Slug (arrowheads). (F) Coinjecting 1:1 mixture of DnWnt1 and Wnt1-expressing cells rescues (G), while 1:1 mixture of DnWnt1:control cells still inhibits Slug (arrowheads). (H) 36 hours post-injection, control cells do not perturb migrating neural crest cells (HNK-1+, green) while injection of DnWnt1 cells (I) disrupts the migratory pattern (white arrow). (**J** and **K**) Nuclear β-catenin in the neural folds. (J) Immunostaining of β-catenin with 5H10 antibody (green) on the open neural plate of stage 10 chick embryos. (K) Higher magnification at a level similar to that indicated by the position of the red arrowhead in (J) shows nuclear localization of β-catenin (white arrowheads), suggesting canonical Wnt signaling pathway activity. Embryos oriented with anterior to the top.

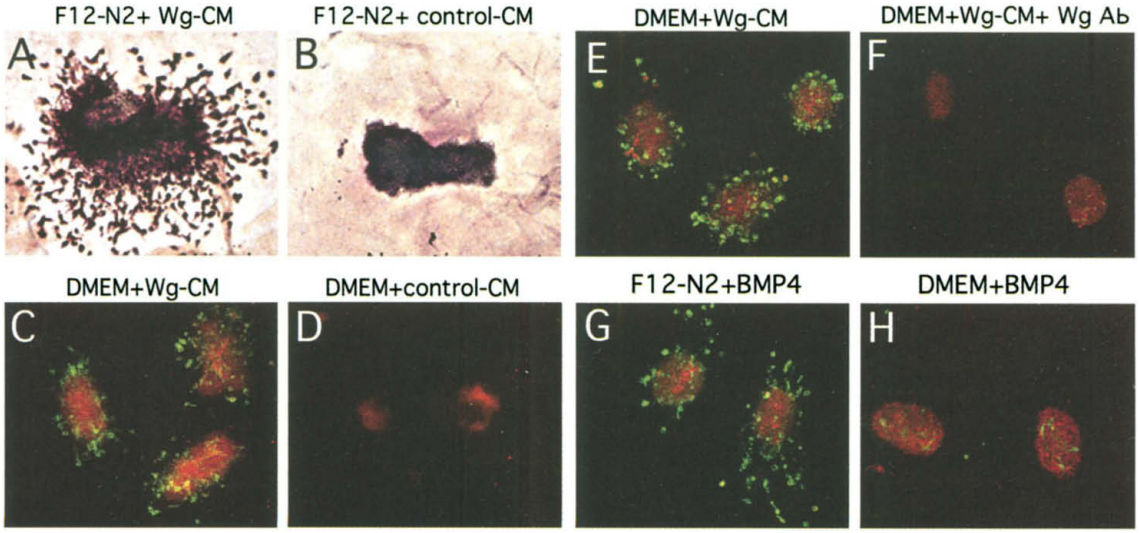

Fig. 2. Wnt signaling induces neural crest formation in vitro. Migratory neural crest cells detected with HNK-1 antibody [brown in (**A** and **B**), and green in (**C** to **H**)] after induction of naïve intermediate neural plates from stage 10 chick embryos. Tissues were 50 to 100-μm long and were explanted into collagen gels (Collaborative Research, Waltham, Massachusetts), cultured (*45*) for 48 hours in 300 μl of defined F12-N2 or DMEM serum-free medium plus 50 μl of conditioned medium from *Drosophila* S_2 (control-CM), or S_2 cells expressing Wingless (Wg-CM), or BM-4 (R&D Systems, Minneapolis, Minnesota). (A) Treatment with Wg-CM induces migratory neural crest cells, unlike (B) control-CM. (C) In a basic medium, DMEM, lacking N_2 additives, Wg-CM still induces neural crest formation. (E) Explants cultured with BMP4 (30 to 650 ng/ml) generated neural crest cells in F12-N2 medium, but not in DMEM alone (F). [(G) and (H)] Addition of 10 to 50 μl of Wg-function–blocking antibody (4D4, DSHB) prevents induction of neural crest cells by Wg-CM.

Table 1. Number and percentage of explants with induced migratory neural crest cells. Induction of neural crest cell formation in intermediate neural plate explants from stage 10 embryos (*45, 46*). BMP4 requires N2 supplement to induce neural crest formation. In contrast, Wg-CM induced neural crest formation without N2.

	Treatment							
	No treatment		BMP-4		Wg-CM		Control-CM	
Medium	Induced explants	%	Induced explants	%	Induced explants	%	Induced explants	%
F12-N2	0 out of 11	0	58 out of 66	88	39 out of 52	75	1 out of 14	7
DMEMsf	0 out of 51	0	2 out of 42	5	103 out of 138	75	4 out of 50	8

studies in *Xenopus* showing that overexpression of β-catenin mimics the effects of Wnt overexpression in causing excess neural crest formation (*18–22*).

We next tested the ability of Wnt signaling to induce neural crest formation from naïve neuroepithelium in vitro. As a Wnt source, we used conditioned medium from *Drosophila* S_2 cells transfected with wingless (*Wg, Drosophila Wnt-1* homolog), known to trigger Wnt signaling in various vertebrate systems (*29–34*). Under conditions used by previous investigators to examine the ability of TGFβs to induce neural crest formation from naïve neural plate (*5–8, 10, 11, 35*), we showed that Wg-conditioned medium, but not control medium, produced a robust induction of neural crest cells after 48 hours in culture (Fig. 2, A and B). The medium used in these assays (F12) contains additives [N2 (Gibco, Life Technologies, Carlsbad, California, USA), a cocktail of factors commonly included in neural cultures], raising the possibility that induction triggered by Wg and/or TGFβ molecules occurs by synergism. Therefore, we used a medium lacking additives [Dulbecco's minimum essential medium (DMEM)]. Wg-conditioned medium induced neural crest formation in naïve neural plates, but BMP-4 did not induce without additives (Fig. 2, C to F) (Table 1). Finally, the inducing effect of Wg-conditioned medium was blocked by adding function-blocking antibodies to Wg (Fig. 2, G and H). These results show that Wnt signaling can trigger neural crest cell induction, even in the absence of factors required to achieve a similar outcome by BMP signaling (Fig. 2).

The mechanisms of neural and neural crest induction in amniotes and anamniotes apparently differ. In amphibians and zebrafish, neural induction occurs in ectoderm in which BMP signaling has been blocked, leading to a "neural default" model (*36–39*) that does not extrapolate well to mouse and chicken. Although the amniote node expresses BMP antagonists (*14, 40, 41*), expression of BMPs is not detected in ectodermal regions before neural induction (*14, 42, 43*). This indicates that signals other than BMP inhibition are required for neural induction in amniotes (*44*), and these may also affect formation of border cell types like the neural crest.

Previous studies have established a role for BMPs in neural crest cell formation by ectopic application and inhibition approaches. However, ectodermal expression of BMPs is weak and transient, whereas expression in the neural folds is robust. Further, BMPs are unable to induce neural crest cells from neural plates in the absence of additives. In contrast, we show that Wnt signaling is both necessary and sufficient to induce avian neural crest cells in the absence of added factors. Wnt6 is expressed in the ectoderm adjacent to neural plate and folds when neural crest cells are being induced. This is the distribution expected for an ectodermal neural crest inducer. Our data suggest that Wnt signaling may be a common mechanism for vertebrate neural crest induction.

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Role of Genotype in the Cycle of Violence in Maltreated Children

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We studied a large sample of male children from birth to adulthood to determine why some children who are maltreated grow up to develop antisocial behavior, whereas others do not. A functional polymorphism in the gene encoding the neurotransmitter-metabolizing enzyme monoamine oxidase A (*MAOA*) was found to moderate the effect of maltreatment. Maltreated children with a genotype conferring high levels of *MAOA* expression were less likely to develop antisocial problems. These findings may partly explain why not all victims of maltreatment grow up to victimize others, and they provide epidemiological evidence that genotypes can moderate children's sensitivity to environmental insults.

Childhood maltreatment is a universal risk factor for antisocial behavior. Boys who experience abuse—and, more generally, those exposed to erratic, coercive, and punitive parenting—are at risk of developing conduct disorder, antisocial personality symptoms, and of becoming violent offenders (*1*, *2*). The earlier children experience maltreatment, the more likely they are to develop these problems (*3*). But there are large differences between children in their response to maltreatment. Although maltreatment increases the risk of later criminality by about 50%, most maltreated children do not become delinquents or adult criminals (*4*). The reason for this variability in response is largely unknown, but it may be that vulnerability to adversities is conditional, depending on genetic susceptibility factors (*5*, *6*). In this study, individual differences at a functional polymorphism in the promoter of the monoamine oxidase A (*MAOA*) gene were used to characterize genetic susceptibility to maltreatment and to test whether the *MAOA* gene modifies the influence of maltreatment on children's development of antisocial behavior.

The *MAOA* gene is located on the X chromosome (Xp11.23–11.4) (*7*). It encodes the *MAOA* enzyme, which metabolizes neurotransmitters such as norepinephrine (NE), serotonin (5-HT), and dopamine (DA), rendering them inactive (*8*). Genetic deficiencies in *MAOA* activity have been linked with aggression in mice and humans (*9*). Increased aggression and increased levels of brain NE, 5-HT, and DA were observed in a transgenic mouse line in which the gene encoding *MAOA* was deleted (*10*), and aggression was normalized by restoring *MAOA* expression (*11*). In humans, a null allele at the *MAOA* locus was linked with male antisocial behavior in a Dutch kindred (*12*). Because *MAOA* is an X-linked gene, affected males with a single copy produced no *MAOA* enzyme—effectively, a human knockout. However, this mutation is extremely rare. Evidence for an association between *MAOA* and aggressive behavior in the human general population remains inconclusive (*13–16*).

Circumstantial evidence suggests the hypothesis that childhood maltreatment predisposes most strongly to adult violence among children whose *MAOA* is insufficient to constrain maltreatment-induced changes to neurotransmitter systems. Animal studies document that maltreatment stress (e.g., maternal deprivation, peer rearing) in early life alters NE, 5-HT, and DA neurotransmitter systems in ways that can persist into adulthood and can influence aggressive behaviors (*17–21*). In humans, altered NE and 5-HT activity is linked to aggressive behavior (*22*). Maltreatment has lasting neurochemical correlates in human children (*23*, *24*), and although no study has ascertained whether *MAOA* plays a role, it exerts an effect on all aforementioned neurotransmitter systems. Deficient *MAOA* activity may dispose the organism toward neural hyperreactivity to threat (*25*). As evidence, phenelzine injections, which inhibit the action of monoamine oxidase, prevented rats from habituating to chronic stress (*26*). Low *MAOA* activity may be particularly prob-

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