Neuron, Vol. 25, 1–5, January, 2000, Copyright ©2000 by Cell Press

Previews

Specificity and Promiscuity among Proneural Proteins

The peripheral nervous system (PNS) is made up of an array of different sensory structures that detect aspects of the environment—for example, the proprioceptive and mechanoreceptive sensory neurons in vertebrates. In *Drosophila*, there is a similar array of sensory organs, including the external sensory bristles (mechanosensory), chordotonal organs (stretch receptors), multidendritic neurons, and olfactory sensillae. The development of these structures involves two processes, the acquisition of neural competence and the specialization appropriate for each sensory organ type. Initially, it appeared that the regulation of these two aspects of development was separate, with proneural genes conferring neural potential and other transcription factors specifying the precise structures to be formed. However, more recently it has become clear that proneural proteins can confer sensory organ identity as well. Two papers in this issue of *Neuron* (Goulding et al., 2000; Huang et al., 2000) further support this, as they describe the isolation of a new *Drosophila* proneural gene, *amos*, that is involved in the development of the olfactory sensillae and multidendritic neurons (MD).

Neural development in both vertebrates and invertebrates involves the establishment of a zone of competent cells, a proneural territory, that subsequently gives rise to one or several neural precursors. Competence is conferred by the expression of proneural genes, such as those of the *achaete-scute* family that encode basic helix–loop–helix (bHLH) transcription factors (Campuzano and Modolell, 1992). These were first identified in *Drosophila* through mutations that disrupt adult expression, leading to the loss of external sensory bristles on the thorax. Subsequently, homologs have been identified in vertebrates and shown to confer similar neural competence (Lee, 1997).

There are two known subtypes of proneural genes in *Drosophila* based on sequence similarities. The *achaete-scute* genes (*achaete*, *scute*, and *lethal of scute*) are required for the external sensory organs as well as for central nervous system development (Campuzano and Modolell, 1992). The second subtype, *atonal*, is involved in development of photoreceptors and chordotonal organs (Jarman et al., 1995). However, even when *atonal* and *achaete-scute* genes are eliminated, some aspects of neural development can still occur, and there has been considerable effort directed at identifying additional proneural genes that account for this.

The *amos* gene encodes a bHLH transcription factor related to Atonal, and was isolated by Goulding et al. (2000) in a degenerate PCR using primers specific for the Atonal subfamily and by Huang et al. (2000) in a yeast two-hybrid screen. Like other proneural genes, *amos* is expressed transiently in the embryo. Expression first appears in clusters of ectodermal cells that correspond to the positions where MD neurons develop, and later this expression becomes restricted to a single cell per cluster. In Notch mutant embryos the expression fails to resolve, demonstrating that, as with the selection of other neural precursors, the resolution of *amos* expression involves lateral inhibition. In the antennal imaginal disc, *amos* mRNA is detected in three semicircular bands that correspond to the sites where olfactory sensillae arise.

Confirmation that *amos* has proneural activity comes from loss- and gain-of-function experiments, although these were hampered by the absence of mutations in *amos*. One approach was to disrupt *amos* function using double-stranded RNA interference. Injection of *amos* dsRNA into embryos resulted in the loss of MD neurons, whereas control experiments using *atonal* dsRNA had no effect on these structures (Huang et al., 2000). Because Amos, like other proneural proteins, dimerizes with Daughterless (the *Drosophila* E12/E47 homolog) to bind to DNA (Huang et al., 2000), another way to assay *amos* function was to see whether defects in PNS development were revealed when the levels of both *daughterless* and *amos* were reduced in heterozygous animals. This was done by making a transheterozygous combination between a chromosomal deficiency that eliminates *amos* and a null allele of *daughterless*. A convincing decrease in olfactory sensillae was observed in these transheterozygotes in comparison to heterozygotes of either mutant chromosome alone (Goulding et al., 2000). The proneural function of *amos* was also evident from targeted misexpression, which resulted in extra MD neurons when *amos* was expressed in stripes in the embryo (Huang et al., 2000) and in a dramatic increase in olfactory sensillae when it was expressed throughout the antennal disc (Goulding et al., 2000). Taken together, these data demonstrate a role for *amos* in promoting sensory organ development.

The results of misexpressing *amos* support the proposal that proneural proteins confer sensory organ specificity as well as neural competence. For example, when *amos* is expressed ectopically in the wing, some of the ectopic sensillae have the characteristics of olfactory sensillae, whereas neither *atonal* nor *scute* misexpression can elicit this class of sensory organs (Goulding et al., 2000). Similar specificities are observed in the embryo. Therefore, even though the Atonal and Amos proteins differ by only a single amino acid in the basic DNA binding domain, they confer specific types of neural differentiation. In vitro, the DNA binding activity of Amos is similar to Atonal, suggesting that other parts of the protein are likely to influence the specificity of the proteins in vivo, probably by mediating specific protein interactions (Huang et al., 2000).

There is evidence for similar specificity among the vertebrate *atonal* and *achaete-scute* homologs. For example, in mice, the *atonal*-related Neurogenins are required for sensory but not autonomic ganglia, whereas the *achaete-scute*-related Mash1 is necessary for the converse (Anderson, 1999; Brunet and Ghysen, 1999). In the *Xenopus* retina, misexpression of two Atonal-related proteins promoted different subsets of cell fates

(Perron et al., 1999). However, there are relatively few experiments in which the comparative potential of different proneural proteins has been analyzed directly. The results from *Drosophila* demonstrate that it is possible to distinguish characteristics between two closely related proteins of the Atonal subfamily. Thus, it would be interesting to extend the studies in vertebrates to test out the specificities of the different homologs in comparable assays.

The misexpression experiments in *Drosophila* demonstrate that individual proneural proteins can have some unique properties. However, these experiments also reveal considerable overlap in their activities, since *scute*, *atonal*, and *amos* can all produce external sensory organs (ES) when misexpressed, even though only *scute* is implicated in normal ES development. The fact that all three proteins elicit ES formation suggests that this is the default route for sensory organ development and that there is a common set of target genes. All three proneural proteins would activate this common contingent, which could include genes such as *asense* (a secondary proneural gene that is expressed in all neural precursors) and *Delta* (encoding a ligand for Notch) and would be sufficient to elicit ES development. The ability to make other types of structures would rely on this basic model being modified through each individual proneural protein regulating some specific targets. One example of such a target is *cut*, which is repressed by Atonal to allow the development of chordotonal organs rather than ES (Jarman and Ahmed, 1998).

The *achaete-scute* complex genes have yet more promiscuous roles, which imply that proneural proteins may act in combination with other factors to elicit responses besides a neural program. For example, proneural genes are required in the selection of precursors for skeletal muscles and midgut (Carmena et al., 1995; Tepass and Hartenstein, 1995). This is consistent with these processes requiring some of the core contingent of genes regulated by proneural proteins, and the example of *Delta* would fit with this broad remit. However, expression of neural-specific targets like *asense* is not induced outside the ectoderm, and other tissue-specific targets are likely to be activated instead. For example, the mesoderm bHLH transcription factor Twist could cooperate with proneural proteins to activate muscle-specific genes and inhibit neural-specific genes in the muscle precursors. It is not clear whether there needs to be a similar "ectoderm" factor that collaborates with the proneural proteins to elicit sensory organ development or whether in the absence of other tissue-specific factors the neural pathway will be the default target.

It is evident that parallel transcription factors must act in combination with the proneural proteins to influence the target genes regulated, not only with respect to neural versus other fates but also with respect to the types of sensory organ formed. For example, Atonal is involved in specifying photoreceptors in the eye and chordotonal organs in the wing. Likewise, Amos is involved in the development of different types of olfactory sensillae. The levels of Lozenge (a Runt domain transcription factor) are important in discriminating which olfactory structures form, and it is proposed that high levels of Lozenge modify the specificity of Amos so that the sensory structures take on one of two alternative fates (Gupta and Rodrigues, 1997; Goulding et al., 2000).

In conclusion, the identification of *amos* highlights the importance of proneural proteins in contributing to sensory organ specificity as well as neural competence. Clearly, one key to our further understanding of neural development will be to identify the targets of proneural proteins, both to find those that are activated by all proneural proteins and those that are specific to each proneural protein and subtype of sensillae. This will allow us to learn what is required to elaborate neural development on the one hand and what is required to dictate the ways that the different types of sensory structures develop on the other.

Sarah Bray
Department of Anatomy
University of Cambridge
Downing Street
Cambridge CB2 3DY
United Kingdom

Selected Reading

Anderson, D.J. (1999). Curr. Opin. Neurobiol. *9*, 517–524.

Brunet, J.F., and Ghysen, A. (1999). Bioessays *21*, 313–318.

Campuzano, S., and Modolell, J. (1992). Trends Genet. *8*, 202–208.

Carmena, A., Bate, M., and Jimenez, F. (1995). Genes Dev. *9*, 2373–2383.

Goulding, S.E., zur Lage, P., and Jarman, A.P. (2000). Neuron *25*, this issue, 69–78.

Gupta, B.P., and Rodrigues, V. (1997). Genes Cells *2*, 225–233.

Huang, M.-L., Hsu, C.-H., and Chien, C.-T. (2000). Neuron *25*, this issue, 57–67.

Jarman, A.P., and Ahmed, I. (1998). Mech. Dev. *76*, 117–125.

Jarman, A.P., Sun, Y., Jan, L.Y., and Jan, Y.N. (1995). Development *121*, 2019–2030.

Lee, J.E. (1997). Curr. Opin. Neurobiol. *7*, 13–20.

Perron, M., Opdecamp, K., Butler, K., Harris, W. A., and Bellefroid, E.J. (1999). Proc. Natl. Acad. Sci. USA *96,* 14996–15001.

Tepass, U., and Hartenstein, V. (1995). Development *121*, 393–405.

Bipolar Cells in the Spotlight: Cause for Excitement

Retinal bipolar neurons are thought to be electrically inexcitable neurons that respond to changes in illumination with graded changes in membrane potential. ON bipolar cells depolarize in response to light, when glutamate release from photoreceptors is decreased, and hyperpolarize in the dark, due to the release of glutamate from photoreceptors and activation of metabotropic receptors on bipolar cell dendrites. The Mb1 neuron of the goldfish retina is an ON bipolar cell that receives photoreceptor inputs primarily from rod photoreceptors. Recent in vitro investigations have revealed that the secretory machinery of this neuron, in addition to

allowing the tonic release of neurotransmitter, can support rapid exocytosis with rates that are comparable to those of conventional fast synapses (Heidelberger et al., 1994; von Gersdorff and Matthews, 1994). There is also evidence that isolated Mb1 neurons can fire Ca^{2+}-based action potentials (Burrone and Lagnado, 1997; Zenisek and Matthews, 1998). If Ca^{2+} were found to enter the synaptic terminal of Mb1 neurons in a regenerative manner in response to changes in illumination, this would revolutionize our view of how this key interneuron of the high-sensitivity rod pathway conveys visual information. Furthermore, it would raise the question of whether this neuron might have specialized features that allow it to safely handle a large, presynaptic Ca^{2+} load.

Protti and colleagues (2000), in this issue of *Neuron*, report that Mb1 bipolar cells are electrically excitable and can make Ca^{2+}-based action potentials in response to light onset. These light-evoked action potentials, observed in the retinal slice preparation, were stereotypic in waveform, specific to the Mb1 class of bipolar cell, and were not observed in cells that lost their synaptic terminals. They could be blocked by a glutamate agonist, consistent with the interpretation that a light-evoked decrease in glutamate release from photoreceptors triggered the regenerative response. The above features were observed only in dark-adapted retinas in response to dim light. However, not all dark-adapted Mb1 neurons fired a Ca^{2+}-based action potential in response to light, but all could fire when treated with a Ca^{2+} channel agonist. Additional experiments will be needed to determine what underlies this variability. In contrast, light-adapted Mb1 neurons typically showed small-amplitude, high-frequency oscillations in membrane potential rather than Ca^{2+} spikes, suggesting that the light-stimulated inhibitory circuitry is generally intact and can curtail spike generation (Zenisek and Matthews, 1998). Taken together, the results of Protti et al. (2000) provide strong, new evidence for the provocative premise that when the high-sensitivity rod pathway dominates the light response, Mb1 bipolar cells transmit visual information in an all-or-none manner.

What advantage would Ca^{2+}-based action potentials have over graded transmission? The rise times of the action potentials were 4-fold faster than the rise times of the graded signals measured in somata without terminals. Thus, Ca^{2+} spikes arising in synaptic terminals may permit Mb1 neurons to release synaptic vesicles in a more rapid and concerted manner than the slower graded responses. Furthermore, the relatively large amplitude of a Ca^{2+} spike compared with a graded response could serve as a signal amplification mechanism. Both features would aid in maintaining the high-sensitivity, low-noise attributes of rod photoreceptor signaling at the synapse between Mb1 bipolar cells and third-order retinal neurons. The intensity of the light stimulus is unlikely to be encoded by spike number due to the long refractory period (1–2 s) between spikes. However, spike latency was found to decrease with stimulus intensity, suggesting that spike latency might be used to encode intensity. This, in turn, requires that there be a downstream convergence of latency information to allow for integration and comparison of signals. This could happen at the level of the ganglion cells, which receive convergent inputs from bipolar cells. Future work will be needed to establish whether this type of latency coding is used by the rod pathway.

The specter of Ca^{2+}-based action potentials in synaptic terminals raises the question of how large presynaptic Ca^{2+} loads are handled. Prior work in neuronal somata and adrenal chromaffin cells indicate that mitochondria play a dominant role in clearing Ca^{2+} from the cytosol (e.g., Thayer and Miller, 1990; Friel and Tsien, 1994; Herrington et al., 1996). Yet, large Ca^{2+} loads taken up by mitochondria may be toxic to cells. Zenisek and Matthews (2000 [this issue of *Neuron*]) provide a detailed, quantitative look at the mechanisms of presynaptic Ca^{2+} clearance in synaptic terminals of acutely isolated Mb1 neurons. Their thorough investigation reveals that both the regulation of presynaptic basal Ca^{2+} and the clearance of presynaptic Ca^{2+} loads evoked by membrane depolarization occur predominantly via a plasma membrane Ca^{2+}-ATPase rather than by mitochondrial uptake or Na^{+}–Ca^{2+} exchange. The authors convincingly argue that because ATP was necessary for Ca^{2+} clearance and a direct role for mitochondria in clearing presynaptic Ca^{2+} was inconsistently observed and small, the primary role of mitchondria is likely to be that of providing ATP for the plasma membrane Ca^{2+}-ATPase. From their estimates, it seems that this ATPase is responsible for the extrusion of $\sim$95% of the Ca^{2+} load resulting from depolarization-induced Ca^{2+} influx. Furthermore, the plasma membrane Ca^{2+}-ATPase was found to have a high capacity for extruding Ca^{2+} and could do so relatively rapidly. These are very desirable features for a synaptic terminal that experiences prolonged periods of Ca^{2+} influx. Presumably, this rapid extrusion helps prevent Ca^{2+}-mediated excitotoxicity caused by mitochondrial overloading by pumping out Ca^{2+} before it can diffuse from sites of influx to the mitochondria, which are clustered some distance away (von Gersdorff et al., 1996). An interesting comparison of terminals and somata reveals that although the amount of Ca^{2+} influx in somata was smaller than in terminals, the time course of somatic Ca^{2+} recovery was slower. The authors do not follow up on this point, concentrating instead on the mechanisms of terminal Ca^{2+} clearance, but the recovery time course reported for somata is similar to that attributed to mitochondrial clearing in adrenal chromaffin cells (Herrington et al., 1996).

This highlights the possibility that individual compartments within a neuron can regulate internal Ca^{2+} via different mechanisms. In total, the data presented by Zenisek and Matthews (2000) indicate that in synaptic terminals of Mb1 bipolar cells, presynaptic Ca^{2+} is regulated primarily by a plasma membrane Ca^{2+}-ATPase. It should be kept in mind that dissociated Mb1 neurons have lost their synaptic inputs and therefore share features, such as minimal inhibitory feedback, with dark-adapted Mb1 neurons. Thus, the conditions under which the high-capacity plasma membrane Ca^{2+}-ATPase has been shown to predominate are comparable to those under which Ca^{2+}-based action potentials may occur. It would be interesting to see whether this mechanism of Ca^{2+} clearance also prevails under conditions of light adaptation, where Ca^{2+}-based action potentials would be unexpected.

Ca^{2+} uptake by mitochondria and its subsequent release has been proposed as a mechanism of activity-dependent changes in neurotransmitter release (Herrington et al., 1996; Tang and Zucker, 1997). Mitochondrial uptake and release of Ca^{2+} was inconsistently observed in Mb1 synaptic terminals, and it typically required high (>800 nM) cytosolic Ca^{2+} (Zenisek and Matthews, 2000). Such high cytosolic Ca^{2+} can be achieved following a Ca^{2+} action potential (Zenisek and Matthews, 1998) or a long depolarization (Heidelberger and Matthews, 1992). However, the amount of Ca^{2+} that was released from mitochondria did not correlate with the magnitude of the Ca^{2+} load. Thus, it would seem that using the release of Ca^{2+} from mitochondria as a way of modulating synaptic efficacy in Mb1 synaptic terminals would be capricious at best, and it is tempting to speculate that if these neurons undergo a meaningful, activity-dependent change in neurotransmitter release, another mechanism must be at work.

In summary, some bipolar cells of the vertebrate retina seem to be electrically excitable, contrary to the commonly held belief that they are only capable of graded responses. Ca^{2+}-based action potentials in the Mb1 bipolar cell are likely to contribute to signal amplification and enhancement of synaptic gain in the high-sensitivity rod pathway. Consistent with the generation of these action potentials, the synaptic terminals of Mb1 neurons are well equipped for effectively handling large presynaptic Ca^{2+} loads. Critical points that remain to be addressed include verifying whether or not Ca^{2+}-based action potentials occur in goldfish Mb1 bipolar cells in situ and determining whether such Ca^{2+}-based action potentials are a universal feature of ON bipolar cells in the rod pathway.

Ruth Heidelberger
W. M. Keck Center for the Neurobiology
of Learning and Memory
Department of Neurobiology and Anatomy
University of Texas
Houston Medical School
Houston, Texas 77030

Selected Reading

Burrone, J., and Lagnado. L. (1997). J. Physiol. *505*, 571–584.

Friel, D.D., and Tsien, R.W. (1994). J. Neurosci. *14*, 4007–4024.

Heidelberger, R., and Matthews, G. (1992). J. Physiol. *447*, 235–256.

Heidelberger, R., Heinemann, C., Neher, E., and Matthews, G. (1994). Nature *371*, 513–515.

Herrington, J., Park, Y.B., Babcock, D.F., and Hille, B. (1996). Neuron *16*, 219–228.

Protti, D.A., Flores-Herr, N., and von Gersdorff, H. (2000). Neuron *25*, this issue, 215–227.

Tang, Y., and Zucker, R.S. (1997). Neuron *18*, 483–491.

Thayer, S.A., and Miller, R.J. (1990). J. Physiol. *425*, 85–115.

von Gersdorff, H., and Matthews, G. (1994). Nature *367*, 735–739.

von Gersdorff, H., Vardi, E., Matthews, G., and Sterling, P. (1996). Neuron *16*, 1221–1227.

Zenisek, D., and Matthews, G. (1998). Vis. Neurosci. *15*, 69–75.

Zenisek, D., and Matthews, G. (2000). Neuron *25*, this issue, 229–237.

Learning to Like Your Voice: Developing Selectivity to Birdsong

A basic principle of functional brain organization is that stimuli in the environment are coded by selective neuronal responsiveness. Classic examples of such neuronal selectivity range from cells in the visual cortex that fire only in response to simple visual stimuli to the more complex cells in the inferotemporal cortex of primates that are selective only for conspecific faces. Although the existence of such neuronal selectivity is axiomatic in cognitive neuroscience, the way in which such striking selectivity develops remains an important topic of research. The classic work of Hubel and Wiesel on the development of neuronal selectivity in the visual system demonstrated that this selectivity is not entirely prespecified or necessarily intrinsic to a neuronal cell type. In fact, severe developmental perturbations can extinguish or profoundly modify the occurrence of cortical neurons that fire selectively to simple visual stimuli. But what types of normal experiences can induce such selectivity? Is learning involved? How enduring are such experiential effects?

Insight into these processes is provided by a paper in this issue of *Neuron* by Solis and Doupe (2000) on the development of selective firing to a bird's own song by neurons in the zebra finch brain. These so-called "song-specific" neurons were discovered by Margoliash and Konishi (1985) when investigating the auditory responsiveness of neurons in the avian song system. The song system consists of a network of interconnected nuclei in the telencephalon, diencephalon, and brainstem of songbirds that regulates the motor production of song (Nottebohm, 1996; see figure). It was therefore of great interest when a key nucleus in this circuit, HVc, was found to exhibit selective auditory responses. In particular, in some neurons the responses were selective not only to features of conspecific song in general but to specific combinations of features unique to the song produced by the male bird himself (Margoliash and Fortune, 1992). Because birdsong is a learned behavior (Marler, 1997), this immediately suggested that specific types of experience were required for the development of such selectivity. Indeed, it was demonstrated by Volman (1993) that such auditory selectivity in white-crowned sparrows only emerges when the sparrows have learned to produce the highly stereotyped song typical of adult birds.

Solis and Doupe (2000) have now demonstrated that selective neuronal firing to the bird's own song is not the result of only a maturational process but rather is also shaped by the learning of song itself. The authors take advantage of the fact that there are two distinct ways in which auditory experience influences song learning in zebra finches and other songbird species (Marler, 1997). Early in ontogeny, young birds must hear conspecific song from a tutor and form an auditory memory of this song that is sometimes referred to as a "template." Subsequently, they gradually match their own

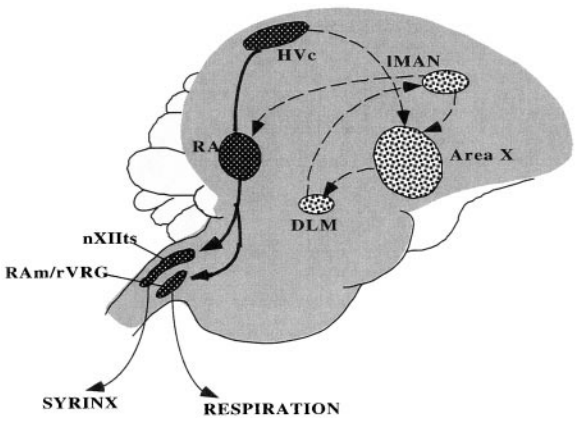

Figure 1. Simplified Diagram of the Song System of Songbirds in the Sagittal Plane

Two pathways can be distinguished, the posterior motor pathway (dark stippling with solid lines connecting the nuclei) and the anterior forebrain pathway (light stippling with dotted lines connecting the nuclei). All recordings were made in the anterior forebrain pathway, a specialized forebrain–basal ganglia circuit. HVc, high vocal center; RA, robust nucleus of the archistriatum; DLM, dorsolateral thalamic nucleus; lMAN, lateral part of the magnocellular nucleus of the anterior neostriatum; RAm, nucleus retroambigualis.

vocal output to this memory or template until they produce the stereotyped or "crystallized" song typical of adult birds. Solis and Doupe selectively altered learning during the second phase by denervating the vocal production organ, the syrinx, prior to the period when the finches would start matching their song to the tutor's song they had heard previously. When the transection heals, the birds have less vocal control, produce some unusual songs, and end up with variable reinnervation of the syrinx. As a result, some adult finches are unable to match the tutor's song at all, although they produce a stereotyped adult-like song, while other individuals learn to produce a reasonable match. This variability allowed the authors to address an interesting question. In the birds that are unable to match their tutor's song, will the selectivity of the neurons in the song system resemble more the tutor's song, which shaped their template, or the song they actually produce?

Solis and Doupe focused on the auditory responses of neurons in nuclei such as Area X and LMAN that are in the anterior forebrain pathway of the song control system (see figure). This pathway is needed for song learning (Bottjer and Johnson, 1997), and neurons in this pathway were shown previously to exhibit selectivity to the bird's own song in close association with the learning of adult-typical song (Solis and Doupe, 1997). In this study, in anesthetized birds that had nerve cuts and whose songs were different from the tutor's song, many neurons in Area X responded equally well to the bird's own song and to the tutor's song. Furthermore, the degree of selectivity to these two stimuli over conspecific and reversed song was considerably less than in normal adults. Also, auditory responses in these birds were exceedingly rare in another song nucleus in the anterior forebrain pathway, LMAN. This is in contrast to the findings for the birds that managed to mimic their tutor in a reasonable manner. Neurons in Area X in these birds developed a normal selectivity to the bird's own song relative to other stimuli, and the neurons in LMAN were also normally selective.

These data tell us something important about how this remarkable neuronal selectivity develops. Clearly, both the tutor's song and the song produced by the bird are important for the development of song-selective neurons. Also, a chronic mismatch between the song the bird produces and the template based on the tutor's song results in a significant alteration in the responsiveness of the neurons in the anterior forebrain pathway of the song system. The dual selectivity of a single neuron that was observed in the adult birds with song incongruent from the tutor's song was observed previously only in young birds that were not yet able to produce adult stereotyped song (Solis and Doupe, 1999). Solis and Doupe speculate that such dual selectivity may be a general feature of the adult song system that was not recognized in the past because the bird's own song is normally close to the tutor's song. Alternatively, such dual selectivity may be a juvenile feature that was maintained in these adults because of their chronic inability to match their song with the tutor's song. Thus, whether the dual selectivity is only needed for the transition to adult song or whether it plays a role in adult song maintenance and/or perception is still unclear.

Overall, this study tells us that deprivation of a very specific type of experience, namely, the matching of vocal output with an auditory memory of the tutor's song, is enough to perturb the development of neuronal selectivity. Further studies on the song system should help clarify the possible adult function of these highly selective neurons. For example, are dual-selective neurons part of the mechanism by which altered song is produced in response to perturbation in auditory feedback (Leonardo and Konishi, 1999)? Is the development of the highly selective neurons in the anterior forebrain pathway essential for the comparison between the bird's own song and the tutor's song that occurs during song learning? In any case, such precision in the development of neuronal selectivity may be a general feature of the vertebrate nervous system. Other examples of complex neuronal selectivity may require highly specific types of sensory feedback during development.

Gregory F. Ball
Department of Psychology
Behavioral Neuroendocrinology Group
Johns Hopkins University
Baltimore, Maryland 21218

Selected Reading

Bottjer, S.W., and Johnson, F. (1997). J. Neurobiol. *33*, 602–618.

Leonardo, A., and Konishi, M. (1999). Nature *399*, 466–470.

Margoliash, D., and Fortune, E.S. (1992). J. Neurosci. *12*, 4309–4326.

Margoliash, D., and Konishi, M. (1985). Proc. Natl. Acad. Sci. USA *82*, 5997–6000.

Marler, P.R. (1997). J. Neurobiol. *33*, 501–516.

Nottebohm, F. (1996). J. Comp. Physiol. [A] *179*, 149–156.

Solis, M.M., and Doupe, A.J. (1997). J. Neurosci. *17*, 6447–6462.

Solis, M.M., and Doupe, A.J. (1999). J. Neurosci. *19*, 4559–4584.

Solis, M.M., and Doupe, A.J. (2000). Neuron *25*, this issue, 109–121.

Volman, S.F. (1993). J. Neurosci. *13*, 4737–4747.