Song-evoked inhibition and its role in vocal plasticity

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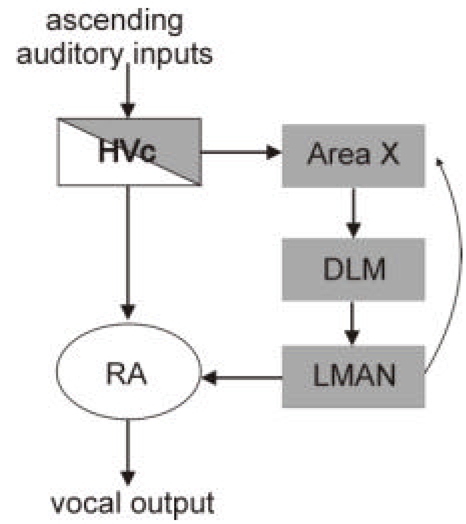
Like humans, songbirds use auditory feedback to learn and maintain species-typical vocalizations. These auditory feedback processes are likely to involve the integration of auditory representations of the learned vocalizations with the pattern-generating circuitry generating the same sounds. The songbird's brain contains a series of interconnected nuclei important to the learning, production and perception of learned song (Figure). Amongst these, the telencephalic nucleus HVc is a major auditory-vocal interface: it is at the apex of a hierarchically-organized central pattern generator for song, and many of its neurons are song-selective, discharging more action potentials to auditory playback of the bird's own song (BOS) than to temporally manipulated versions of the BOS (i.e., reverse BOS) or the songs of other birds. HVc is structurally heterogeneous, containing two relay cell types and interneurons, and functionally heterogeneous, as its two relay cell types give rise to anatomically separate pathways implicated in either audition-dependent vocal plasticity or vocal control (Figure). This anatomical arrangement places HVc in a pivotal position where auditory information could influence vocal quality, as occurs during song learning and adult song maintenance. Our interests are to identify the cellular (i.e., synaptic and intrinsic) mechanisms through which song-selectivity is generated, determine if song-evoked activity is transmitted through either or both of the two functionally distinct pathways of the song system, and explore how such auditory-evoked activity could affect vocal plasticity.



The song system: HVc contains two relay cell types, one that innervates the song motor nucleus RA, and the other that innervates the basal ganglia homologue area X. Area X, the thalamic nucleus DLM and the anterior telencephalic nucleus LMAN form the anterior forebrain pathway (AFP; *gray*), which is implicated in auditory feedback and song perception, and which is essential to audition-guided vocal plasticity in juvenile and adult birds.

Birdsong is complex in both its spectral and temporal features, and thus song-selectivity is likely to develop through several auditory processing stages. Nonetheless, HVc is a useful site to begin to examine the cellular mechanisms underlying heightened song-selectivity, because its neurons show markedly greater song-selectivity than do neurons in areas presynaptic to the nucleus. We have started to explore the synaptic and intrinsic mechanisms that underlie song-selective action potential discharge in HVc using sharp microelectrodes to make *in vivo* intracellular current clamp recordings from identified HVc neurons in urethane-anesthetized adult male zebra finches, an Australian songbird species. These recordings reveal that both HVc relay cell types exhibit similar suprathreshold song-selectivity, characterized by phasic action potential discharge to forward (but not reverse) BOS playback. Therefore, HVc relay cells are sensitive to temporal features in the BOS and provide direct auditory drive to the two functionally distinct branches of the song system.

Despite their suprathreshold similarities, the BOS-evoked action potential responses in the two HVc relay cell types arise via different subthreshold processes. RA-projecting neurons display purely excitatory song-selective subthreshold responses, while the subthreshold responses of X-projecting neurons comprise less-selective depolarizing and highly selective hyperpolarizing components. Even though action potential responses in both relay cells are usually elicited only by BOS playback, an interesting feature of the subthreshold depolarizations seen in RA-projecting neurons is that they can be elicited by a wide range of acoustical stimuli, including the reverse BOS, as well as noise and tone bursts. This observation suggests that the neurons presynaptic to the impaled cell fire action potentials to a much broader range of stimuli than do individual HVc neurons, and shows that RA neurons generate highly song-selective action potential discharge in part by thresholding their more broadly responsive synaptic inputs.

We were interested to know whether the broad subthreshold responses seen in RA-projecting neurons are due to synaptic inputs arising from neurons outside of HVc, or instead stem from the convergence of many narrowly (but distinctly) tuned neurons local to HVc. To address this question, we coupled intracellular recordings from RA-projecting neurons with local inactivation of HVc, achieved by applying concentrated GABA acutely via a puffer pipette. In the absence of local circuit activity, persistent and prolonged (i.e., not phasic) subthreshold depolarizations were still evoked in RA-projecting neurons by song playback. These responses remained biased to forward BOS, indicating that neurons extrinsic to HVc must already exhibit a degree of less phasic song-selectivity. Similar experiments performed while recording from X-projecting neurons produced an unexpected result: inactivating the local circuit unmasked prolonged subthreshold depolarizations in X-projecting cells resembling those normally seen in RA-projecting neurons from the same bird. Therefore, it appears that the subthreshold differences that we have seen in HVc reflect a local circuit contribution, specifically involving common excitatory inputs to the two relay cells coupled with restricted interactions between inhibitory HVc interneurons and X-projecting neurons. Further evidence that the two relay cell types receive common excitatory input comes from simultaneous dual intracellular recordings from the two relay cell types, which reveal spontaneous and BOS-evoked synchronous excitatory inputs. Sequential and simultaneous dual intracellular recordings from interneurons and X-projecting cells also revealed a strong positive correlation between interneuron spike rates and maximal membrane potential negativity in X-projecting cells, consistent with the idea that interneurons drive song-evoked inhibition seen in X-projecting cells. Intracellular dialysis experiments and paired interneuron-X-projecting neuron recordings are currently underway to further dissect the mechanisms underlying song-evoked inhibition onto X-projecting cells. In summary, local inactivation experiments show that extrinsic inputs to both HVc relay cell types are song-selective, and that activity within HVc “inverts” song-related auditory activity in the X-projecting neuronal population.

We are in the process of exploring the function of auditory-evoked inhibition in X-projecting neurons. One possible role for inhibition is to restrict suprathreshold activity in X-projecting cells to distinct times in the BOS, perhaps after certain note combinations that distinguish the BOS from the songs of other birds. Indeed, playback of single notes and note combinations from the BOS indicate that in certain X-projecting neurons, particular note combinations presented in the same order as they appear in the BOS are required to drive suprathreshold responses. When the constituent notes are played either in isolation or in abnormal combinations, only subthreshold responses are elicited, and these often consist of predominantly inhibitory components. Another distinct although not mutually exclusive role for inhibition relates to the means by which X-projecting neurons activate neurons in the anterior forebrain pathway (AFP; see Figure). David Perkel and his colleagues have shown that the AFP functionally resembles the mammalian extrapyramidal system, in that the output neurons of area X make inhibitory GABAergic synapses onto the thalamic neurons in DLM, which when released from inhibition fire high frequency action potential bursts. Because DLM axons make excitatory synapses on LMAN neurons, inhibition onto X-projecting cells could serve to indirectly activate LMAN neurons, via the disinhibition of DLM neurons. We presently are using dual intracellular recordings from identified HVc relay cells and LMAN neurons to test this idea, and will discuss data from these experiments in more detail. Ultimately, these experiments can address whether song-evoked inhibition in HVc is crucial to engage portions of the song system essential to vocal plasticity. Such a mechanism could drive auditory-guided vocal plasticity, perhaps in part by leading to the coincident activation of synaptic inputs onto neurons in the song motor nucleus RA, the direct and indirect targets of HVc's two relay cell populations (see Figure). In summary, I will present data showing that HVc receives song-selective afferent drive and that the local circuit in HVc effectively "inverts" song-evoked activity in one of HVc's two relay cell types, and I will discuss the consequences of this inversion for selectivity to note combinations present in the BOS and for the activation of the two functionally distinct song system pathways.