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Editors

TANIA SIH
ALBERTO CHINSKI
ROLAND EAVEY
RICARDO GODINHO

Coordinator
TANIA SIH

Modulation of Auditory Processing by Intracortical Inhibition

Liisa A. Tremere, Ryan F. Kovaleski and Raphael Pinaud

Introduction

It is not surprising that animals like the zebra finch, a highly social songbird species that relies heavily on vocal communication between conspecifics, show anatomical and functional refinements of their central auditory pathways. In this animal, song is learned from a tutor in a critical period in post-natal development, and subsequently remains stable throughout life. This vocal learning ability is unusual in the animal kingdom and depends on intact hearing function: deafness or interference with normal auditory feedback largely disrupts vocal learning, a behavior thought to provide a basis for human speech acquisition. The perceptual processing of songs is also required for many behaviors in adult songbirds including individual identification within a flock, assessment and access to a potential mate, and for delineating and defending territory. As such, the detection and faithful neural representation of auditory communication signals in songbirds remains critical throughout adulthood of both sexes. Because intact hearing function is required for a number of behaviors in the social biology of songbirds, significant efforts have been directed at understanding the anatomo-functional organization of central auditory pathways in both developing and adult songbirds.

Central Auditory Processing

The general organization of the ascending central auditory pathways is similar in most vertebrates including songbirds. Acoustic signals are transduced in the cochlea and progress to forebrain centers through multi-synaptic circuits. In particular, the ascending system comprises a series of pontine, mesencephalic and thalamic structures that have been reviewed elsewhere and, consequently, will not be further discussed in this article (Vates et al., 1996; Mello et al., 1998; Mello and Pinaud, 2006). Importantly, at the level of the forebrain, information is received via a set of thalamic auditory projections to a structure named Field L2, the functional counterpart of the thalamorecipient layer IV in mammals (Vates et al., 1996; Mello and Pinaud, 2006). This area then sends information to a number of adjacent auditory forebrain areas including the caudomedial nidopallium (NCM) and the caudomedial and caudolateral mesopallium (CMM and CLM, respectively) (Vates et al., 1996; Mello et al., 1998; Mello and Pinaud, 2006). NCM is the focus of this review. Importantly, the present article does not provide a comprehensive review of NCM's anatomy and function, but rather focuses on data from our group presented during the annual IAPO meeting. For additional reading see (Vates et al., 1996; Mello et al., 1998; Mello, 2002; Mello et al., 2004; Mello and Pinaud, 2006). NCM neurons have been shown to have complex response properties with rich informational value. These cells often have multi-frequency and dynamic receptive fields. Interestingly, slightly over half of the neurons in NCM are inhibitory (GABAergic) with the other half presumably being composed of excitatory (glutamatergic) cells (Pinaud et al., 2004; Pinaud et al., 2008b). These findings suggested that inhibitory transmission may be critical to the physiology of NCM and, consequently, to the auditory processing of behaviorally-relevant communication cues.

GABAergic Neurons in the Auditory Forebrain

In the auditory system, a primary function identified for GABAergic transmission was to suppress neuronal responses to frequencies that flank a neuron's preferred frequency. Benefits of this form of inhibition in sensory processing include the sharpening of receptive fields and improved efficiency in the coding of sensory signals. Subsequent research specifically revealed the importance of GABAergic inhibition in determining sound location as well as other more complex processing like sensitivity to harmonics or directionally restricted sweeps in frequency.

Surprisingly, the organization and contributions of inhibition to the auditory processing of songs in songbirds had not been systematically explored until recently.

In our initial studies we aimed to identify inhibitory neurons in the songbird auditory forebrain. To this end we cloned the zebra finch homologue of the 65 kDa glutamic-acid decarboxylase gene (zGAD65), one of the synthetic enzymes for GABA, and utilized this cDNA to generate zebra finch-specific riboprobes for in-situ hybridization (Pinaud et al., 2004). Importantly, we also characterized an antibody that recognizes GABA in zebra finch tissue (Pinaud et al., 2004; Pinaud and Mello, 2007; Pinaud et al., 2008b). The molecular profile generated with in-situ hybridization for zGAD65 mirrored results obtained with immunocytochemistry directed at GABA allowing the confirmation GABA production by cells in NCM. Our histological studies revealed that, based on soma size, there are at least two categories of GABAergic cells in NCM, small (3.3–10 µm) and large diameter neurons (15–20.8 µm). While not empirically tested, smaller neurons typically support local circuit functions. This cell type accounted for the majority of inhibitory neurons within NCM (Pinaud et al., 2004; Pinaud et al., 2008b). A second type of neuron revealed with our studies was a large GABA-positive neurons, that were only occasionally found in NCM and may be projection neurons (Pinaud et al., 2004; Pinaud et al., 2008b).

In order to address the extent to which inhibitory neurons are activated as part of song processing, we developed a double-fluorescence in-situ hybridization protocol for the detection of two mRNA species, at single cell resolution (Pinaud et al., 2004; Pinaud et al., 2008a; Jeong et al., 2010; Jeong et al., 2011). With this method, we were able to use an activity-dependent gene named *zenk*, to map song-responsive neurons, and our zGAD65 probes to identify inhibitory neurons. We determined that GABA producing neurons in NCM are directly activated by auditory experience in freely-behaving birds (Pinaud et al., 2004). These observations are consistent with the notion that local inhibitory transmission during auditory processing is physiologically relevant and part of the neural basis of vocal communication.

Tonic GABAergic Synaptic Activity Regulates the NCM's Excitatory Circuits

Using a slice preparation containing NCM, we initiated efforts aimed at determining how inhibition shapes the physiology of NCM neurons (Pinaud et al., 2004; Pinaud et al., 2008b). We observed that all NCM neurons have high levels of spontaneous synaptic activity that was, on average, 3.1 Hz. Of these events most were inhibitory, mediated by the $GABA_A$ receptor and were abolished by bath application of bicuculline (BIC), a selective $GABA_A$ receptor antagonist (Pinaud et al., 2004; Pinaud et al., 2008b). Spontaneous inhibitory post-synaptic currents (sIPSCs) were observed in nearly all cells in our preparation and synaptic events averaged 48 pA in amplitude with a decay time constant (τ) of 6.9 ms. These findings are consistent with reported observations for $GABA_A$ receptor properties from a variety of comparable preparations. Pharmacological blockade of spontaneous $GABA_A$-mediated synaptic events was used to address the potential functional importance of on-going inhibition. Release from inhibition revealed large, powerful bursts of excitatory synaptic activity (average: 232 pA) that occurred at very low frequencies (average: 0.08 Hz) (Pinaud et al., 2008b). The events were completely blocked by bath application of 6,7-dinitroquinoxaline-2,3-dione (DNQX) indicating that they are mediated by non-NMDA glutamatergic transmission. Thus, one of the functional roles for tonic GABA release in NCM is to suppress the spontaneous activity of the excitatory network.

Inhibition Shapes Song Coding in the Awake Brain

We next investigated how inhibitory transmission in NCM shapes song processing in the awake brain. To this end we combined bilateral, multi-electrode extracellular recordings with local pharmacological manipulations in the NCM of adult, restrained zebra finches. Song driven responses occur throughout NCM and song driven spike trains typically capture much of the temporal organization of the stimulus structure (Pinaud et al., 2008b). Blockade of GABAergic inhibition dramatically altered the temporal precision between the stimulus and spike response relationship. At the level of individual song syllables, the information bearing unit of song, sustained responses were instantaneously converted into highly phasic, on/off-like responses. The result of this change was that coded song (spike trains) reflected fewer features of song and was on occasion not aligned with the stimulus due to changes in the temporal relationship between stimulus and response. Importantly, total voltage changes integrated over the period of the played song did not change so the results did not reflect increased activity at disinhibited neurons but rather inconsistent shifts following presentation of the adequate stimuli. Surprisingly, BIC occluded the responsiveness of NCM neurons to certain song syllables (Pinaud et al., 2008b). Importantly, attenuations in coding fidelity caused by antagonism of $GABA_A$ receptors were not due to the inability, by NCM neurons, to discriminate between frequencies, as frequency-tuning curves were not affected by $GABA_A$ receptor blockade.

Concluding Remarks

The findings presented in this review show a strong presence of putative local GABAergic interneurons in a key anatomical station in the songbird ascending auditory pathway, the NCM. In addition, NCM houses a low number of large, GABAergic neurons likely to be projection neurons. Physiological studies mentioned in this review indicate that GABAergic synapses in NCM are critical to maintaining baseline inhibition to suppress spontaneous excitatory network activity. In naturalistic states of activation, as is observed for the recording of song driven neuronal responses made in awake animals, the roles of GABAergic synaptic transmission in song coding are robust. Local blockade of inhibitory transmission in NCM caused considerable deterioration in the temporal organization of song coding with a complete loss of representation for certain song elements. Consistencies in the anatomical and functional organization of inhibitory circuits in the auditory system, observed between songbirds and most vertebrate species, underscore the importance of GABAergic transmission in sensory information coding and processing.

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