主論文の要約

Excitatory-Inhibitory Synaptic Coupling in Avian Nucleus Magnocellularis

（トリ蝸牛神経核におけるフィードフォーワード抑制性投射による出力制御機構）

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【Introduction】

NM neurons receive excitatory input from auditory nerve fibers (ANFs) and transmit phase-locked spikes bilaterally to coincidence detectors to calculate interaural time differences for sound localization. The level and timing of NM output are critical for this calculation, and therefore must be tuned across sound frequency and intensity in multiple mechanisms.

One of the mechanisms is the differentiation of excitatory input according to the neuronal characteristic frequency (CF). Low-CF (<0.5 kHz) neurons receive small bouton terminals from multiple ANFs, each of which causes a small response insufficient to generate a spike, whereas higher-CF neurons receive a few end-bulb terminals, each of which causes a large response sufficient to generate a spike. This differentiation ensures precise neuronal output across frequencies, despite that fluctuation of spike timing (jitter) is larger in ANFs tuned to low-frequency sound.

Another mechanism is related to the feedforward polysynaptic inhibitory input from ANFs via superior olivary nucleus (SON). The effects of this inhibitory input were studied in high-CF neurons and showed that inhibition reduces the level and jitter of spike output specifically for intense sounds through an increase of a shunting conductance. However, it remains unknown how the balance between the excitatory and inhibitory inputs changes according to the activity of ANFs and how that balance shapes the output of NM across frequencies.

【Material and Methods】

Chickens (Gallus domesticus) of either sex at post-hatch days 0–7 were used in this study. We prepared a thick slice (2.5 mm thickness) involving both excitatory and inhibitory circuits in the auditory brainstem (Fig. 1). Voltage-clamp recordings were performed in either whole-cell or cell-attached condition using a pipette filled with Cs^+-based solution. Ipsilateral ANFs were stimulated by applying electrical pulses via a bipolar tungsten electrode placed within the distal part of the bundle of ANFs (Fig. 1, red arrow). In some experiments, DNQX (60–80 μM) and SR-95531 (100 μM) were applied to the bath to block excitatory and inhibitory transmission, respectively.

【Results】

We stimulated ANFs and examined the intensity dependences of EPSCs and IPSCs in NM neurons under whole-cell clamp (Fig. 2). In low-CF neurons, both EPSCs and IPSCs had similar threshold and increased in parallel with intensity, making them balanced for wide intensity ranges particularly at a later part of the train (20 stimuli). In higher-CF neurons, on the other hand, IPSCs had extremely higher threshold and were kept smaller than EPSCs for intensities.

We then examined spike responses of these neurons to the ANF stimulation under cell-attached clamp (Fig. 3). In low-CF neurons, the number of spikes increased gradually with intensity toward the later part of the train and it required strong stimuli for saturation. In addition, spike jitter decreased with intensity at each stimulus, which would reflect the balanced EPSCs and IPSCs in the neurons. In higher-CF neurons, spikes persisted and the jitter remained small throughout the train even at the lowest intensity, which were consistent with the large unitary EPSCs in the neurons. These made dynamic range of responses wider in low-CF neurons than in higher-CF neurons.

We finally examined the effects of inhibition on these spike responses in the low-CF neurons (Fig. 4). The application of a GABA blocker (SR-95531) increased the number of spikes particularly at the later part of the train, which caused a prominent leftward shift in the intensity dependence of firing probability; it lowered both the threshold intensity (single arrowheads) and the saturating intensity (double arrowheads), thus narrowing the dynamic range of responses.

【Discussion】

IPSCs were more readily triggered in low-CF NM neurons than in higher-CF NM neurons, suggesting a greater importance of inhibition in control of responses to low-frequency sound. Elevation of sound intensity increases the number of active ANFs and the firing rate in each fiber. This elevation of intensity should increase the size of EPSPs rather evenly at each cycle for low-frequency sound (Figs. 5A, B). In this situation, EPSPs may already reach a level close to spike threshold at a majority of cycles even at auditory thresholds, and hence a slight increase in the sound intensity may easily cause saturation of spike responses and narrowing the dynamic range without inhibition. Thus, the tight coupling between activation of EPSCs and IPSCs is particularly suited for the low-CF NM neurons. For high-frequency sound, many sound cycles fail to trigger spikes in ANFs due to the short period of the sound, although the number of these failed cycles decreases at higher sound intensity (Figs. 5C, D). Because higher-CF neurons receive only one or a few ANFs, and individual EPSPs are large enough to generate a spike, it is expected that elevation of sound intensity may increase the number of cycles with EPSPs rather than the size of EPSPs at each cycle.

【Conclusion】

In this study, we developed a thick-slice preparation of the chicken brainstem, and showed that the relationship between excitatory input and feedforward inhibitory input was expressed differentially at each tonotopic region; the relationship was linear in neurons tuned to low frequency, expanding dynamic range by preventing saturation of spike generation; by contrast inhibitory input remained much smaller than excitatory input in

neurons tuned to higher frequency, thus ensuring high-fidelity transmission. These tonotopic regulations of feedforward inhibitory input optimized the output across frequencies and intensities, playing a fundamental role in the timing coding pathway in the auditory system.