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Neuronal specializations for the processing of interaural difference cues in the chick

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Sound information is encoded as a series of spikes of the auditory nerve fibers (ANFs), and then transmitted to the brainstem auditory nuclei. Features such as timing and level are extracted from ANFs activity and further processed as the interaural time difference (ITD) and the interaural level difference (ILD), respectively. These two interaural difference cues are used for source localization by behaving animals. Both cues depend on the head size of animals and are extremely small, requiring specialized neural properties in order to process these cues with precision. Moreover, the sound level and timing cues are not processed independently from one another. Neurons in the nucleus angularis (NA) are specialized for coding sound level information in birds and the ILD is processed in the posterior part of the dorsal lateral lemniscus nucleus (LLDp). Processing of ILD is affected by the phase difference of binaural sound. Temporal features of sound are encoded in the pathway starting in nucleus magnocellularis (NM), and ITD is processed in the nucleus laminaris (NL). In this pathway a variety of specializations are found in synaptic morphology, neuronal excitability, distribution of ion channels and receptors along the tonotopic axis, which reduces spike timing fluctuation in the ANFs-NM synapse, and imparts precise and stable ITD processing to the NL. Moreover, the contrast of ITD processing in NL is enhanced over a wide range of sound level through the activity of GABAergic inhibitory systems from both the superior olivary nucleus (SON) and local inhibitory neurons that follow monosynaptic to NM activity.

Keywords: brainstem auditory nucleus, interaural difference cues, SON, tonic inhibition, phasic inhibition

INTRODUCTION

The auditory nervous system is highly sensitive to changes in acoustic signals both in the frequency and the level (Dooling et al., 2000; Klump, 2000). Activity of ANFs codes the sound timing as the phase-locked-firing and the level as the firing-rate. Anatomically separate, and physiologically distinct pathways process these two auditory features (Oertel, 1999; Carr and Code, 2000). Anatomical separation is particularly distinct in the avian auditory system (Figure 1), where the pathway starting from NM carries the temporal information, and ITD is processed in NL. The pathway starting from NA carries the intensity information and ILD is processed in LLDp (Sullivan and Konishi, 1984; Takahashi et al., 1984). These two interaural differences inherent in auditory signals are used as cues for sound source localization (Moiseff, 1989). ITDs are generally used for processing low frequency sounds, while ILD is a cue used for high frequencies (Rayleigh, 1907).

Sharpening of ITD selectivity by GABAergic inputs has been demonstrated in higher auditory nuclei such as the inferior colliculus of the barn owl (Fujita and Konishi, 1991). In mammals, neurons in medial superior olive receive glycinergic inhibitory innervation from the medial and the lateral nucleus of the trapezoid body (Kuwabara and Zook, 1992; Grothe and Sanes, 1994). We are therefore interested in the presence and the roles of such inhibitory innervations in the ITD processing of NL. GABAergic innervations in NL are mostly from neurons in SON and some from the GABA positive interneurons located near NM and NL (Code et al., 1989; von Bartheld et al., 1989; Yamada et al., 2013). SON receives excitatory inputs from ipsilateral NA and NL, and inhibitory inputs from the contralateral SON, and makes projections to the ipsilateral NL, NM, NA and to the contralateral SON (Figure 1; Lachica et al., 1994; Yang et al., 1999; Monsivais et al., 2000; Burger et al., 2005).

In this review article I will first discuss the possible interplay between ILD and ITD, then I will detail the specializations found in the timing processing pathway, and the role of inhibition to make the ITD tuning tolerant to the sound level.

ILD PROCESSING IS AFFECTED BY INTERAURAL PHASE DIFFERENCE

Timing and level information is processed in separate neuronal pathways originating in the cochlear nuclei but ultimately merge in the midbrain, mesencephalicus lateralis dorsalis (avian homolog of the inferior colliculus; Pena and Konishi, 2001; Konishi, 2003). However, they are not processed in total separation even at lower levels. They influence one another at multiple steps of encoding and processing. Sound level affects processing of ITD under certain conditions (Viete et al., 1997; Dasika et al., 2005; Nishino et al., 2008), and sound timing affects processing of ILD (Sato et al., 2010; in mammals see Finlayson and
and the ipsilateral dominant sound across 0 ILD indicates the direction selectivity of LLDp units. This IPD effect on ILD processing in LLDp neurons may compensate for the small ILD cue available to the animal (Sato et al., 2010). The balance of excitation and inhibition changes with sound location, and in the barn owl LLDp, it is reported that the reliability of the response to spectrottemporal feature of LLDp neuron is enhanced by temporally delayed inhibition of LLDp neurons through gain modulation of the input-output function of the neuron (Steinberg et al., 2013).

**SYNAPTIC SPECIALIZATIONS IN NM**

Neurons in NM do not have appreciable dendrites, and ANFs make synapses on the cell soma. ANFs form enfolding end-bulbs of Held around the cell body in the high and middle characteristic frequency (CF) neuron but not in low CF neurons. Accordingly the EPSCs recorded in the high-middle CF NM neurons are large and generated in all-or-none manner with a small number of amplitude steps when the intensity of electrical stimulation applied to the ANFs bundle is changed, while the EPSCs recorded in the low CF neurons are small and the size gradually increases depended on the intensity of electrical stimulus (Fukui and Ohmori, 2004). NM neurons express low-voltage-activated K\textsubscript{v}1.1 channels with a gradient along the tonotopic axis. High CF neurons have stronger K\textsubscript{v}1.1 channel expression and conductance, resulting in more negative resting membrane potential and higher spike threshold. Blocking these channels by dendrotoxin depolarizes the resting membrane potential and reduces the spike threshold (Fukui and Ohmori, 2004). Dendrotoxin is known to block low-voltage-activated K\textsuperscript{+} channels of K\textsubscript{v}1.1, K\textsubscript{v}1.2, and K\textsubscript{v}1.6 subtypes (Hopkins et al., 1994; Harvey, 2001). Synaptic transmission during on-going stimuli is robust in the high-middle CF synapse but is depressed quickly in low CF synapses (Oline and Burger, 2014). A large readily releasable pool size in the high-middle CF terminals could maintain the reliable transmission. This may function to maintain the suprathreshold EPSCs in high CF neurons while enabling summation to enhance phase-locking in low CF neurons as it is discussed below.

NM neurons are specialized to encode temporal information of sound from ANFs activity. The low frequency sound information is strongly phase-locked, however it is actually encoded with a large timing jitter in ANFs. This timing fluctuation is reduced during transmission from ANFs to NM neurons (Fukui and Ohmori, 2004; Fukui et al., 2006; see Joris et al., 1994). Here, the mechanism is explained by the temporal integration of small EPSPs. Because the low frequency NM neuron is innervated by a large number of small bouton shaped synapses, single EPSPs are so small that multiple EPSPs are required to summate in order to reach spike threshold (Fukui and Ohmori, 2004; Kubo and Ohmori, 2009). Therefore, only those synaptic inputs arriving within a limited time window could contribute to NM spike; NM activity becomes more precisely phase-locked than ANF activity. However, the integration makes the depolarization of the NM neuron slow, which increases the level of inactivation of Na\textsuperscript{+} channels. Axon initial segment (AIS), the site of action potential initiation, is extended longer in the axon of low CF NM neurons than the high-middle CF NM neurons. Clustering of a large number of Na\textsuperscript{+} channels at the AIS would allow sufficient
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FIGURE 2 | Responses of NA unit and LLDp units to binaural stimuli.
(A) Response of NA units to contralateral tone of 200 Hz. Binaural stimulus was applied in-phase (solid black line) or 180° out of phase (dotted line) with a constant ipsilateral tone of 53 dB; 20 dB above the threshold. Responses induced by contralateral monaural stimulus are included (solid gray line).
(B) Rate-ILD relationship of LLDp units. ILD was defined as ipsi-contra SPL [dB]. Different symbols indicate different cells. Solid lines are for in-phase and broken lines are for 180° out-of-phase relationships. (C,D) IPD modulation of rate-ILD relationship of LLDp units with strong ipsilateral inhibition (C) with weak ipsilateral inhibition (D). The inset indicates IPDs applied to both (C,D).
The slope of rate-ILD relationship across zero ILD is affected by IPD in (C) but not in (D). Reproduced with permission from Sato et al. (2010).

SPECIALIZATIONS OF ITD ENCODING IN THE NL
Somas of NL neurons have bipolar tufted dendrites and an axon emerges from the cell body. Dendrite morphology changes systematically along the tonotopic axis. Dendrites are short, relatively unbranched, occur in large numbers in high CF NL cells. The number of dendrites decreases in the middle-CF neurons but they become thicker and longer. Only a few primary dendrites extend away from the soma in the low-CF neurons, and they have extensive branching (Smith and Rubel, 1979; Kuba et al., 2005; Sanchez et al., 2010).

ITD depends on head size, and in most birds, the physiological maximum ITD is smaller than 100 μs. Considering the maximum firing rate of most neurons is less than or equal to 1 kHz, this maximum available ITD cue is extremely small; thus the auditory system needs specialization to process ITDs accurately.

During embryonic development, NMDA receptor currents increase in the NM-NL synapse, however it decreases dramatically before hatching. AMPA receptor currents increase during the embryonic development, particularly in the high CF NL cells. The EPSC kinetics becomes faster with development and rectifies in all CF regions, suggesting the exclusion of GluR2 receptor subunits from the synapse (Sanchez et al., 2010). Kinetics and amplitude of EPSCs are symmetrical in single NL neurons between inputs of two sides (Lu, 2009). Moreover, tonotopic gradients are matched
between the EPSC time course and the feature of postsynaptic band-pass filtering in single NL neurons (Slee et al., 2010). These are consistent with the faster EPSC and mEPSC kinetics in NL neurons after hatching (Kuba et al., 2005).

**LOW-VOLTAGE-ACTIVATED K⁺ CHANNELS ENHANCE COINCIDENCE DETECTION, AND MAKE ITD DETECTION MOST SENSITIVE FOR MID-FREQUENCY SOUND**

The best sensitivity to ITD or the smallest error of sound source localization was observed in the mid-audible frequency range in the avian species (Klump, 2000). Consistent with this observation, we found that the coincidence detection of bilateral NM spikes was most accurate in the middle-CF NL neurons. In brainstem slice experiments of the post-hatch chicks conducted at body temperature, 40°C, the time window of coincidence detection was 1700, 300, and 600 ms, for the low, middle, and high CF neurons, respectively; the time window is defined as the time separation of bilateral stimuli applied to projection fibers from NM, which generates spikes in more than 50% trials (Kuba et al., 2003). Moreover, we found that the time course of EPSP measured as the half amplitude width have a significant positive correlation with the time window of coincidence detection (Figure 3); therefore, NL neuron with fast EPSPs has temporally sharp coincidence detection. The time course of EPSC is progressively faster toward high CF neurons. However, the time course of EPSP is fastest in mid-CF neurons, which is almost the same or sometimes faster than the time course of EPSP recorded in the same neuron (Kuba et al., 2005). The falling phase of the EPSP was accelerated due to strong activation of low-voltage-activated K⁺ channels caused by EPSPs. Application of dendrotoxin prolonged the falling phase of EPSP. The expression of Kv1.2 channels is confirmed immunohistochemically in the NL, and the density of immuno-reactivity is the highest in the mid-CF region, where the time window for the coincidence detection is most precise (Kuba et al., 2005). These findings are consistent with the idea that Kv1.2 channels accelerate EPSP time course in the middle-CF NL neurons.

**Na⁺ CHANNEL DISTRIBUTION IN AIS MAKES SPIKE-GENERATION STABLE IN WIDE FREQUENCY RANGES**

We have been puzzled for a long time by the observation that the spikes and Na⁺ currents were small in the high and middle CF NL neurons than those of low CF NL neurons (Kuba et al., 2003, 2005, 2006). By immunohistochemical observations we found that the AIS is extended in length and located close to the cell soma in low CF NL neurons while short and located distant in the high CF NL neurons. The significance of this Na⁺ channel distribution is interpreted by a computer simulation using a NEURON model under an assumption that NL neurons receive excitatory synaptic inputs at the frequency that closely matches with their CF; namely the frequency of synaptic inputs is high in the high CF NL neurons and low in the low CF NL neurons. Simulations demonstrated that the depolarization of the cell soma is greater in high CF NL neurons than the low CF NL neurons during sound inputs. This depolarization would inactivate Na⁺ channels and prevent spike generation if the AIS, thus Na⁺ channel, is located close to the cell soma. By displacing the AIS to a distance where the level of steady depolarization is small because of the electro-tonic property of the axon, the level of Na⁺ channel inactivation should be reduced; however the reduced level of membrane depolarization may also reduce the activation level of Na⁺ channels at a distance. Consequently, the balance of activation and inactivation of Na⁺ channels is achieved, and the spike generation is optimized by controlling the spatial distribution of Na⁺ channels for each NL neuron depending on its CF. This is likely the underlying mechanism for the stable processing of ITD in each NL neuron (Kuba et al., 2006; see also Ashida et al., 2007).

**HCN CHANNELS MODIFY THE COINCIDENCE DETECTION**

Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels have a reversal potential around −30 mV, are activated by membrane hyperpolarization, and the voltage-sensitivity is modulated by cyclic nucleotides. Channel gating is shifted in the positive direction when the cytosolic concentration of cyclic nucleotides is high, and the sensitivity to cyclic nucleotide is greater in HCN2 than in HCN1 channel subtype (Pape, 1996; Santoro and Tibbs, 1999; Biel et al., 2009). In the chicken NL, both HCN1 and HCN2 channels are expressed along the tonotopic axis with a gradient (Yamada et al., 2005). Expression of HCN1 is graded extensively toward the low CF region of the nucleus, while the expression of HCN2 is less graded across the nucleus. The membrane depolarization of NL neurons was confirmed when the level of cyclic AMP was raised either by incubation of slices with 8-Br-cAMP or by photo-illumination of the cell that was loaded with a caged compound of cyclic AMP through the patch electrode, which likely reflected an increased level of activation of HCN channels (Yamada et al., 2005). The membrane depolarization improved the coincidence detection by accelerating the time course of EPSPs, presumably because of the activation of low-voltage-activated K⁺ channels. The relatively high density of HCN2 channels over HCN1 channels in the...
SUSTAINED GABAergic INHIBITION IMPROVES ITD PROCESSING

Firing rates of ITD processing neurons alternates periodically as ITD changes during a tonal stimulation, and the period of the ITD tuning curve was determined by the CF of the neuron (Goldberg and Brown, 1969; Carr and Konishi, 1990; Yin and Chan, 1990). The sound pressure level affected the contrast between the peak and trough firing rates (Pena et al., 1996). Loud sound was expected to increase the firing rate both at the peak and the trough of ITD tuning curve, and to reduce the peak-trough contrast (or ITD sensitivity, Dasika et al., 2005). However, the peak-trough contrast was actually maintained rather than reduced at high sound pressure level in in vivo recordings from the barn owl (Pena et al., 1996). Pena and colleagues proposed that inhibition from SON controls the ITD tuning in NL, making it tolerant to sound pressure level.

By recording single unit activity in NL in vivo, ITD tuning was found dependent both on the sound frequency and the sound pressure level (Nishino et al., 2008). The peak-trough contrast in mid-to-high CF NL units (higher than 1 kHz) was maximal at intermediate sound pressure levels. The peak-trough contrast was practically lost when a very loud sound was applied because of the increased firing rate both at the peak and the trough of ITD tuning curve (90 dB or louder sound). In low CF NL units (lower than 1 kHz), neural activity was temporally suppressed after a loud sound. The peak-trough contrast became larger as the sound became louder. This is because the trough-firing rate decreased with the sound pressure level, even to the level lower than 1 kHz.

**FIGURE 4 | Modulation of peak-trough contrast of ITD tuning curve of low-CF NL neurons by inhibition.** Peak-trough contrasts of ITD tuning curve are calculated by including the sustained inhibition of weak (gray line), strong (black line), and phasic inhibition (dotted gray line) separately, and by including both the strong sustained and the phasic inhibition (dotted black line). Modified from Yamada et al. (2013).
than the spontaneous firing rate. These observations are consistent with the sustained SON inhibition of low CF NL neurons. Consistently after electrical lesioning of the ipsilateral SON, the contrast of ITD tuning in the low CF NL neuron collapsed at low sound (Nishino et al., 2008), and the tolerance of ITD tuning to the sound pressure level became similar to that of the mid-to-high CF NL units. The sound pressure level dependence of ITD processing of the mid-to-high CF NL neurons was not virtually affected by lesioning of the SON. SON receives sound pressure level information through NA (Figure 1), and GABAergic projection from SON to NL is robust in the low CF region of NL but becomes less prominent toward the high CF region. The density of the SON projection along the tonotopy is correlated with the magnitude of the response to SON lesions across the tonotopic axis in NL (Nishino et al., 2008). We conclude, accordingly, that the dense inhibitory projection from SON to NL makes the ITD tuning tolerant to the sound pressure level in NL (Nishino et al., 2008).

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