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Binaural Spectral Processing in Mouse Inferior Colliculus

Xi Bie

zoebiexi@gmail.com

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Binaural Spectral Processing in Mouse Inferior Colliculus

Xi Bie, Ph.D

University of Connecticut, 2015

ABSTRACT

Frequency tuning is a basic property of neurons in the auditory system since individual neurons respond to part of the spectrum of audible sounds. Sound localization relies on binaural cues and is a second basic property of the system. Yet, little is known about how frequency tuning is affected by binaural stimulation. Since the inferior colliculus (IC) in the midbrain integrates virtually all of the binaural and monaural information ascending from the lower auditory brainstem nuclei, it is an ideal location in which to study the influence of binaural (diotic) stimulation on spectral processing. To do so, we recorded from single neurons in the mouse IC to binaural and monaural tones delivered sequentially in each trial. Neurons were analyzed according to how the monaural frequency response area (FRA) was altered by binaural stimulation. Overall, the maximum firing rate, Q10, Q40, best frequency (BF), characteristic frequency (CF), and thresholds for the population were similar to contralateral and binaural stimulation. However, not all neurons reacted similarly, and the types of changes resulting from binaural stimulation were identified using cluster analysis to separate neurons based on the ratio of the binaural/contralateral responses. About a 40% of the neurons had maximum firing rates and bandwidths that were unchanged by binaural stimuli, but the remaining 60% showed some combination of change in maximum firing rate, bandwidths, or threshold. The remaining cells all have rates changed by binaural

stimulation, either increased or decreased, while the effects on the bandwidth were different. When neurons had maximum firing rates that were suppressed by binaural stimulation, this resulted in sharper tuning, and the thresholds to binaural stimuli were either higher or lower. Fewer neurons had firing rates enhanced by binaural stimulation but were unchanged in bandwidth or threshold. The inhibitory FRA generated by binaural two-tone stimulation of the ipsilateral ear was usually similar in BF to the FRA from contralateral excitation, but some neurons showed sideband inhibition generated by the ipsilateral stimulation. We conclude that binaural stimulation can alter the spectral processing of many neurons in the IC, and this might facilitate processing of the sound localization and sound texture.

Binaural Spectral Processing In Mouse Inferior Colliculus

Xi Bie

B.S., Wuhan University, 2009

A Dissertation

Submitted in Partial Fulfillment of the

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Xi Bie

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APPROVAL PAGE

Doctor of Philosophy Dissertation

Binaural Spectral Processing In Mouse Inferior Colliculus

Presented by

Xi Bie, B.S.

Major Advisor

Douglas L. Oliver, Ph.D

Associate Advisor

Richard Mains, Ph.D

Associate Advisor

Shigeyuki Kuwada, Ph.D

Associate Advisor

Srdjan Antic, Ph.D

Associate Advisor

Monty Escabi, Ph.D

University of Connecticut
2015

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CHAPTER 1: INTRODUCTION

1.1 Sound and auditory system

Sound is characterized as a vibration of mechanical waves of pressure and displacement, which are transmitted through a medium, such as water, air, etc. Sound waves exhibit 4 major features: frequency, amplitude, waveform, and phase. The frequency (cycle per second) of sound is measured in Hertz (Hz), where 1 Hz is defined as one cycle per second. The amplitude of sound corresponds to the sound level in hearing physiology and is related to the perception of loudness. It is measured from a reference level in log units, the decibel (dB). The waveform of sound is the amplitude plotted against time. A pure tone can be visualized as a sinusoidal wave. However, in the natural environment, sounds usually consist of acoustically complex waveforms. Those waveforms can be modeled as the sum of sinusoidal waves of varying amplitudes, frequencies and phases. Phase represents the particular point in the cycle of a waveform, which is measured as an angle in degrees. The auditory system is the sensory system for the sense of hearing. It consists of the peripheral auditory sensory organ, the ear, and the central auditory system which is located in the brain. The ear collects sound from the environment, transduces the mechanical waves of sound into neural signals, and finally sends it to the central auditory system in the brain.

The inner ear transfers the mechanical information of the sound into neuronal electrical signals in the auditory system. When airborne sound reaches the subject, the external and middle ears collect the waves, amplify them, and then transmit the mechanical waves to the fluid-filled cochlea of the inner ear. The basilar membrane

within the cochlea vibrates in response to sound, and the vibration varies systematically at different positions as a function of the frequency of the sound (Von Békésy, 1952; Kiang et al., 1967; von Békésy, 1970). Instead of pure tones, sound in nature are usually very complex. A Fourier transform can decompose a complex waveform into a combination of single sinusoidal waves. In the auditory system, the cochlea acts as the mechanical frequency analyzer that decomposes the complex sound into sinusoidal components. Each point on the basilar membrane has a characteristic frequency (CF) to which it is most sensitive. The base of the basilar membrane responds best to high frequency, while the apex responds to low frequency (Békésy, 1951). Thus, as the position on the basilar membrane changes from the apex to the base, the CF systematically changes from low to high. All in all, this creates a tonotopic organization.

An inner hair cell transforms the mechanical vibration into an electrical signal by the bending of stereocilia, stretching of the filamentous tip-links that attach cilia to the excitable membrane, and this leads to the depolarization of the hair cell membrane, also known as receptor potential. In particular, hair cells at each point along the basilar membrane respond to the CF of that point. The depolarization of the hair cell membrane leads to release of glutamate, eventually leading to an action potential in the auditory nerve fibers.

The auditory nerve is a part of the VIIIth cranial nerve whose fibers innervate the inner hair cells of the cochlea. The nerve fibers come from the spiral ganglion cells reside in the cochlear, who also send axons into cochlear nucleus. Several fibers synapse onto one hair cell. As a result, these nerve fibers have the same CF as the hair cell they innervate, and the spectral sensitivity (frequency information of the sound) of the hair cell

is transmitted to the nerve fiber (Evans, 1972). The nerve fibers then project to the cochlear nucleus in the brainstem and carry the neural signals with auditory information into the central auditory system.

1.2 The auditory pathway in the brain

1.2.1 Auditory pathway in cochlear nucleus. The auditory nerve fibers carry the spectral information from the peripheral auditory system into the cochlear nucleus in the brainstem. Each auditory nerve fiber bifurcates, one branch descends to enter the posteroventral cochlear nucleus (PVCN) and the dorsal cochlear nucleus (DCN). The other branch ascends to enter the anteroventral cochlear nucleus (AVCN) (Cant, 1992). At that location, they synapse on the neurons of the cochlear nucleus.

The cell types and organization in the three parts of the cochlear nucleus differ tremendously. The T-stellate cells which have a radial, star-like dendritic tree, and they are the main source of direct inputs to the IC from the AVCN (Osen, 1969). Outputs of T-stellate cells can directly project to the integration center in the midbrain --the inferior colliculus (IC), or project indirectly to the IC from the superior olivary complex or the nuclei of the lateral lemniscus (Oliver, 1987). Bushy cells have several short branches of dendrites that look like a “bush”, and reside mainly in the AVCN, mainly in the ventral portion of the AVCN. They are categorized as spherical bushy cells or globular bushy cells depending on the location and shape. The endbulbs of Held are large terminals of the auditory nerve fibers that synapse on the large, low-frequency spherical bushy cells. The axons of these cells project to the medial superior olive (MSO) on both sides (Fig.1.1). The small spherical bushy cells are tuned to higher frequencies and their axons project to the lateral superior olive (LSO) on the ipsilateral side. The axons of globular

bushy cells project to the contralateral medial nucleus of the trapezoid body (MNTB), and, in turn, sends an inhibitory output to the LSO (Osen, 1969; Ryugo and Parks, 2003). As a result, the bushy cells in the AVCN project to the IC via the superior olivary complex, while the stellate cells send direct and indirect outputs to the IC.

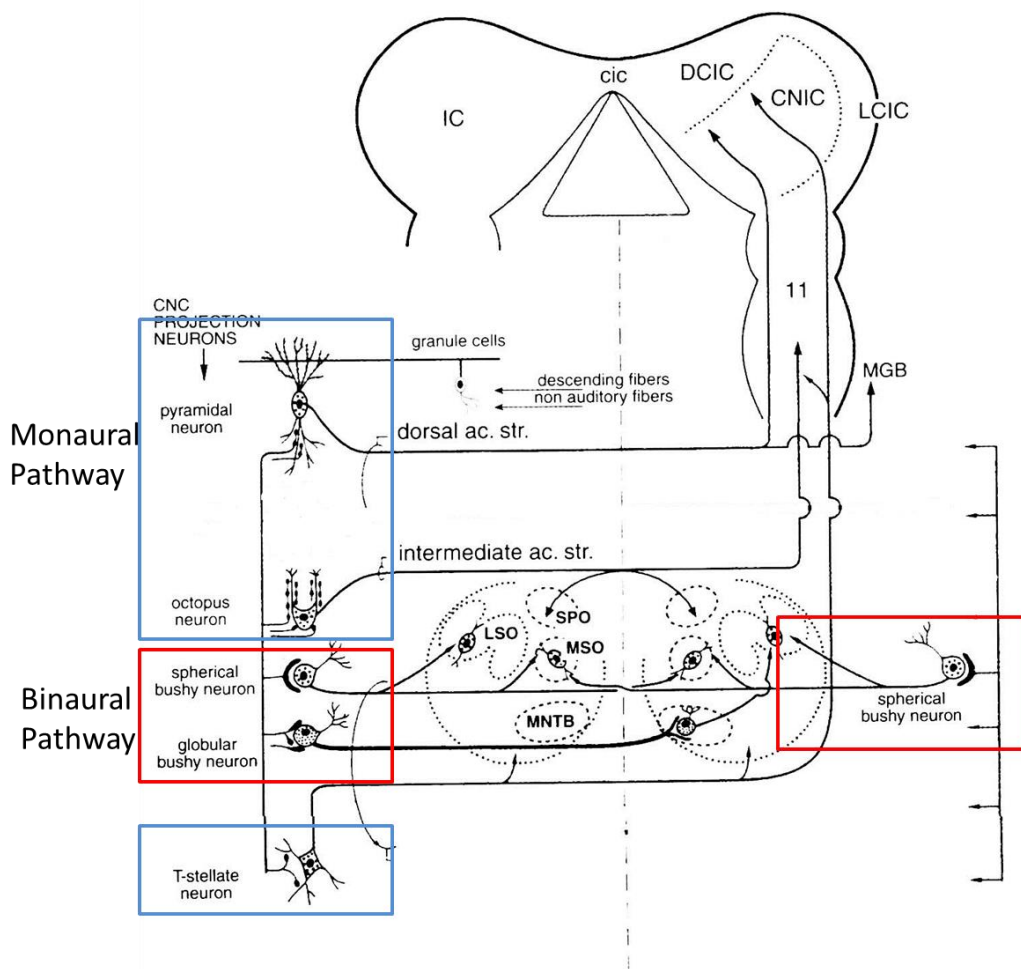


Figure 1.1. The outputs of cells from cochlear nucleus (adapted from Moore and Osen, 1979). The monaural pathways are indicated as the blue box that have direct projection to the IC, while the red box indicate the indirect pathways project to the superior olivary complex that process information from both ears.

Octopus cells are exclusive to the PVCN. They have large and irregularly shaped cell bodies with several large dendrites emerging from one side, much like the tentacles of an octopus (Osen, 1969; Kane, 1973; Adams, 1986). The octopus cells project to the

contralateral superior paraolivary nucleus (SPO) and the ventral nucleus of the lateral lemniscus (VNLL) (Godfrey et al., 1975; Rhode et al., 1983; Rhode and Smith, 1986; Smith et al., 1993; Rhode and Greenberg, 1994; Rhode, 1998; Bal and Oertel, 2000).

In the DCN, the fusiform cells project directly to the IC (Adams, 1979). The fusiform cell receive inputs from the auditory nerve fibers and the stellate cell outputs from VCN (Rose et al., 1959; Osen, 1969; Ryugo and May, 1993), as well as inputs from other cell types in DCN. The deep layer of the DCN contains a variety of multipolar cells including the giant cells that project to the contralateral IC (Adams and Warr, 1976).

1.2.2 Auditory pathway in Superior Olivary Complex. The auditory system processes both monaural and binaural information. The information carried by the auditory nerve fibers to the cochlear nucleus conveys monaural information processing. When the outputs leave cochlear nucleus and converge in the superior olivary complex, information from both ears will be integrated and this conveys binaural information processing. There are several circuits for processing the binaural information in the superior olivary complex (Boudreau and Tsuchitani, 1968; Goldberg and Brown, 1969) .

The outputs of spherical bushy cells in AVCN terminate in the medial superior olive (MSO) on both sides. The MSO cells have long dendrites which extend to the medial and lateral sides. The lateral dendrites receive inputs from the ipsilateral AVCN while the medial dendrites receive inputs from the contralateral AVCN (Cant and Benson, 2003). Therefore, the information from the two ears is integrated in the same MSO neuron. The outputs from spherical bushy cells are all excitatory outputs with glutamate as the main neurotransmitter. At the same time, the MSO receives inhibitory inputs from the medial and the lateral nuclei of the trapezoid body (MNTB and LNTB)

(Cant and Hyson, 1992; Kuwabara and Zook, 1992), and these inputs use glycine as the main neurotransmitter.

The LSO receives excitatory inputs from the axons of the spherical bushy cells and stellate cells in the AVCN on the ipsilateral side (Stotler, 1953; Warr, 1966; Shneiderman and Henkel, 1985; Cant and Casseday, 1986; Smith et al., 1993). The axons from the globular bushy cells of the AVCN synapse in the medial nucleus of the trapezoid body (MNTB) on the contralateral side. The neurons in the MNTB receive a very large ending from the contralateral globular bushy cell called the calyx of Held (Held, 1893; Borst and Soria van Hoeve, 2012). The large size of the axon makes a synaptic response with a short time delay. The MNTB then sends an inhibitory output to the ipsilateral LSO (Harrison and Warr, 1962; Elverland, 1978; Tolbert et al., 1982; Glendenning et al., 1985; Spangler et al., 1985; Friauf and Ostwald, 1988; Bledsoe et al., 1990).

In rodents, the superior paraolivary nucleus (SPO) is another distinct nucleus in the superior olivary complex. It receives excitatory inputs from the contralateral VCN and inhibitory input from the ipsilateral MNTB (Osen et al., 1984; Schofield and Cant, 1991; Schofield, 1995). The neurons in the SPO are large GABAergic cells that send inhibitory inputs to the ipsilateral IC.

The MSO sends excitatory inputs to the ipsilateral IC and the dorsal nucleus of lateral lemniscus (DNLL) (Adams, 1979; Glendenning et al., 1981). The LSO sends excitatory outputs to the contralateral IC as well as inhibitory outputs to the ipsilateral IC (Brunso-Bechtold et al., 1981; Saint Marie and Baker, 1990; Glendenning et al., 1992). The outputs of the nuclei in the superior olivary complex ascend to synapse on other

nuclei that in turn synapse in the IC, or directly from the nuclei of the superior olivary complex to the IC.

1.2.3 The nuclei of lateral lemniscus. The axons of octopus cells from the PVCN exit the cochlear nucleus via the intermediate acoustic stria and terminate in the ventral nuclei of the lateral lemniscus (VNLL). The LL is the main tract that connects the superior olivary complex with the IC. The axons from many neurons of the ventral cochlear nucleus and superior olive also terminate in the nuclei of the LL: the ventral (VNLL), the dorsal (DNLL), and the intermediate nuclei (INLL) (Oliver and Shneiderman, 1991; Helfert, 1997).

The inputs to the VNLL mainly originate from the contralateral VCN and ipsilateral MNTB. In that way, the information is from the contralateral side. The VNLL projects to the ipsilateral IC. In contrast, the DNLL receives inputs from both ears. Specifically, it receives collaterals from axons that also innervate the IC: contralateral outputs from the VCN and DNLL, ipsilateral outputs from MSO, VNLL and bilateral outputs from LSO. The DNLL sends a strong inhibitory projection to the contralateral and ipsilateral IC (Ross et al., 1988; Shneiderman et al., 1988; Malmierca, 2003).

In conclusion, the auditory pathways in the lower brainstem have several circuits to process information that is sent to the midbrain. Some circuits convey monaural and others binaural information to the IC. The inputs to the IC from these circuits can be excitatory, inhibitory, or both. With the convergence of those excitatory and inhibitory inputs carrying binaural and monaural information, the IC can process all the information about sound — its location, spectral content, and intensity level.

1.2.4 Inferior Colliculus. The IC is the integration center for all of the auditory outputs from the lower auditory nuclei, and it is subdivided into two regions, a central nucleus (CNIC) and a surrounding cortex. The cortex of the IC can be divided into the dorsal cortex (DCIC), the lateral cortex (LCIC) and the rostral cortex (RCIC). The CNIC receives most of the afferent inputs from the lower auditory nuclei, while the DCIC is mostly influenced by the descending pathways.

The CNIC is defined by the presence of fibrodendritic laminae that are tonotopically organized (Oliver and Morest, 1984). The fibrodendritic laminae are composed of disc-shaped principle cells with their dendrites aligned in parallel to each other and with the afferent lemniscal fibers. Stellate cells are the remaining cells in the central nucleus, which often run in a mediolateral direction and cross several fibrodendritic laminae.

The probability that responses of IC neurons may be related to their input sources is increased by the synaptic domain hypothesis (Oliver, 2000; Loftus et al., 2010). Inputs to the IC are segregated into functional zones that are smaller than individual frequency band laminae. Within such a zone, the IC neurons will tend to be dominated by only a subset of the ascending inputs to the IC.

The inputs to the IC are well known, but how the inputs get integrated largely remains a mystery. When different circuits are activated in the ascending auditory pathways to the IC, will the physiological response be the same?

1.3 Spectral processing in the auditory system

Frequency is a basic property of sound. The spectral information plays a major role in recognizing different sounds. For example, the enjoyment of music requires distinguishing the frequencies of sound created by different musical instruments. As mentioned above, the basic function of the ear is to decompose complex sound wave forms into a combination of tones. Spectral processing is a fundamental property of neurons throughout the auditory system, and the ability of the system to analyze the spectral composition of sounds is integral for the identification of sound objects.

The tonotopic organization in the cochlea is transmitted by the auditory nerve to the brain. From there, tonotopic organization and spectral sensitivity are inherited by all structures of auditory system. Neurons throughout the auditory system have a sensitivity to a part of the audible frequency spectrum, and the sensitivity varies as the sound level changes.

However, spectral information processing is not the same in all parts of the system (Young, 1980; Winter and Palmer, 1990). The study of spectral processing by neurons in the inferior colliculus may shed light on how information from the lower nuclei is integrated in the auditory midbrain.

Electrophysiological recordings from single auditory nerve fibers reveal how the spectral information is processed (Kiang et al., 1967). One way to measure the frequency response is to determine the threshold for each frequency, that is, the lowest sound level that evokes an action potential. A plot of these thresholds over the neurons frequency range is called a tuning curve. The auditory nerve fibers have tuning curves that resemble an asymmetrical letter “V”, in that neural threshold is lower on its low frequency side of the CF, whereas the threshold increases more rapidly on its high frequency side. The CFs of the nerve fibers cover the audible range, but the shape of the tuning curves for auditory nerve fibers is consistently the asymmetric V type.

1.4 Spectral processing in the brain

1.4.1 Frequency tuning in cochlear nucleus. The auditory nerve fibers carry the spectral information from the peripheral auditory system into the cochlear nucleus in the brainstem. Each auditory nerve fiber bifurcates, one branch descends to synapse on neurons in the PVCN and DCN, the other branch ascends to synapse on neurons in the AVCN (Cant, 1992). One way to measure the frequency tuning of these neurons is to plot the neuronal response as a function of frequency and intensity, the so called frequency response area (FRA). FRA's have been measured in all three parts of the cochlear nucleus.

In general, for some neurons in the cochlear nucleus, the spectral responses do not mimic those of the auditory nerve fibers and there is an integration of excitatory and inhibitory inputs to create new types of FRA. The cell types and organization in the three parts of the cochlear nucleus are distinct and show different types of spectral processing. Those spectral processing patterns have been categorized in previous research (Young, 1980; Winter and Palmer, 1990). T-stellate cells have type I, III, or I/III FRAs (Shofner and Young, 1985; Winter and Palmer, 1990). Both excitatory and inhibitory responses were visualized in those studies because of the presence of spontaneous activity. Type I neurons have purely excitatory responses but very little sideband inhibition. They resemble the frequency responses of the auditory nerve fibers, namely the asymmetric V type with a steep high frequency side (Kiang et al., 1967). The type III neurons have a simple asymmetric V-shaped excitatory area, similar to the type I, but they also have inhibitory sidebands at higher and lower frequencies than the BF. The type I/III neurons are an intermediate type between types I and III in that they also have V-shaped excitatory areas, but they lack spontaneous activity, so the inhibitory sidebands cannot be detected.

Spherical bushy cells respond mainly to low frequencies and they project to the MSO and LSO. The globular bushy cells respond to high frequencies and project to the LSO and the MNTB. The tuning curves of the bushy cells are like ones in the auditory nerve, asymmetric V type with little inhibition on the side (Rhode and Greenberg, 1992).

The neurons in the DCN have either type II, III or type IV FRAs and are characterized by large areas of inhibition (Shofner and Young, 1985; Joris, 1998). The shape of the type III in the DCN is similar to the type III in the AVCN, that is, an

asymmetric V type. The type IV cells have strong inhibition with an island of excitation at frequencies near the BF and stimulus levels near threshold. Consequently, there is a non-monotonic response, meaning the neuronal responses decrease at high levels, and the whole excitatory region is encapsulated in a closed area. The type II neurons have nonmonotonic rate-level functions, low spontaneous activity, and show no response to wideband noise.

1.4.2 Frequency tuning in superior olivary complex. The frequency responses of the neurons in the MSO have asymmetric V-shaped tuning curves similar to those in the auditory nerve and those of the spherical bushy cells in the AVCN that provide the MSO input (Goldberg and Brown, 1969). The bushy cells retain the spectral information from the auditory nerve very little. The axons from the spherical bushy cells in the AVCN also synapse in the lateral superior olive (LSO) on the ipsilateral side. However, the tuning in the LSO differs from that in the MSO since some of the neurons show a narrowly tuned response flanked by inhibition (Boudreau and Tsuchitani, 1968; Caird and Klinke, 1983). The frequency ranges of those LSO cells do not expand when the intensity increases. This narrowing could be due to inhibitory inputs from the MNTB. The MSO also receives inhibitory inputs from the lateral nucleus of the trapezoid body (LNTB) and MNTB, and these use glycine as the main neurotransmitter (Smith, 1995). It has been found that the temporal dynamics of the excitatory and inhibitory inputs to the MSO are well balanced (Couchman et al., 2010).

A detailed analysis of the tuning curves in the MNTB was carried out by Guinan (Guinan et al., 1972). Besides the asymmetric V type, other less common types were observed. These include a V-shaped tuning curve with an extra peak, a flat bottomed

curve, a symmetric V type with an expansion of the frequency tuning on both high and low frequency sides, and narrowly tuned neurons with an inward slope (like the letter “T”). Although the number of those other types was much lower than the number of asymmetric V type neurons, this is evidence that spectral processing becomes more complicated as the information about sound progresses to the upper nuclei in the auditory pathway.

1.4.3 Frequency tuning in the nuclei of LL. A large part of the outputs from the cochlear nucleus and superior olivary complex terminate in the nuclei of LL (Oliver and Shneiderman, 1991; Irvine, 1992; Helfert, 1997). In general, the tuning is broader in VNLL than in the auditory nerve (Batra and Fitzpatrick, 2002). Batra and Fitzpatrick showed that the tuning curves of VNLL were typically asymmetric V shaped for the sustained and transient cells, while the onset cells had a broader symmetrical V type that was expanded on both sides (Batra and Fitzpatrick, 1999, 2002). The cells in the INLL also have broad tuning curves (Casseday and Covey, 1992). The frequency tuning curves in DNLL are either displayed as narrowly tuned or asymmetrical tuning curves with a flat slope above the best frequency (Metzner and Radtke-Schuller, 1987).

1.5 Spectral processing in the inferior colliculus

Virtually all of the lower auditory centers have outputs that terminate in the IC. Spectral information is transmitted to the IC from the VCN, DCN, MSO, LSO, DNLL, VNLL and INLL. Thus, spectral processing in the IC reflects a synthesis of many inputs.

The spectral processing in IC has been studied in many species, such as the cat, rabbit, rat, gerbil, chinchilla, and bat (Hind et al., 1963; Evans, 1972; Aitkin et al., 1975; Ehret and Merzenich, 1988; Casseday and Covey, 1992; Yang et al., 1992; Palombi and

Caspary, 1996; Ramachandran et al., 1999; LeBeau et al., 2001; Hernandez et al., 2005; Bandyopadhyay et al., 2007; Atencio and Schreiner, 2008; Palmer et al., 2013). For example, extracellular recordings in the cat show that there are several different shapes of FRAs in the IC. For example, a broad “V-shaped” FRA is seen in the IC is similar to that seen in the brainstem (Fig.1.3). This type of tuning describes monotonic neurons whose frequency range broadens with increasing stimulus intensity and is similar to the type I response in the cochlear nucleus. “I-shaped” FRAs are also observed in the IC. In this type, the neuron maintains a constant, narrow bandwidth across sound levels (Ramachandran et al., 1999). This is similar to cells in the LSO that have inhibitory side bands. The O type response pattern in the cat exhibits non-monotonic rate level functions and shows inhibition of the response when the sound levels increase. The O type response is similar to the type IV cells in the DCN.

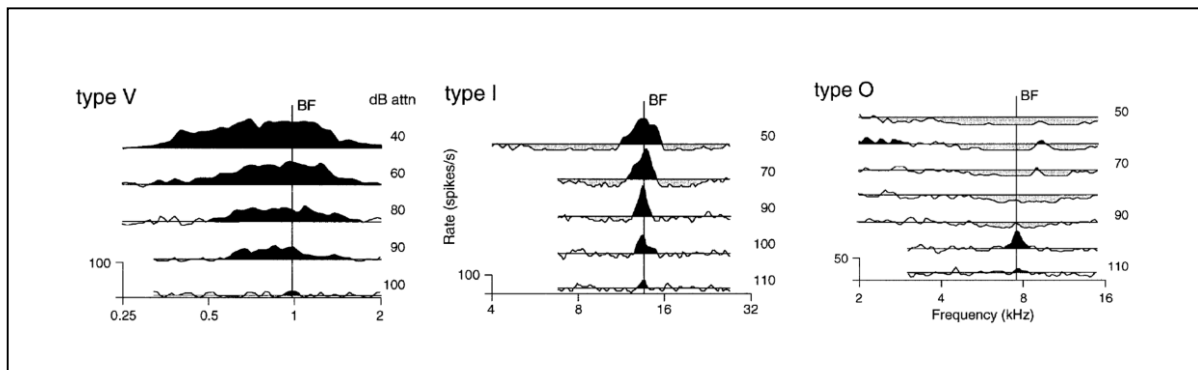


Figure 1.3 (Ref. Ramachandran et al., 1999, black-excitatory response areas, grey-inhibitory response areas)

More detailed classification have been made in other species. In the IC of the guinea pig, seven types were defined: V-shaped, non-monotonic V types (VN, the response does not increase with level), N type (narrowly tuned, like I type), C type

(closed, no response at high intensity, like O type), TD type (tilt down, the CF tilt to the low frequency), TU type (tilt up, the CF tilt to the high frequency) and D type (double-peaked) (Palmer et al., 2013). In addition to these, there were many neurons of the intermediate type that were difficult to match to one of these exact FRA types.

In rats, V-shaped FRAs were in the majority (69% of the neurons, Hernandez et al., 2005). The remaining neurons were classified as narrowly tuned, closed, low tilt, high-tilt, multi-peaked, U-shaped (the broadly tuned), mosaic (several islands of responses without clear CF), and inhibitory (high level of inhibition was observed). The multi-peaked types were the most frequently observed non-V type neuron.

Egorova et al. (Egorova et al., 2001) classified FRAs in the mouse as class I, II, III and IV. The major types observed in that study were the class I, II, and III. Class I was a very asymmetric V-type with a steep slope on the high-frequency side, while class III was a symmetrical V-type. Class II was similar to the I-type in the cat in that it was narrowly tuned and had inhibitory side bands. The O-type response and multi-peaked FRAs were all included in the class IV type.

Despite various types and nomenclatures of FRAs found in different species, most types are quite similar to each other. In particular, the main difference primarily lies in the classification of FRA's rather than the species specific differences. However, the percentage of each type of FRA is different across species. The V-shaped type (both symmetric and asymmetric) represents 69% of neurons in the rat, 84% in the guinea pig, 66% in the mouse, but only 12-30% in cat in two different research group (Kuwada et al., 1984; Ehret and Merzenich, 1988; Ramachandran et al., 1999; Egorova et al., 2001; LeBeau et al., 2001; Hernandez et al., 2005). The non-V-shaped neurons represents 29%

of neurons in the rat, 16% in guinea pig, 34% in mouse, but 88% in cat. These differences could originate from minor species variations since rodents have a similar distribution. However, it could also come from sampling or experimental differences. For example, the studies may differ in the number of neurons or the location of the neurons recorded. The use of anesthesia in the preparation and recording process could also be a factor creating those differences.

According to the description and the comparison of the FRAs across different species, we can conclude that although research groups have distinctly named the FRA types in different species, most types are quite similar to each other. In particular, the main difference primarily lies on the interpretation of those differences rather than the differences due to species itself. However, the percentage of each type in those species can be different. The V-shaped type (both symmetric and asymmetric) represents 69% of neurons in the rat, 84% in the guinea pig, 66% in the mouse, but only 12% in cat (Ehret and Merzenich, 1988; Ramachandran et al., 1999; Egorova et al., 2001; LeBeau et al., 2001; Hernandez et al., 2005). At the same time, the non-V-shaped neurons constitute 29% of neurons in the rat, 16% in guinea pig, 34% in mouse, but 88% in cat. These differences could originate from minor species variations since rodents have a similar distribution. However, it could also come from sampling or experimental differences. For example, the studies may differ in the number of neurons or the location of the neurons recorded. The use of anesthesia in the preparation and recording process could also be a factor creating those differences.

To further complicate the discussion of FRA types and species differences, there is some debate as to whether FRA types actually form distinct groups in the IC. The

evidence from the Palmer and Malmierca groups (Malmierca et al., 2008; Palmer et al., 2013) suggests that distribution of FRA's type form a continuous distribution rather than separated into distinct groups. Although the different iconic types are real, there are many intermediate types. This complicates the sorting of spectral responses into different types.

All the previous research with spectral processing in the IC was conducted with either monaural stimulation or free field stimulation. The link and comparison between the monaural spectral processing and binaural spectral processing is missing. Yet, to our knowledge, there is no systematic study to compare spectral processing when sound comes from two ears versus just one ear. Does the frequency tuning of the neuron change? To address this question, this project will use both monaural and binaural sound stimulation in the same neuron to determine if spectral tuning is changed.

1.6 Binaural processing

One important function of the auditory system is the localization of the position of a sound source. Differences in the arrival time and the intensity of the sound in the two ears are the physical cues that help to identify the location of a sound. When sound from the cochlea arrives into the cochlear nucleus in the brainstem, the pathway only carries information from one ear. The outputs from cochlear nucleus merge in the superior olivary complex and information from the two ears is integrated. This is binaural processing.

1.6.1 Interaural time difference. When the sound source is positioned off the midline, there is a difference in the arrival time of the sound to the left ear and right ear.

This difference is called interaural time difference (ITD), and it is an important cue for localizing low frequency sounds.

1.6.2 The tuning of ITD at MSO. When the sound source moves around the head, the ITD cue varies, and the MSO is the first part in the brain sensitive to differences in ITD. The discharge rates of the MSO neurons systematically varies with ITD. However, not all MSO neurons respond maximally to the same ITD which means that MSO neurons are tuned to different ITDs. It has been proposed that the MSO neurons act as coincidence detectors and fire maximally when two excitatory inputs from both sides arrive simultaneously (Jeffress, 1948; Goldberg and Brown, 1969; Yin and Chan, 1990; Spitzer and Semple, 1995). In addition to the excitation from two sides, the MSO also receives inhibitory inputs from the MNTB and the LNTB. The excitatory and inhibitory interaction also contributes to the ITD sensitivity since the application of a glycinergic antagonist--strychnine may affect ITD processing (Brand et al., 2002).

Extracellular recordings in the MSO showed that cells have low characteristic frequencies, and most neurons responded to sounds from each individual ear and both ears together (Yin and Chan, 1990; Spitzer and Semple, 1995; Batra et al., 1997). Both the monaural response to the individual ear and the binaural response to both ears were phase locked. The recordings showed that, relative to the monaural responses, most cells exhibited a facilitated response in the firing rate at their most favorable ITDs and a suppressed firing rate at unfavorable ITDs. The sample of individual neurons recorded in the MSO showed a spatial distribution of the ITDs along the anterior-posterior axis. Cells sensitive to low ITDs (around 0 μ s) were located anteriorly, while more positive ITDs were located posteriorly. This systematic representation of ITDs represents a place code

that is sent to the IC. This concept is based on a limited number of cells and has created considerable controversy (e.g., McAlpine et al. 2001).

The projections of the MSO terminate to the dorsal nucleus of lateral lemniscus (DNLL) and the IC (Adams, 1979; Glendenning et al., 1981). The cells in the DNLL are also tuned to different ITDs (Brugge et al., 1970; Markovitz and Pollak, 1994; Yang and Pollak, 1994; Kelly et al., 1998; Kuwada et al., 2006). The tuning of the ITD in the DNLL is sharper than MSO (Palmer and Kuwada, 2005; Kuwada et al., 2006). The MSO also sends excitatory inputs to the IC, while at the same moment of time, the DNLL sends inhibitory inputs to the IC.

1.6.3 ITD tuning in IC. The spatial information about the ITD cue is carried by the axons of the MSO via the lateral lemniscus to the IC. Studies of ITD sensitivity in the IC have been conducted extensively. Characteristic delay is defined as the ITD where a cell responds at the same relative amplitude at different frequencies of the sound stimulus (Rose et al., 1966). Characteristic phase is defined as the interaural phase, where the characteristic delay happens. Composite peaks are the peaks of averaged delay curves and represent the “best” ITD. The tuning of ITD in the IC largely resembles the ITD tuning in the MSO. However, the distributions of characteristic delay, characteristic phase and composite peaks in the IC are broader than in the MSO (Yin and Chan, 1990; Kuwada et al., 2006). One important difference in the binaural interactions in the MSO and the IC is that there may be additional binaural processing in the IC. The direct inputs from the cochlear nucleus are independent of the superior olivary complex and may contribute to the broader tuning of ITD in IC (Grothe et al., 2010).

1.6.4 Interaural level difference. When a sound is closer to one ear than the other, the shadowing of the sound wave by the head makes the sound louder at the near ear. The difference between two sounds is the interaural level difference (ILD, also called interaural intensity difference), and it is the primary cue for sound localization in high frequency neurons. The lateral superior olive (LSO) is the primary site for ILD processing.

1.6.5 The tuning of ILD in LSO. The excitatory and inhibitory interaction in the LSO allows the neurons to detect interaural intensity differences. When the sound comes from a lateral position, e.g., 90° from the midline of the head, the excitation from the ear on the same side as the sound source will be the strongest, while inhibition from the opposite ear will be the lowest. Thus, the response of the LSO neurons on the same side as the sound will be the strongest. The maximal response cannot exceed that to stimulation of the ipsilateral ear alone. Thus the mechanism is subtractive in the LSO (i.e., cannot exceed the monaural rate) whereas it is considered multiplicative in the MSO (i.e., can exceed the sum of the firing rates to monaural stimulation).

The relative strength of excitation and inhibition can vary among the neurons in LSO, thus creating different responses to the same ILD (Goldberg and Brown, 1969; Sanes and Rubel, 1988; Joris and Yin, 1995; Irvine et al., 2001). The ILD response can be plotted as response rate against the ILD. For the excitation-inhibition (EI) neurons, the binaural response is lower than the ipsilateral response, indicating an inhibition from the contralateral side. The individual neurons do not exhibit sharp tuning to a specific ILD, but since every neuron has sensitivity to a range of ILDs, the ILD behaves like a low pass filter where the cut off is at different ILDs and the population of LSO neurons acts

together to code ILD information. The LSO sends excitatory outputs to the contralateral IC as well as inhibitory outputs to the ipsilateral IC (Brunso-Bechtold et al., 1981; Saint Marie and Baker, 1990; Glendenning et al., 1992).

1.6.6 ILD tuning in IC. There are many cells in the IC that are sensitive to ILD (Wenstrup et al., 1986; Semple and Kitzes, 1987; Irvine and Gago, 1990). The ILD could be inherited from the LSO inputs, but it could also be generated through other circuits (Casseday and Covey, 1992; Li and Kelly, 1992; Vater et al., 1992; Faingold et al., 1993; Klug et al., 1995). For the cells that receive inputs from LSO, the application of the GABA receptor antagonist bicuculline in the IC was found to have no effect on the ILD processing and the spatial receptive field, indicating that the information was processed in the lower nuclei (Park and Pollak, 1993, 1994).

However, the convergence of excitatory and inhibitory inputs in the IC also plays an important role in ILD processing. For some IC cells, the application of bicuculline disrupted the ILD tuning dramatically (Park and Pollak, 1993, 1994). The inhibitory inputs to those neurons could come from GABAergic neurons in DNLL. The injection of the excitatory amino acid antagonist kynurenic acid or the GABA-A agonist into DNLL shifted the ILD curves for the neurons in contralateral IC, and this indicates an inhibitory role for DNLL in binaural processing in the contralateral IC (Li and Kelly, 1992; Faingold et al., 1993).

A third pathway could also contribute to the excitatory-inhibitory interactions in the IC. Glycinergic neurons in the LSO provide an uncrossed projection to the IC (Saint Marie et al., 1989a; Saint Marie and Baker, 1990; Glendenning et al., 1992). These neurons would be excited by stimuli in the ear ipsilateral to the LSO and, thereby,

provide inhibition in the ipsilateral IC. The application of the glycine antagonist strychnine reduced the ipsilaterally-evoked inhibition for 36% of the neurons in the IC, and the function of the ILD processing was greatly influenced by those neurons (Klug et al., 1995).

In conclusion, the ILD processing in the IC could be inherited from the lower brainstem nuclei such as the LSO, but the convergence of excitatory and inhibitory inputs in the IC also may directly contribute to the ILD processing.

1.7. Spectral cues to locate sound

In addition to ITD and ILD cues, sound spectrum is also important for sound localization. It is thought to be essential for detecting changes in the elevation (Musicant and Butler, 1984). The spectral cues are based on the pinnae of the outer ear. When animals move the position of their head or ear, the angle of the sounds coming to the ear is dramatically altered. The pinnae selectively amplify or attenuate certain frequencies, so that the spectral content transmitted to the inner ear will be different when the sound is at different locations. This is seen as notches or peaks in the spectrogram of the sound (Roffler and Butler, 1968; Blauert, 1969; Hebrank and Wright, 1974; Oldfield and Parker, 1984).

The fusiform cells in the DCN are excited by wideband noise and they are good at detecting the spectral notches produced by the pinnae (Rhode, 1992). Information from these neurons is transmitted directly to the IC. Thus, binaural processing by IC neurons involves the integration of monaural pinnae based spectral cues with binaural ITD and ILD information in the localization of sound.

The binaural processing of sound enables animals to locate the sound source positions, help them avoid danger and find sources in the natural environment. Spectral processing is a fundamental property of the auditory system that is preserved at every level of the system. Despite this, there is no systematic study that compares how binaural cues affect spectral processing. Would the perception of pitch be altered by a change in the sound location? How do binaural cues affect frequency perception? This thesis presents an analysis of how binaural stimuli affect frequency tuning in neurons of the IC.

IC neurons receive numerous inhibitory as well as excitatory. Both must play an important role in spectral processing. However, visualizing the inhibitory inputs is difficult when recoding neural activity in the IC, especially in animals under anesthesia when spontaneous activity is absent.

Neurons of the MSO and LSO in the superior olive provide the main excitatory binaural inputs to the IC. Those with projections to the IC from the ipsilateral MSO and contralateral LSO use glutamate as a main neurotransmitter (Ito and Oliver, 2010; Ito et al., 2011) (Saint Marie et al., 1989a; Ito and Oliver, 2010).

Binaural inhibitory projections to the IC come from the LSO and the DNLL. The LSO sends a glycinergic input to the ipsilateral IC (Saint Marie et al., 1989a; Ito and Oliver, 2010; Ito et al., 2011). The DNLL receives collaterals from afferents that also innervate the IC and then sends a strong GABAergic inhibitory projection to the contralateral IC (Goldberg and Brown, 1969; Shneiderman et al., 1988).

Monaural excitatory inputs to the IC come from contralateral AVCN, DCN and INLL. Monaural inhibitory projections to the IC come from the VNLL and SPO (aka

DMPO). These use glycine and GABA, respectively, as their main neurotransmitter (Ito and Oliver, 2010; Ito et al., 2011). The VNLL receives inputs from the contralateral AVCN and PVCN (either directly or indirectly via MNTB) that are mainly driven by sound in the contralateral ear (Metzner and Radtke-Schuller, 1987). The SPO is a monaural nucleus in the superior olive that is especially large in rodents and has projections to the IC (Schofield, 1995; Ito and Oliver, 2010). It consists of GABAergic multipolar cells that are the largest in the SOC. The predominant response of the SPO neurons is suppression to sound stimulation followed by a robust off response when the sound is turned off (Kuwada and Batra, 1999; Kulesza et al., 2003).

Although most if not all cells in the IC receive inhibitory inputs, they are not always evident in extracellular recordings. In many studies of monaural spectral coding, two-tone stimulation is used to examine the inhibitory FRAs that may flank the excitatory ones (Ehret and Merzenich, 1988; Vater et al., 1992; Fuzessery, 1994; Saitoh and Suga, 1995; Fuzessery and Hall, 1996; Lu and Jen, 2001). Two tone stimulation is usually played in the same speaker either monaurally or in the free field. However, in the analysis of binaural processing, sounds are delivered to both ears and there is often inhibition induced by the stimulation from the ipsilateral side. That inhibition is usually studied only at the CF or BF of the neuron. In order to fully investigate binaural spectral processing, it is necessary to identify inhibition driven by the ear ipsilateral to the IC under binaural conditions. To do so, we developed a new approach. An unvaried probe stimulus is presented at the BF of the neuron to the contralateral ear, while the response to random frequency and intensity combinations presented to the ipsilateral ear is recorded. Consequently, this thesis presents an analysis of the spectral processing of the

inhibitory inputs to the IC driven by the ipsilateral ear that may be compared to the binaural and monaural frequency responses.

CHAPTER 2: BINAURAL STIMULATION CHANGES FREQUENCY RESPONSES OF IC NEURONS IN THE RATE AND SHAPE

Authors: Xi Bie and Douglas L. Oliver

*Department of Neuroscience, University of Connecticut Health Center, Farmington, CT, USA
06030-3401*

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Corresponding author
Dr. Douglas L. Oliver
Department of Neuroscience
University of Connecticut Health Center
Farmington, CT, 06030-3401 USA
E-mail: doliver@neuron.uchc.edu

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2.1 ABSTRACT

Frequency tuning is a basic property of neurons in the auditory system since individual neurons respond to part of the spectrum of audible sounds. Sound localization relies on binaural cues and is a second basic property of the system. Yet, little is known about how frequency tuning is affected by binaural stimulation. Since the inferior colliculus (IC) in the midbrain integrates virtually all of the binaural and monaural information ascending from the lower auditory brainstem nuclei, it is an ideal location in which to study the influence of binaural (diotic) stimulation on spectral processing. To do so, we recorded from single neurons in the mouse IC to binaural and monaural tones delivered sequentially in each trial. Neurons were analyzed according to how the monaural frequency response area (FRA) was altered by binaural stimulation. Overall, the maximum firing rate, Q10, Q40, best frequency (BF), characteristic frequency (CF), and thresholds for the population were similar to contralateral and binaural stimulation. However, not all neurons reacted similarly, and the types of changes resulting from binaural stimulation were identified using cluster analysis to separate neurons based on the ratio of the binaural/contralateral responses. About a 40% of the neurons had maximum firing rates and bandwidths that were unchanged by binaural stimuli, but the remaining 60% showed some combination of change in maximum firing rate, bandwidths, or threshold. The remaining cells all have rates changed by binaural stimulation, either increased or decreased, while the effects on the bandwidth were different. When neurons had maximum firing rates that were suppressed by binaural stimulation, this resulted in sharper tuning, and the thresholds to binaural stimuli were either higher or lower. Fewer neurons had firing rates enhanced by binaural stimulation

but were unchanged in bandwidth or threshold. The inhibitory FRA generated by binaural two-tone stimulation of the ipsilateral ear was usually similar in BF to the FRA from contralateral excitation, but some neurons showed sideband inhibition generated by the ipsilateral stimulation. We conclude that binaural stimulation can alter the spectral processing of many neurons in the IC, and this might facilitate processing of the sound localization and sound texture.

2.2 INTRODUCTION

The inferior colliculus (IC) is the principle auditory structure in the midbrain, and it receives inputs from virtually all of the lower auditory centers, as well as from the auditory cortex (Oliver, 2005). The IC integrates these inputs and sends auditory information to the auditory cortex via the thalamus. Thus, it serves as one of the main integrative centers for auditory information in the mammalian brain (Oliver and Morest, 1984; Irvine, 1992; David R. Moore, 2010). It is an ideal location to study the interaction of spectral and binaural processing since it is the first location in the pathway where monaural and binaural pathways carrying spectral information converge.

Spectral processing is the property of the auditory system to respond to the audible frequencies of sound. The frequency response area (FRA) is one measurement of spectral processing since it is a map of the neuronal response to frequencies at different sound levels. Many different types of FRAs have been identified in the IC over decades of study (Hind et al., 1963; Evans, 1972; Aitkin et al., 1975; Ehret and Merzenich, 1988; Casseday and Covey, 1992; Yang et al., 1992; Palombi and Caspary, 1996; Ramachandran et al., 1999; LeBeau et al., 2001; Hernandez et al., 2005; Alkhatib et al., 2006; Atencio and Schreiner, 2008; Palmer et al., 2013). However, little is known about how spectral processing is related to binaural processing since there has not been a direct comparison of FRAs of single neurons collected under monaural and binaural conditions.

Binaural processing takes advantage of the two ears and the binaural cues in the natural environment that enable the localization of sound in space. Binaural cues include the interaural level difference (ILD), a major cue for high-frequency sound localization (Feddersen, 1957) and interaural time difference (ITD), a major cue for localization of

low-frequency sounds (Klumpp, 1956; Zwislocki, 1956; Fitzpatrick and Kuwada, 2001). Although there have been many previous studies of binaural processing in the IC (Erulkar, 1972; Kuwada, 1997; Palmer AR, 2005), how binaural cues affect spectral processing is unknown, since studies of binaural processing rarely include sounds with a wide range of frequency and intensity. For example, does the spectrum of sound seen by individual neurons change when a sound moves from a highly lateralized sound location on one side to a position directly in front of the animal?

Previous studies have suggested that certain types of FRA may be related to binaural processing. Neurons with a V-shaped FRA in the IC were often found to be sensitive to ITD (Ramachandran and May, 2002; Palmer AR, 2005), while neurons with an I-shaped FRA are thought to process ILD (Semple and Aitkin, 1979; Irvine and Gago, 1990). Other studies found that interaural phase may change the iso-intensity response area dramatically, but there is little change in the best frequency when responses to monaural or binaural ITD stimuli are compared (Kuwada et al., 1984). In general, it is not clear to what extent the FRA may change when the sound changes from monaural to binaural stimulation.

Both excitatory and inhibitory inputs are activated by a monaural stimulus and integrated by IC neurons to produce a FRA (Yang et al., 1992; Palombi and Caspary, 1996; Ramachandran et al., 1999; Egorova et al., 2001). Likewise, excitatory and inhibitory inputs combine for binaural processing and this is particularly obvious in ILD processing where the ipsilateral ear provides inhibition (reviews by (Grothe, 2003; Kandler and Gillespie, 2005). However, it is unclear how the inhibition from the ipsilateral ear interacts with contralateral stimulation to produce the integrated FRA. In

order to address this issue, we have developed a binaural two-tone stimulation protocol to visualize the inhibitory response induced by sounds from the ipsilateral ear.

In this study, we compared spectral processing in the mouse IC in response to monaural versus binaural stimulation to see to what extent ipsilateral ear stimulation alters monaural contralateral frequency tuning. Responses of a single neuron to contralateral and ipsilateral stimulation were compared directly to those in response to binaural stimulation, and all three stimuli were presented sequentially in the waveform of a single trial. In addition, a novel two-tone binaural stimulation protocol was devised to show the ipsilateral inhibitory input to a neuron that was constantly firing in response to a contralateral suprathreshold stimulus at CF.

2.3 MATERIALS AND METHODS

2.3.1 *Animals*

In the present study, 60 GAD67-GFP knock-in Swiss Webster mice (Tamamaki et al., 2003) and 37 CBA/J mice (P21-P45, female; #000656, Jackson Laboratory, Bar Harbor ME) were used. The transgenic mice were maintained by breeding hemizygous and wild type animals. Both hemizygous transgenic (Tg/+) and homozygous (+/+) wild-type offspring were used in the experiments. Previous research showed there was no difference in the peak time, peak amplitude, half width, charge, or ratio of early and late charge of EPSCs or IPSCs between the two genotypes (Ono and Oliver, 2014). All experiments were performed under protocols approved by the University of Connecticut Health Center and in accordance with NIH guidelines for the care and use of laboratory animals. All efforts were made to minimize the number of animals used and their suffering.

2.3.2 *Surgical Preparation and Anesthesia*

Animals were anesthetized with an intramuscular injection of a mixture of ketamine (100 mg/kg), xylazine (20mg/kg) and acepromazine (10mg/kg). Surgery and recording took place in a double-walled sound attenuation chamber (Industrial Acoustics Company, NY, NY). The animal was held with a bit bar and a nose piece used to deliver gas anesthesia (isoflurane, 0.5-1%) during the surgery and recording. The head was further stabilized during surgery with bilateral maxillary pressure. A craniotomy was performed posterior to the lambdoid suture on the right side to visualize the dorsal surface of the right inferior colliculus (IC). The heart rate, breathing rate, and blood SpO₂ were monitored with a non-invasive vital sign monitor (MouseOx Plus, Starr Life

Science Corp, PA). The body temperature was monitored with a rectal probe coupled to a digital thermometer and maintained at $> 35^{\circ}\text{C}$ with an electrical heating pad (FHC DC temperature controller, FHC Inc, ME). Leg withdrawal reflexes were also monitored.

2.3.3 Acoustic Stimulation and Electrophysiological Recording

Acoustic stimuli were generated with a TDT System 2 or 3 (TDT, Tucker Davis Technologies, Gainesville, FL) under the control of a personal computer with custom Matlab software (Brian Bishop, University of Connecticut Health Center; Marcel van der Heiden, University of Utrecht, Netherlands). Sounds were delivered by electrostatic speakers (TDT EC1, Tucker Davis Technologies, Alachua, FL). The earphone was coupled to a small metal tube that fit snugly in the ear. The sound delivery system was calibrated for amplitude and phase from 100 to 100,000 Hz. The calibration was taken 1 mm away from the end of the metal tubes with a 1/4" microphone (Type 4135, Brüel & Kjaer, Naerum, Denmark). The duration of tones or noise was 50 - 250 ms depending on the experiment, and the linear rise and fall times were 5 ms.

Prior to single unit recording, the auditory brainstem response was used to determine the auditory threshold of each ear. Differential electrodes were placed at the vertex and at the back of the neck. Click stimuli (0.5 ms, 1000 clicks) delivered to each ear separately. Responses were filtered between 500 Hz to 3 kHz and averaged.

For single unit recordings, glass patch pipettes with an outer diameter of 2-3 μm were used and filled with 0.9% saline ($\pm 2\%$ Fluorogold). The pipette was advanced into the IC and the movement of the electrode was controlled with a manipulator (In-vivo Manipulator, Scientifica, East Sussex UK). After a single unit was isolated, a Bak window discriminator (Bak Electronics, Sanford FL) was used to isolate single spikes.

Extracellular recordings in response to broadband white noise bursts (10 kHz-50 kHz) were used as a search stimulus to locate neurons with short latency (12.8 ± 3.12 ms; mean \pm SD, $n=101$), presumed to be in the central nucleus of the IC (ICC). Tonal frequency sweeps at 70 dB SPL were used to determine the best frequency (BF) of the neuron. The threshold and rate level function of the neuron were determined at BF by delivering sounds of different intensity (5 - 10 dB steps) in random order. Finally, the neuron was tested with tone bursts at randomized frequencies from 0.25 to 72 kHz and intensities from threshold to 80 dB SPL in 10 dB steps. Each repetition of a tone included binaural, ipsilateral, and contralateral stimulation played in that order. The binaural stimuli were diotic with identical tones played in both ears at the same time. After recording, the location in ICC was verified in 21 recordings by iontophoresis of Fluorogold from the injection pipette and histology.

In order to visualize these potential inhibitory inputs from the ipsilateral ear and examine their frequency responses, we used a binaural variation of two-tone inhibition in which a constant probe stimulus at BF, 10 dB above threshold, was delivered to the contralateral ear, while random pure tones at random intensities were played in the ipsilateral ear. This binaural two-tone stimulation was presented in each waveform along with the contralateral and ipsilateral stimuli alone. To construct an inhibitory frequency response area (iFRA), the response to the binaural stimulus was subtracted from the response to contralateral alone in the same trial for each frequency-intensity combination. A negative number indicates a response inhibited by the ipsilateral ear stimulus. Only binaural responses that differed from monaural response more than one standard deviation above the background firing rate were considered as significantly different.

2.3.4 Data analysis

A FRA was constructed in response to the sound stimuli with custom Matlab software (Brian Bishop, University of Connecticut Health Center; Monty Escabi, University of Connecticut). In order to analyze the changes of the frequency response more systematically, we extracted several parameters from the FRAs produced by binaural, contralateral, and ipsilateral stimulation (Table 2.1). Maximal firing rate is the highest firing rate at any frequency or intensity. Best frequency (BF) is the calculated as the centroid tuning frequency at the highest sound level used (80 dB SPL). Characteristic frequency (CF) is the calculated centroid tuning frequency at or near the threshold of the neuron (Escabi et al., 2007). The standard deviation of the centroid tuning in octaves was calculated to represent the spread of the FRA, and twice the standard deviation was used as an estimate of bandwidth (Escabi et al., 2007). The quality factor (Qn) is the ratio of the centroid frequency divided by the tuning bandwidth, and is used to estimate the width of tuning at a particular sound level above threshold. For example, Q10 is the quality factor at 10 dB above threshold. The ratio between BF and CF (BF/CF) is an indicator of the change in tuning created by sound intensity.

Parameter	Binaural	Contra	B/C Ratio
Max firing rate	Binaural max rate	Contra max rate	B/C max rate
BF	Binaural BF	Contra BF	B/C BF
CF	Binaural CF	Contra CF	B/C CF
BF/CF	Binaural BF/CF	Contra BF/CF	B/C BF/CF
Q10	Binaural Q10	Contra Q10	B/C Q10
Q30	Binaural Q30	Contra Q30	B/C Q30
Q40	Binaural Q40	Contra Q40	B/C Q40

Table 2.1. The major parameters extracted from FRAs.

Cluster analysis (SPSS Statistics, IBM, NY) was used to sort the cells with similar properties into groups. The ratio of the binaural response divided by the contralateral response (B/C ratio) for each parameter was used in the cluster analysis, and the K-means algorithm partitioned the cells into separate clusters on the basis of the similarities of the response. The B/C ratios for maximal firing rate, Q values, and BF/CF parameters were each normalized to the maximum value then used to cluster the cells into subgroups. To compare clusters, the spike counts of the FRA for each cell were normalized to the maximum contralateral firing rate, and binaural FRAs were normalized to the contralateral FRAs. The normalized FRA was in a matrix with the largest point at 1. Then, the frequency was converted to an octave scale with 0 set to be the CF. After normalization, all the FRAs in one cluster were averaged. The B/C ratios for specific parameters were compared across groups with a two-tailed T test; and the t value, p value, and degree of freedom were used to describe the significance level.

2.4 RESULTS

In order to determine whether spectral processing with binaural stimuli differs to that with monaural stimuli, we obtained the neural responses to binaural and monaural (contralateral and ipsilateral) stimulation from 209 neurons in the ICC. Figure 2.1 shows an example of our stimulation paradigm at an intensity of 80 dB SPL and the response of an ICC neuron. Each frequency is presented three times. From such responses to different sound levels (10-80 dB SPL) a FRA for each of the three sound stimulus conditions was constructed.

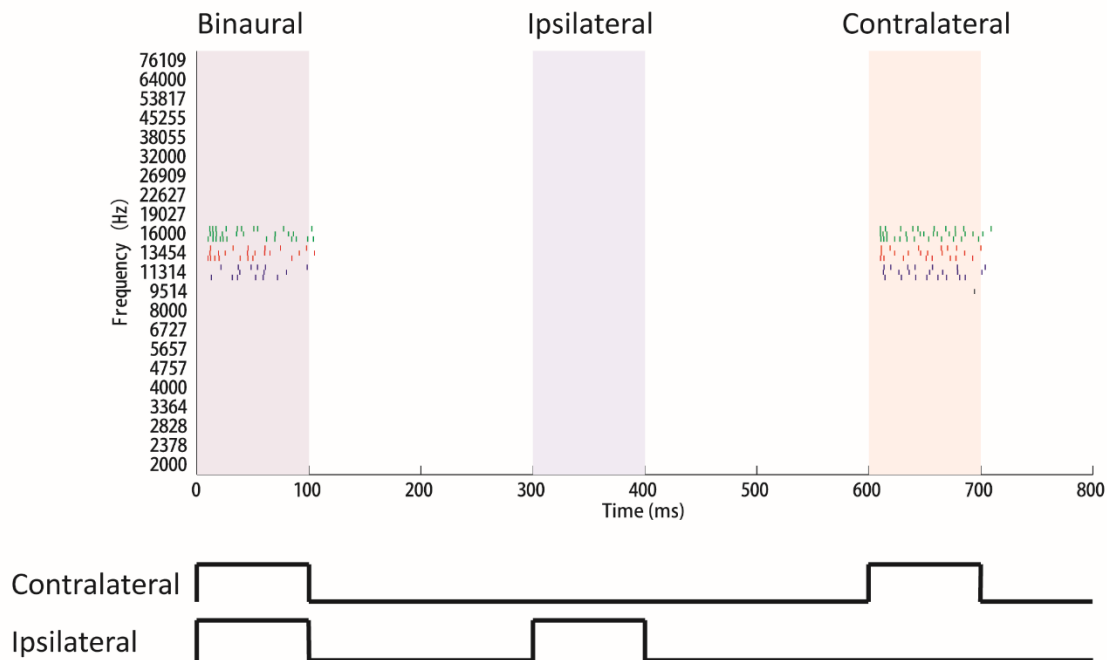


Figure 2.1. Responses to binaural, ipsilateral and contralateral stimuli are presented as a part of the raster plot for a single frequency. The binaural stimulation is at 0 ILD. Each raster point indicates a single spike in the extracellular recording. This plot shows the response of a single neuron to an 80 dB SPL stimulus. The responses to sound presented to each ear is shown under the timeline. The stimuli are 100 ms duration followed by an interval 200 ms or longer.

2.4.1 Spectral patterns with contralateral stimulation

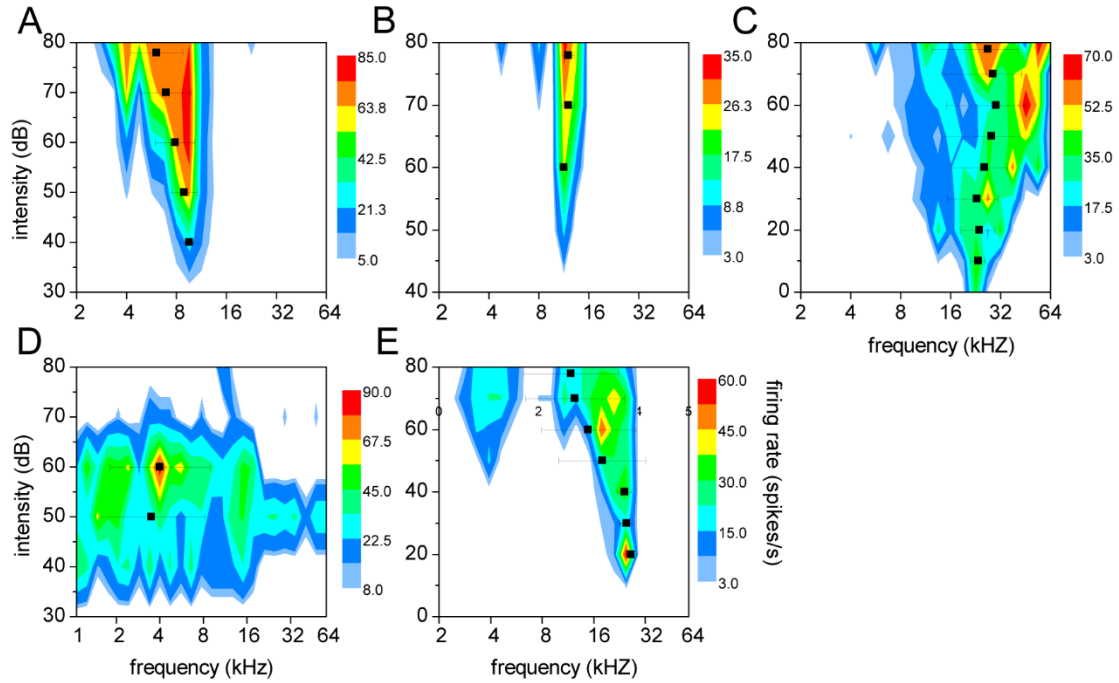


Figure 2.2. Five types of frequency response areas (FRA) were observed in the mouse IC in response to contralateral monaural stimuli. Each FRA is represented as a three dimensional pseudo-color plot. The X axis represents frequencies from low to high, the Y axis represents sound level from low to high, and the color indicates the firing rate. The black square at each intensity level represents the centroid frequency, and the horizontal lines show the tuning bandwidth. A: The class I asymmetric V-type FRA. B: Class II I-type FRA (the bandwidth is narrower than the frequency marker). C: The class III symmetric V-type. D: The class IV O-type showing a non-monotonic response. E: The class V multi-peaked FRA.

We observed five characteristic types of FRA in response to contralateral ear stimulation. The asymmetric V-type, exhibited a FRA that broadens at the low frequency side but remained steep on the high frequency side (Fig. 2.2A). The I-type had a sharply tuned FRA (Fig. 2.2B). The symmetric V-type showed a FRA whose frequency range broadens symmetrically at both high and low frequencies (Fig. 2.2C). The O-type showed broad tuning and non-monotonic response that indicated more inhibition at higher sound levels (Fig.2.2D). Finally, the multi-peaked type FRA had more than one peak in the response (Fig. 2.2E). The first three types were the most common in the present study and

similar to class I, II, and III types seen previously in the mouse IC (Egorova et al., 2001). Multi-peak neurons were rare in our sample. Although these are iconic types, there were many cells with FRAs that show intermediate properties (Palmer et al., 2013).

2.4.2 FRA changes with binaural stimulation

Does binaural stimulation alter the frequency tuning of IC neurons? To answer this question we compared the FRAs in response to binaural stimulation to those to contralateral or ipsilateral stimulation.

Under binaural stimulation some neurons showed an increased firing rate accompanied often with a broadening of its frequency range. An example of this broadening is shown for a neurons with a symmetric V-type FRA in Figure 2.3A. The tuning became broader at both low and high frequencies when the intensity increased and its threshold was ~35 dB. In contrast, the FRA to contralateral monaural stimulation was more narrowly tuned and its threshold increased to ~40 dB. The shape of the FRA to ipsilateral stimulation was similar to the binaural FRA, albeit with a lower firing rate and a higher threshold. For this particular neuron, the FRA shape under binaural stimulation looked similar to the combination of the FRAs evoked by contralateral and ipsilateral stimulation. In other neurons, the maximum firing rate at the best frequency increased with binaural stimulation, but the tuning shape did not change.

In other neurons, binaural stimulation suppressed the firing rate and narrowed the tuning bandwidth (e.g., Fig. 2.3B). In this example, there was no response to ipsilateral stimuli. This suggests that the inputs from ipsilateral ear inhibited frequencies on either side of this neuron's BF.

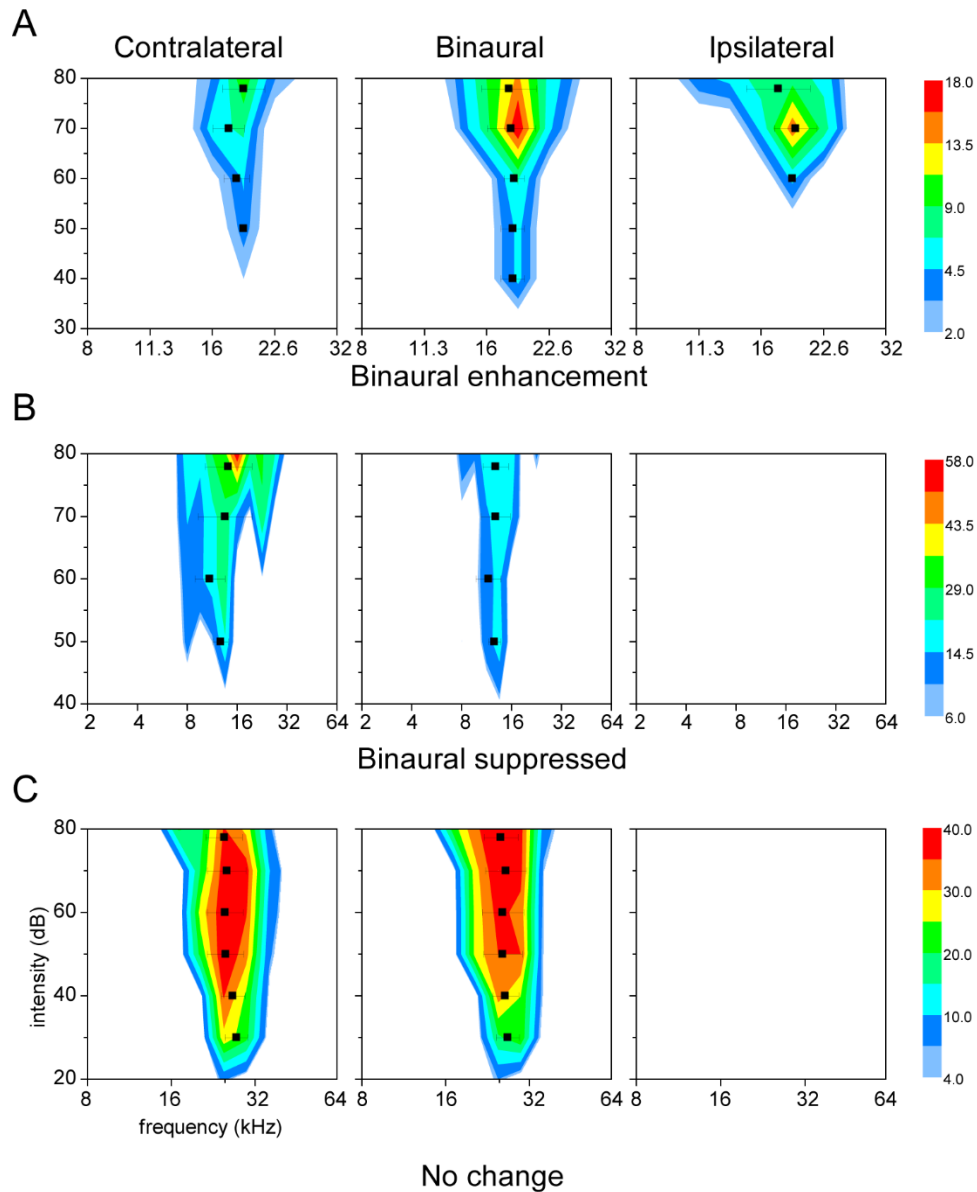


Figure 2.3. Responses to binaural and monaural stimulations obtained in the same neuron. A: Example of a neuron where binaural stimulation expanded the shape of the FRA seen with contralateral stimulation and increased the firing rate. The response of this neuron to contralateral stimuli was a sharply tuned FRA, but with binaural stimulation, the FRA was more broadly tuned class I V-type. B: Example of a neuron where binaural stimuli narrowed the tuning bandwidth and reduced firing rate. The tuning was much narrower under binaural stimulation when compared with contralateral stimulation, and the firing rate was much lower at binaural stimulation. The binaural stimulation made the frequency tuning sharper. C: Example neuron showing little difference in frequency tuning under binaural and contralateral monaural conditions.

Numerous neurons had very similar FRA's to binaural and contralateral stimulation. In one example (e.g., Fig. 2.3C), the tuning bandwidth, CF, BF, and firing

rates were similar to binaural and contralateral ear stimulation. This type of neuron could be classified as monaural because binaural stimulation evoked responses nearly identical to that evoked by monaural (i.e., contralateral) stimulation. In still other neurons, ipsilateral ear stimulation created FRA's similar to binaural stimulation, again suggesting only monaural inputs.

The examples presented above showed that binaural stimuli can have diverse effects on the frequency tuning of cells in the IC. In order to analyze the changes of the frequency response more systematically, we compared the firing rate, Q10, Q40, BF, CF, and the ratio of BF to CF to binaural and contralateral ear stimulation. These measures are plotted in Figure 2.4. In general, the responses to contralateral and binaural stimuli were well correlated. The maximum firing rate under both types of stimulation was strongly correlated ($r = 0.91$, Fig. 2.4A), but contralateral stimulation evoked significantly more spikes (12.6%) than binaural stimulation ($t = -4.812$, $df = 192$, $p < 0.000005$). The Q10 (Fig. 2.4 B) could vary dramatically with binaural and monaural stimulation. Overall, the correlation was low ($r = 0.31$), but excluding the outliers the correlation was improved to 0.72. Application of the Q40 metric dramatically reduced the number of outliers and resulted in a higher correlation ($r = 0.88$) with no significant differences between binaural and contralateral stimulation (Fig. 2.4C). The CFs and BF's were also strongly correlated (BF, $r = 0.94$; CF, $r = 0.95$) again with no significant differences between binaural and contralateral stimulation (Fig. 2.4D, E). Consistent with this strong relationship, the ratio of BF to CF also showed a strong correlation, ($r = 0.89$), again with no significant differences between binaural and contralateral stimulation. We also compared the thresholds to binaural and contralateral stimulation (Fig. 2.4G). The

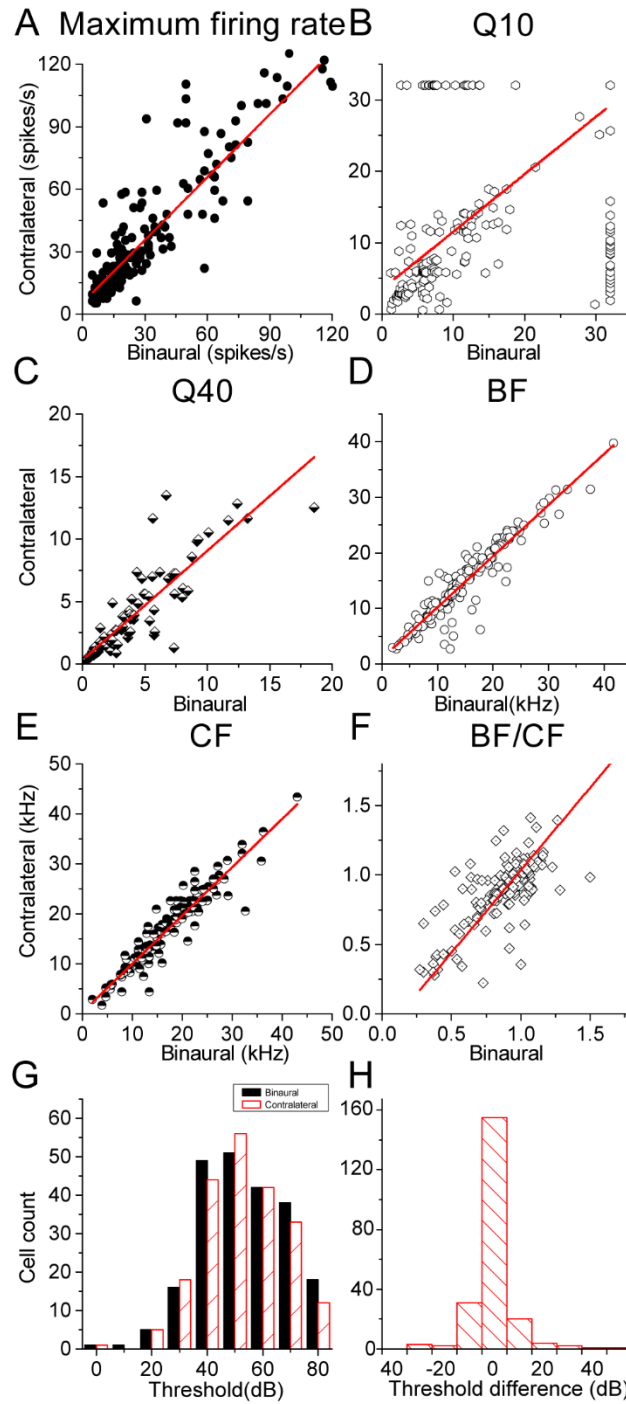


Figure 2.4. Comparison of binaural and contralateral responses from all neurons in our sample. Each symbol represents one neuron. The X axis is the response to binaural stimulation, while the Y axis value is the contralateral response. A: Maximum firing rates (n=193). Values less than 5 were removed. B: Q10 values (n=184). Values >32 were set to 32. C: Q40 values (n=101). D: Best frequency (BF) (n=180). E: Characteristic frequency (CF) (n=180). F: The comparison of the binaural BF/CF ratio to contralateral value to illustrate a change in the tuning direction (n=183). G: The comparison of the binaural and contralateral FRA threshold distribution. H: The distribution of the difference between binaural and contralateral threshold.

distribution of thresholds was not significantly different ($\chi^2 = 227$, $df = 8$, $p > 0.05$) between the two types of stimulation. In order to provide a one-to-one comparison, we subtracted the contralateral threshold from the binaural threshold of each neuron. This distribution showed that the bulk of the neurons (71%) had the same threshold (Fig. 2.4H).

2.4.3 Cluster analysis

Binaural stimuli had different effects on the spectral tuning of cells in the IC (e.g., Fig. 2.3). To analyze these effects, we performed a cluster analysis to group the cells with similar properties (Fig. 2.5). We calculated the ratio of the binaural to contralateral (B/C) responses for each metric.

We first divided the cells into three groups based on threshold (Fig. 2.5, middle column): those with low thresholds (≤ 40 dB SPL, Q40, $n = 94$), those with moderate thresholds (≤ 50 dB SPL, Q30, $n = 34$), and those with high threshold (≥ 60 dB SPL, $n = 75$). The Q30 in the high threshold group could not be measured, so this group was omitted from further analysis.

Next, the neurons were clustered using the normalized B/C ratios for maximum firing rate, Q10, Q40 or Q30, and the ratio of BF to CF. Q40 and Q30 groups were clustered separately. In general, both the Q40 and Q30 groups could be subdivided into enhancement and suppression groups that together represent ~40% of the neurons (Fig. 5, right column, 51/128). For the Q40, low threshold group, 10 neurons were enhanced (cluster 1), 16 were suppressed and separated into two groups (cluster 2, $n=9$; cluster 3, $n=7$), and 56 unchanged neurons (cluster 4). The Q40 unchanged group alone accounted for 44% (56/128) of the sample. Three singleton neurons represented the remainder of the

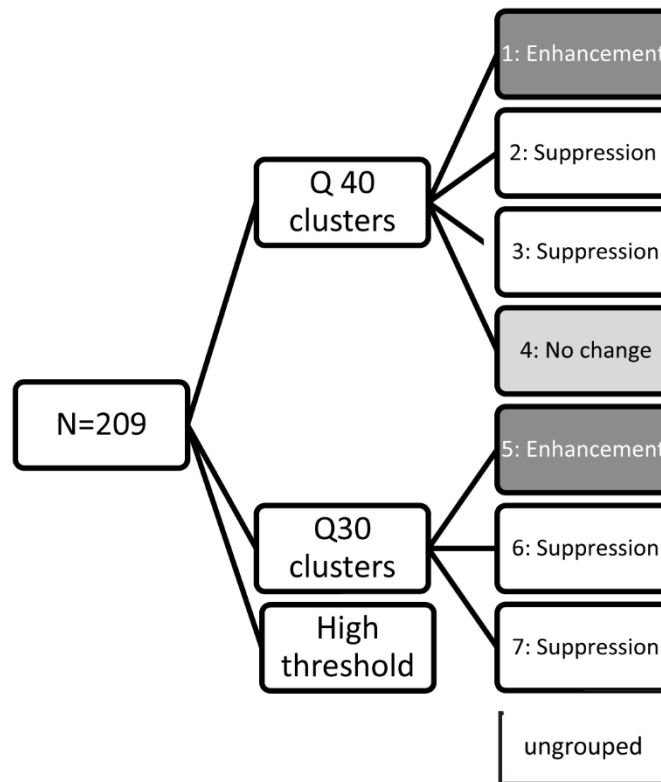


Figure 2.5. Grouping of neurons based on the properties of monaural and binaural FRAs. Middle column: Groups with low, medium or high thresholds. **Right column:** For the low and medium threshold groups, the neurons were clustered based on the B/C ratios of the maximum firing rate, the Q10, BF/CF, and Q40 (or Q30). **Numbers of neurons in each cluster are:** 1: n=10; 2: n=9; 3: n=7; 4: n= 56; 5: n=9; 6: n=11; 7: n=5.

Q40 group. For the Q30, moderate threshold group, nine neurons were enhanced (cluster 5), 17 were suppressed and divided into two groups (cluster 6, n=11; cluster 7, n=5). Due to the small sample size, there was no unchanged group in the Q30 clusters, and there was one singleton neuron. In the Q40 and Q30 groups, we did not cluster multi-peaked neurons (n=6) and neurons with threshold shifts (n=17) since the B/C ratio could not be calculated in the latter group due to a missing Q40 or Q30 value. These neurons are presented in a following section.

The separation of the clusters can be verified in the scatter plots of Figure 6. The best separation of the clusters in the Q40 group is shown when ratios of maximum rate

and Q10 are compared (Fig. 2.6A). Clusters 1, 2, and 3 separated along the maximum rate axis with the no change group straddling the ratio of 1. Cluster 4 separated due to larger relative binaural Q10 values and maximum rates <1 . This separation was not seen when Q40 and max rate are compared (Fig. 2.6C). For the Q30 groups, the maximum rate and Q30 were the major factors in clustering and these are shown best in Figure 2.6D. Cluster 6 and 7 differed in their Q30 ratios, but that separation was not seen for Q10 (Fig. 2.6B).

Evoked excitatory responses were observed in all groups: binaural enhanced, suppressed and no change. Interestingly, all groups had neurons that showed excitatory responses to ipsilateral ear stimulation: 40% (4/10 cells) and 33.3% (3/9 cells) of cells in the Q40 and Q30 enhanced group, respectively; 28.5% (16/56 neurons) in the Q40 no change group, respectively; and 25% (4/16 neurons) and 12.5% (2/16 cells) in the Q40 and Q30 suppressed group, respectively. This indicates that binaural spectral responses were not a simple summation of responses from both ears. These findings are consistent with the finding that that most cells in the IC have excitatory and inhibitory synaptic currents to contralateral and ipsilateral ear stimulation (Ono and Oliver, 2014). The distribution of sampled neurons in each group is shown in table 2.2.

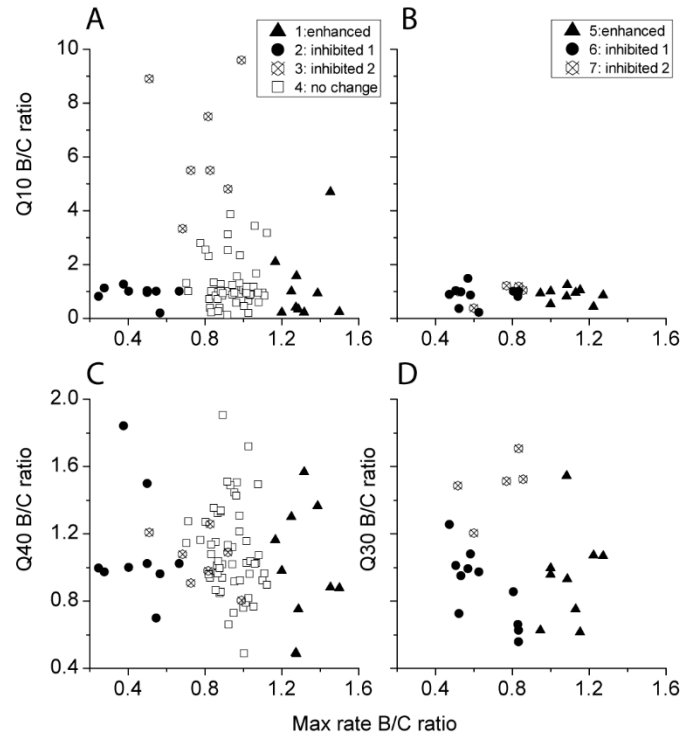


Figure 2.6. Raster plots used for the parameter ratio comparison of different clusters as a validation of cluster numbers. A: Cells with Q40 were divided into 4 groups, and the symbols with different distribution showed that the properties were—one group with enhanced firing rate, one group with inhibited rate, one group with inhibited rate and narrower Q10, one group with no change in rate. B: Cells with Q30 were divided into 3 groups, which differs in firing rate as suppressed and enhanced. C: Same group of cells from A but with comparison of Q40. D: Same groups from B but with comparison of Q30, which shows two groups have narrowed tuning at binaural condition and one group has enhanced tuning.

N=209	Q40 clusters n=94	Cluster 1. Enhancement	n=10
		Cluster 2. Suppression	n=9
		Cluster 3. Suppression	n=7
		Cluster 4. No change	n=56
		Q40 Threshold shift	n=9
		Q40 Singleton clusters	n=3
		Q40 Multi-peaked	n=3
	Q30 clusters n=34	Cluster 5. Enhancement	n=9
		Cluster 6. Suppressed	n=11
		Cluster 7. Suppressed	n=5
		Q30 Threshold shift	n=8
		Q30 Singleton clusters	n=1
		Q30 Multi-peaked	n=3
	High threshold n=75		

Table 2.2. Distribution of sampled neurons.

2.4.4 Cluster groups show effects of binaural stimulation

To show how the cells in the same cluster responded to binaural and monaural stimuli, we normalized the individual FRAs and calculated the mean to obtain a group FRA. In these group FRAs, the frequency was normalized to an octave scale and the CF set to zero.

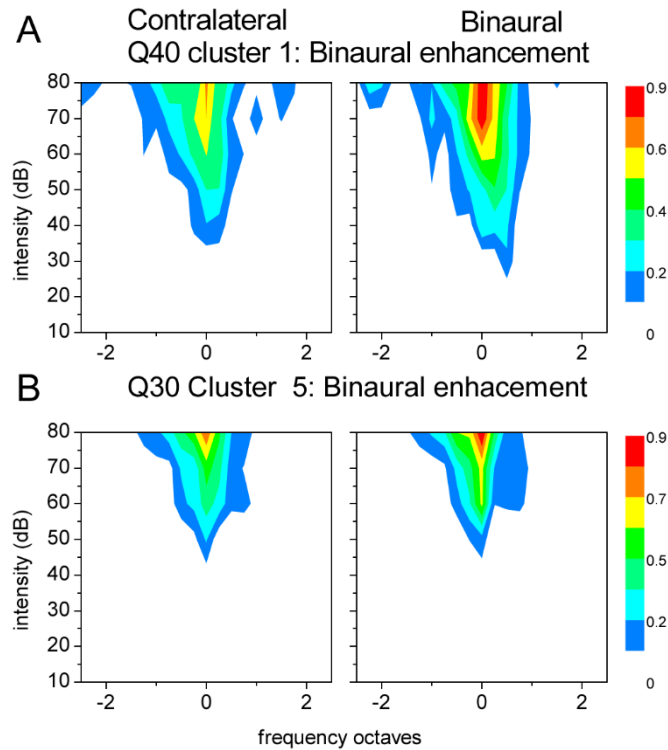


Figure 2.7. The normalized group FRAs from the two clusters that showed binaural enhancement. The mean values of the group data are shown. The frequency is normalized to an octave scale with the CF at zero. Results from low- and medium-threshold groups were similar. **A:** Low-threshold FRAs from Q40 cluster 1. **B:** Middle threshold Q30 cluster 5.

The binaurally enhanced Q40 CLUSTER 1 (Figs. 2.5-2.6) showed that binaural stimulation significantly increased the maximum firing rate around BF ($t = 4.01$, $df = 8$, $p = 0.004$), but the shape and the thresholds were similar (Fig. 2.7A). The Q30 binaurally enhanced CLUSTER 5 (Figs. 2.5-2.6) showed normalized FRA's (Fig. 2.7B) similar to

those in CLUSTER 1 except the mean thresholds were higher (~45 dB SPL). Binaural firing rate at BF was significantly higher compared to monaural firing rate ($t = -2.0$, $df = 8$, $p = 0.04$).

Two main trends emerged from the binaurally suppressed neurons (Figs. 2.5-2.6). One trend is illustrated by Q40 CLUSTER 2 and Q30 CLUSTER 6. Binaural stimulation made the tuning sharper, and the threshold higher. The mean firing rate of cluster 2 neurons (Fig. 2.8A) to binaural stimulation was about half that to contralateral stimulation. In contrast to the enhanced cluster 1, the tuning was narrower to binaural stimulation and suppressed on the low frequency side. The binaurally suppressed Q30 cluster 6 (Fig. 2.8B) was similar to the binaurally suppressed Q40 CLUSTER 2 with significant suppression at BF ($t = -3.23$, $df = 10$, $p = 0.009$). Thus, for both the Q40 and Q30 suppressed groups, binaural stimulation raised the threshold and sharpened the tuning.

The second trend is represented by the binaurally suppressed Q40 CLUSTER 3 (Fig. 2.8C). It was similar to that of CLUSTER 2 in that the binaural firing rate was lower and the tuning was narrower, but the thresholds were similar (~45-50 dB). CLUSTER 3 also differed from CLUSTER 2 since the B/C Q10 ratio were significantly different ($t = -5.85$, $df = 9$, $p = 0.0002$), and the B/C max rate ratios were also different ($t = -4.31$, $df = 9$, $p = 0.001$). Neurons in the binaurally suppressed Q30 CLUSTER 7 (Fig. 2.8D) were similar to CLUSTER 3 except that the thresholds were higher and the overall tuning was narrower. In all binaurally suppressed clusters, binaural stimulation produced a more sharply tuned response.

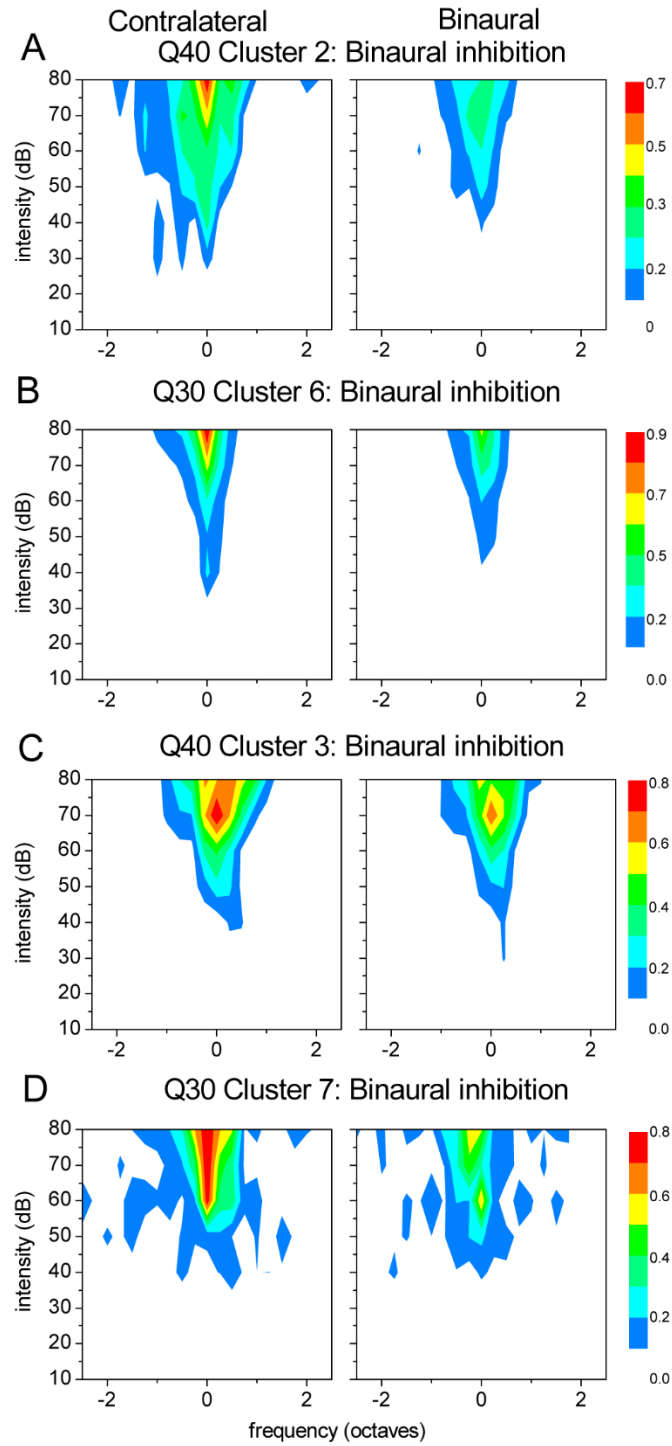


Figure 2.8. The normalized group FRAs from the clusters that showed binaural inhibition. **A:** CLUSTER 2 from the Q40 group where both the firing rate and bandwidth were reduced with binaural stimulation. **B:** CLUSTER 6 from the Q30 group where both the firing rate and bandwidth were reduced with binaural stimulation. **C:** CLUSTER 3 where the binaural tuning was sharper at low intensities and tuning was tilted to the high frequency side. **D:** CLUSTER 7 showed little change in bandwidth.

The Q40 CLUSTER 4 was classified as “no change” due to the similar binaural and monaural maximum firing rates (Figs. 2.5-2.6). For this large group, there was strong similarity in the tuning width and FRA shape (Fig. 2.9) to binaural and contralateral stimulation.

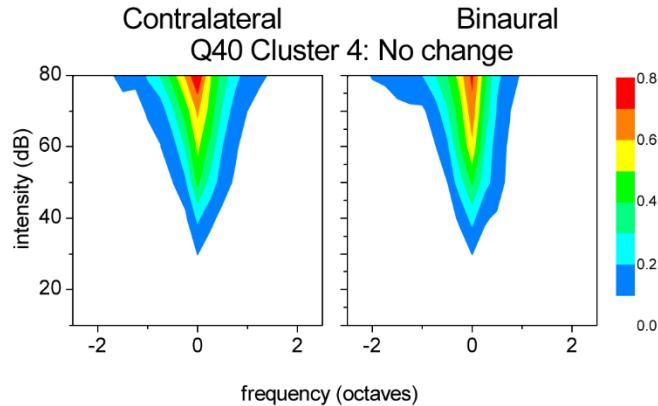


Figure 2.9. The normalized group FRA of CLUSTER 4 that showed little change in firing rates and FRA shape with binaural stimulation.

2.4.5 Large threshold shifts

Threshold shifts were seen in 29% of the neurons (60/209, Fig. 2.4H). Some of these were large enough so that the measurement of binaural or contralateral Q40 or Q30 was not possible. Because the B/C ratio could not be performed, these neurons (13%, 17/128) could not be included in the cluster analysis. In the Q40 threshold shift group (Fig. 2.10A, n=9), binaural stimulation produced a higher threshold (10 dB on average) than contralateral alone, and there was no measurable binaural Q40. This group exhibited suppression and sharper tuning with binaural stimulation in addition to the higher threshold. Thus, these neurons were similar to cluster group 2. The Q30 threshold shift group (Fig. 2.10B, n=8) showed the opposite threshold shift. Binaural stimulation

resulted in a lower threshold than contralateral alone (average -11.25 dB). Despite this, the binaural firing rate was suppressed and the binaural tuning was sharper. Consequently, this group closely resembled cluster group 3. Thus, these groups appear to be further examples of the two main modes of binaural suppression seen in the cluster analysis.

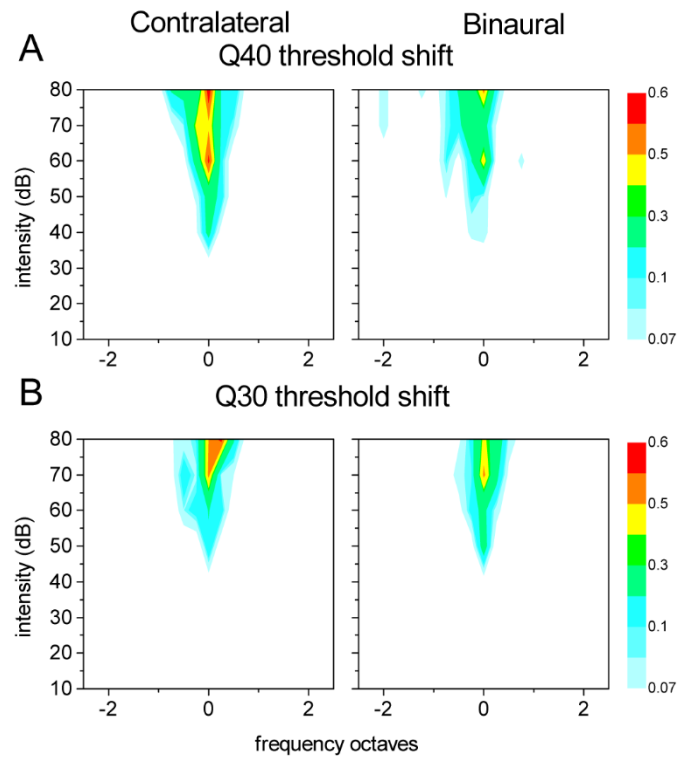
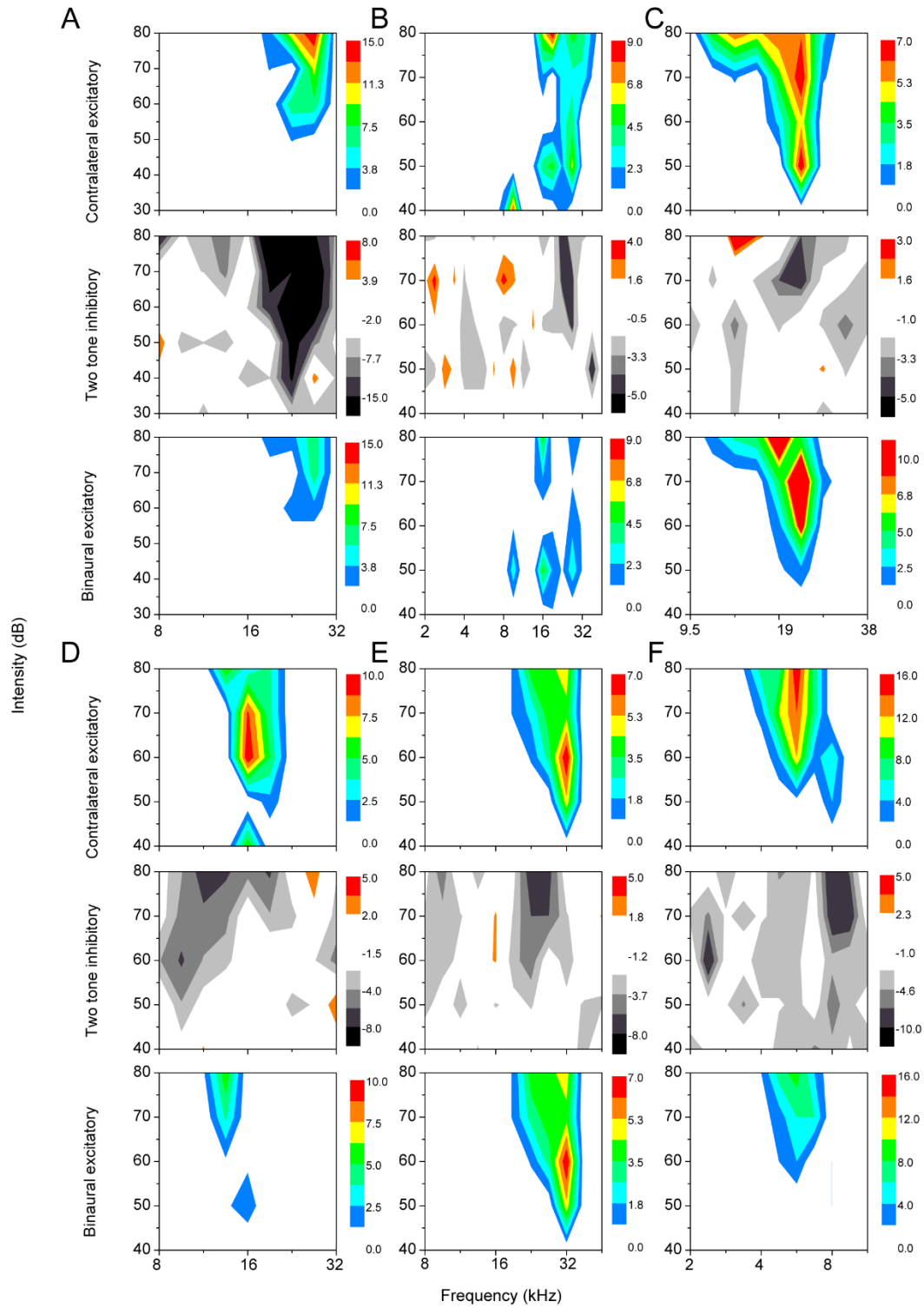


Figure 2.10. Threshold shifting. A: Q40 threshold shift group: the threshold of binaural response was higher than contralateral response, and the tuning was narrower. B: The Q30 threshold shift group: The binaural stimulation has a lower threshold than contralateral alone. Despite this, the binaural firing rate was suppressed and the binaural tuning was sharper.

2.4.6 Two-tone inhibitory FRAs

Ipsilateral stimulation may evoke inhibitory inputs to the IC neurons that is difficult to detect in extracellular recordings. To identify inhibitory effects evoked by ipsilateral ear stimulation, we recorded from 45 neurons using a binaural two-tone



cFRA. B: The sharply tuned iFRA covered similar frequency area with the cFRA, but it was more narrowly tuned. C: The iFRA had similar CF with cFRA, but the threshold was higher. D: iFRA had an inhibition at lower frequency side than cFRA. E: iFRA were stronger at low frequency side and high threshold. F: iFRA had an inhibition at higher frequency side than cFRA.

paradigm. In this sample, 78% of the neurons showed a tuned inhibitory frequency response area (iFRA). We then compared the iFRA to the contralaterally evoked excitatory FRA. We also compared contralateral FRA and iFRA to the binaural FRA to determine whether the summation of the two can predict the binaural response. Two main patterns emerged.

In 15 neurons, the iFRA displayed a similar frequency range as the contralaterally measured FRA (Fig. 2.11A, B, C). The CF and BF of the excitatory and inhibitory responses were similar. In neuron shown in Figure 2.11A, the inhibitory tuning was much broader and had a much lower threshold than the contralateral, excitatory FRA. This resulted in a narrowed and higher threshold binaural FRA as compared to the contralateral FRA. For the neuron in Figure 2.11B, the inhibitory tuning was narrower with a higher threshold. This created a binaural FRA that was suppressed at the CF where inhibition is the strongest, but lateral excitation was preserved. The prominent feature of neuron Figure 2.11C was its higher inhibitory threshold. However, the binaural FRA showed a spike rate enhancement, but no change in the shape related to ipsilateral inhibition.

In 12 neurons, there was evidence of sideband inhibition (Fig. 2.11 D, E, F). The neurons in Figures 2.11D and 11E had a CF in the iFRA that was tuned to lower frequencies than its excitatory FRA. The cell in D showed excitation where the ipsilateral inhibition was the strongest and a significant frequency shift. In contrast, the cell in E cells showed a similar shape of FRA for binaural and contralateral stimuli and little effect

of the inhibition illustrated by the iFRA. The iFRA of the neuron in Figure 10F was tuned to higher frequencies than its excitatory FRA, and this resulted in a sharpened FRA with a decreased firing in the binaural condition. Sideband inhibition from the ipsilateral ear may be the mechanism used by some neurons that showed sharper tuning to binaural stimulation.

The remaining 8 neurons showed more complicated patterns of inhibition. In some cases, a combination of different inhibitory inputs might have created a complex inhibitory response, such as a broad inhibition with multiple peaks.

2.5 DISCUSSION

The present study shows that in most cases binaural stimulation changes the spectral processing in the mouse IC. The population responses to monaural and binaural stimuli are highly correlated; but, in individual neurons, the change from monaural to binaural conditions may alter the response. In about 44% (56/128) of neurons with low or moderate thresholds, changing the binaural conditions did not alter the firing rate, bandwidth, BF/CF ratio, or threshold significantly. However, the majority of neurons showed one or more change in these parameters. Neurons that had suppressed firing with binaural stimulation represented 38% (49/128) of the sample when both the cluster results and the threshold shift units are combined. In these neurons, tuning was narrower and there was a threshold shift in response to binaural stimulation when compared to contralateral monaural stimulation, but the direction of the shift distinguished the two groups. The smallest group in our sample had enhanced firing to binaural stimuli (15%, 19/128), and the bandwidth and threshold remained the same. These data suggest the absence of a general rule for how binaural stimulation alters frequency tuning in the IC. Binaural changes in firing rate and FRA shape may interact with spectral characteristics imposed by the pinna to create the spectral cues used in sound localization.

2.5.1 Comparison with previous studies

This study is one of few that look at spectral coding in the IC with both binaural and monaural stimuli in the same neuron. Monaural frequency tuning has been studied in the auditory system in the midbrain of the cat, rabbit, rat, gerbil, chinchilla, and bat (Hind et al., 1963; Evans, 1972; Aitkin et al., 1975; Ehret and Merzenich, 1988; Casseday and Covey, 1992; Yang et al., 1992; Palombi and Caspary, 1996; Ramachandran et al., 1999;

LeBeau et al., 2001; Hernandez et al., 2005; Bandyopadhyay et al., 2007; Atencio and Schreiner, 2008; Palmer et al., 2013). In most cases, a closed sound system was used with the stimulus delivered only to the contralateral ear (Hind et al., 1963; Aitkin et al., 1975; Ehret and Merzenich, 1988; Casseday and Covey, 1992; Yang et al., 1992; Palombi and Caspary, 1996; Ramachandran et al., 1999; LeBeau et al., 2001; Hernandez et al., 2005; Palmer et al., 2013). In the present study, we used a closed sound system with independent speakers to deliver stimuli to either contralateral or ipsilateral ears or both ears together. Several studies used an open field stimulation to generate FRAs in the IC (Egorova et al., 2001; Alkhatib et al., 2006). In the mouse study by Egorova et al. (2001), the speaker was located in the anterior quadrant at 45 degrees relative to the mouse sagittal plane and contralateral to the recording electrode in the IC. That arrangement would produce an invariant, non-zero interaural level difference (ILD) with the sound in the contralateral ear loudest. In the present experiment, the binaural stimuli had an ILD of 0, similar to a speaker directly overhead or in front of the animal as in Alkhatib, et al. (2006). Despite the use of both contralateral monaural and binaural stimuli in previous studies, none of these previous studies systematically compared the monaural and binaural conditions in the same neurons.

In the literature, several schemes for the categorization of FRAs have been used in the IC. One commonly used system was devised in the cat: the V-type, I-type and O-type FRA (Ramachandran et al., 1999). Similar types have been observed in the mouse IC. Egorova et al (2001) classified FRAs in the mouse as class I, II, III and IV and class I, II, III were the major types observed in that study. Class I was a very asymmetric V-type with a steep slope on the high-frequency side, and class III was a symmetrical V-type.

Class II was similar to the I-type in that it was narrowly tuned and had inhibitory side bands. The O-type response was not reported by Egorova et al. (2001), but they did observe a multi-peaked FRA that they called their class IV. In the present study, we found examples of each of the Egorova FRA types. In addition, we observed a few O-type FRA in units that had a non-monotonic rate intensity function. We found multi-peaked FRAs rarely since our recordings avoided the dorsal cortex of ICC where they are more common (Malmierca et al., 2009). The difference between our results and those of Egorova et al. (2001) may be attributed to the larger sample size in the present study. Portfors et al. (Portfors et al., 2011) observed class IV neurons more frequently than the present study, and that might be related to differences in the methodology since we used anesthesia but Portfors et al (2011) did not. Anesthesia can alter firing patterns in the IC (Kuwada et al., 1989).

Recently, a comprehensive study of frequency tuning in over 2800 neurons from the guinea pig showed that many FRAs in the IC fall outside of discrete classes (Palmer et al., 2013). That study concluded that the types of FRAs identified in most studies, while clearly iconic, are part of a continuous pattern with many intermediate examples. This is also likely to be true for the mouse. Although we can identify the same iconic types of FRA in the mouse seen by others, we chose not to use those categories in our analysis of the impact of binaural stimulation on frequency tuning. Rather, we compared the binaural and monaural responses directly on a number of parameters without reference to the type of FRA.

2.5.2 Binaural cues and spectral processing

Binaural cues, such as ILD, may change the spectral processing for some neurons. Previously, internal time differences (ITD) were reported to change the frequency response of some low frequency neurons in the cat (Kuwada et al., 1984), and this provides evidence that monaural spectral processing may be changed by binaural stimulation. However, the comparison of monaural and binaural CF showed a high correlation of the two values for the majority of neurons (Kuwada et al., 1984). The ILD, rather than the ITD, is a more effective binaural cue for the mouse because of the small head size and poor sensitivity to low frequency stimuli (Lauer et al., 2011). In the present study, the ILD was the major binaural cue varied. Indeed, binaural diotic stimulation with 0 dB ILD could change the monaural frequency responses of some neurons in our sample (Figs. 2.3, 2.4). However, as in the case with ITD, the overall trend was that CF and BF under monaural and diotic binaural conditions were highly correlated and usually the same (Fig. 2.6).

The neurons in IC prefer some ILDs more than others (Irvine and Gago, 1990; Heffner et al., 2001; Ono and Oliver, 2014). Most neurons have a contralateral preference. That is, the highest spike rate is in response to stimuli when the level in the contralateral ear is higher than in the ipsilateral ear. This is true when the monaural ILD classifications EE, EI, and EO are used, where EE is bilateral excitation, EI is contralateral excitation with ipsilateral inhibition, and EO is contralateral excitation with no response to ipsilateral. Previous studies show that stimulation of the ear ipsilateral to the IC enhanced, suppressed or had no effect on the firing of IC neurons (Roth et al., 1978; Semple and Aitkin, 1979; Wenstrup et al., 1988; Irvine and Gago, 1990). The EE

group may correspond to our binaural enhanced group; however, other neurons in addition to that group showed evidence of ipsilateral excitation. The EI and EO groups would seem to correspond to the binaural suppression and no change groups in the present analysis, except that binaural suppression can occur in multiple ways – with bandwidth narrowing, strong or weak rate suppression, and strong or weak threshold shifts. It is unlikely that there are many truly monaural neurons EO in the mouse IC since almost all IC neurons displayed both EPSCs and IPSCs to stimuli in either ear, although stimulation in the contralateral ear evokes larger currents (Ono and Oliver, 2014).

When ILD is studied with stimuli with an average binaural level (ABL), the ILD with the highest response might be considered the “best” ILD. Most neurons have the best ILD where the level in the contralateral ear is the highest relative to that in the ipsilateral ear (e.g., >40 dB than the level in the ipsilateral ear) (Ono and Oliver, 2014). Our monaural stimulation of the contralateral ear alone is most similar with this ABL stimulus.

However, in natural, open sound field conditions, the stimulus in the ipsilateral ear is probably never 0 dB. Our data are consistent with the previous finding that the binaural synaptic effect is seen best as the ABL moves away from the best ILD (Ono and Oliver, 2014). Most neurons in our sample show some change in the binaural FRA at 0 ILD when compared to the contralateral monaural stimulus. Fewer neurons have two best ILDs in a U-shaped response to ABL, one on each side, at highly lateralized positions (Ono and Oliver, 2014). These may correspond to neurons in our sample that fire to ipsilateral monaural stimuli. Even fewer neurons have the best ILD in the center where the ILD is 0 (Ono and Oliver, 2014). These may corresponds to the binaurally enhanced

group in the present experiment. In some neurons, we determined the best ILD with ABL stimulation after collecting the FRAs for the monaural and 0 ILD stimuli. Subsequently, the FRA was collected again using the best ILD. Those results showed no major difference from the 0 ILD condition (data not shown).

2.5.3 Most inputs to the IC have similar spectral coding

Part of the explanation for the diversity of FRAs is that ICC receives ascending afferents that originate from many sources (Willott et al., 1985; Frisina et al., 1998; Loftus et al., 2004; Cant and Benson, 2006; Ito et al., 2011). Inputs terminate in ICC from the anteroventral (AVCN), posteroventral (PVCN), and dorsal (DCN) cochlear nucleus contralaterally. Other inputs arise in the ipsilateral ventral nucleus of the lateral lemniscus (VNLL) and medial superior olive (MSO). Some nuclei in the auditory brainstem have bilateral inputs to IC such as the lateral superior olive (LSO) and dorsal nucleus of the lateral lemniscus (DNLL). In the mouse, the intermediate nucleus of the lateral lemniscus (INLL) also has bilateral inputs to IC.

Direct, monaural, excitatory inputs to ICC will come from neurons in the cochlear nucleus. These neurons provide monaural excitatory inputs to the IC since the axons bypass the superior olivary complex (Kuwada et al., 1997; Xiong et al., 2013; Ono and Oliver, 2014), and they use glutamate as a neurotransmitter (Ito and Oliver, 2010). A number of neuron types in the cochlear nucleus provide excitatory inputs to the IC (Oliver, 1984, 1987), but they may provide different types of FRA input.

AVCN stellate cells exhibit FRAs that are similar to the asymmetric V type response in IC (Winter and Palmer, 1990). In contrast, the principle cells in the DCN

usually have a FRA comparable to O type in the IC (Young, 1980; Joris, 1998). Thus, cochlear nucleus may provide inputs with dissimilar excitatory FRAs.

The major binaural excitatory input to the IC in the mouse is the LSO. The MSO is quite small and mouse is most sensitive to high frequency sound, the ILD plays a more important role than ITD in sound localization.

LSO neurons send excitatory information to the contralateral IC (Oliver et al., 1995; Ito and Oliver, 2010). The spherical bushy cells in the ipsilateral cochlear nucleus send excitatory outputs to the LSO, which converge with inhibitory inputs that arise from the globular bushy cells in the opposite cochlear nucleus via the principal neurons of the medial nucleus of the trapezoid body (MNTB). This excitation-inhibition innervation in the LSO creates the binaural responses. Thus, the LSO has a higher firing rate to ipsilateral monaural stimulation than to binaural stimulation (Tsuchitani, 1977). The ipsilateral spherical bushy cell inputs is primary-like (asymmetric V-type) and has a frequency selectivity that matches the input from the MNTB driven by the globular bushy cells on the opposite side. The inhibitory frequency tuning curves in LSO had shapes and characteristics that were similar to the excitatory tuning curves (Boudreau and Tsuchitani, 1968; Tsuchitani, 1977; Caird and Klinke, 1983). Most likely, LSO inputs provide the IC with an asymmetric V-type excitatory input.

Another source excitatory input to IC in the mouse is from the INLL (Frisina et al., 1998; Ito and Oliver, 2010; Ito et al., 2011). Little is known about the spectral processing of these neurons or their inputs. Most likely, the primary input to INLL is from the contralateral cochlear nucleus.

IC neurons also receive inhibitory synaptic inputs (Kuwada et al., 1997; Xiong et al., 2013; Ono and Oliver, 2014). These can be monaural and strictly driven by stimuli in the contralateral ear, or they can reflect binaural interactions. The primary monaural inhibitory input to IC is from the VNLL (Saint Marie and Baker, 1990; Ito and Oliver, 2010). In the rat VNLL, most neurons have V-shaped FRAs, but multi-peaked responses were also frequently observed (Zhang and Kelly, 2006). Most inputs to VNLL arise from the stellate neurons of the contralateral AVCN and PVCN that also have similar FRAs.

Binaural stimulation may activate inhibitory inputs to the IC from the LSO. Glycinergic neurons in the LSO project to the ipsilateral ICC and make inhibitory synapses (Saint Marie et al., 1989b; Oliver et al., 1995). The responses of glycinergic neurons in LSO have not been identified, and they would provide ILD-sensitive asymmetric V-type inhibition to the IC in response to stimulation in the ear ipsilateral to an IC neuron. This inhibition also may show ITD-sensitivity to the envelope of high-frequency sounds.

A third binaural inhibitory input to IC is from the DNLL, a nucleus with only GABAergic neurons (Adams and Mugnaini, 1984; Ito et al., 2011). It receives input from most auditory brainstem nuclei, notably the MSO and LSO but not DCN (Shneiderman et al., 1988). Thus, its binaural properties are due to its inputs rather than from a direct binaural interaction (Xie et al., 2005; Pecka et al., 2007; Pecka et al., 2010). DNLL then projects bilaterally to the IC, and its neurons would convey ILD- and ITD-sensitive binaural inputs with asymmetric V-type FRAs.

The most important point to be gleaned from this discussion is that almost all inputs to the ICC are asymmetric V-type except for those from the DCN. This lack of

diversity suggests that the different FRAs in ICC must be due to the integration of these inputs rather than the replication of different types of input. In particular, inhibition driven by the ipsilateral ear may be important in shaping the FRA under binaural conditions.

2.5.4 The role of ipsilateral inhibition in shaping the FRA

Our use of binaural two-tone stimulation has several advantages. The two tone inhibition is usually studied using two tones produced by the same speaker (e.g. Egorova et al., 2001; Jen et al., 2002; Alkhatib et al., 2006; Felix and Portfors, 2007). For our study, the probe stimulation was played by separate speakers, one contralateral to the recording side and the other on the ipsilateral side. This separates the waveforms of the two tonal stimuli and avoids the creation of distortion products. Moreover, since the constant stimulus is contralateral and the roving stimulus is ipsilateral, it allows the identification of inhibitory or excitatory inputs induced by the stimuli in the ipsilateral ear. When we detected there was an inhibitory effect from the ipsilateral stimulation in the FRA experiment, the two tone stimulation will be played to the neuron to check the ipsilateral inhibition.

The comparison of the monaural contralateral FRA with the inhibitory FRA evoked by ipsilateral stimulation showed that most of the time the iFRA covered a similar frequency and intensity (42.9%). Less often, sideband inhibition was seen (34.3%). For most of the neurons, even when there was sideband inhibition, the CF of the iFRA were very similar to the contralateral evoked FRA. These results are similar to those single-speaker two-tone stimulation, except that in this study the ipsilateral stimulus is certain to evoke the inhibition. Ipsilateral inhibition was also clearly demonstrated with binaural

whole cell recording in the mouse IC (Ono and Oliver, 2014). Thus, the ipsilateral inhibition can shape the FRA in two ways. In most cases, it may play a gain control function since it matches the frequency and intensity range of the contralateral FRA. Less often, ipsilateral inhibition will shape the tuning by means of sideband inhibition.

The comparison of binaural FRA to the contralateral and iFRA showed that a binaural FRA is not always a summation of excitatory contralateral FRA and the ipsilateral iFRA. This is consistent with the notion that the binaural FRA reflects both excitation and inhibition driven by each ear.

2.5.5 Interaction of spectral and binaural processing

Both spectral and binaural processing in IC are based on the integration of all the excitatory, inhibitory, ipsilateral and contralateral inputs.

From the comparison of the FRA evoked by contralateral monaural and binaural ILD 0 stimuli, we showed that binaural stimulation changes either the firing rate or the shape of the FRA in the majority of the IC neurons. In some cases, the activation of binaural inputs may not provide any additional spectral information to the IC than that provided by one ear alone. Alternatively, the binaural inputs may modulate the rate or shape of the response.

In the largest single cluster (cluster 4, Fig. 2.9), the firing rates to the contralateral monaural and binaural stimulus conditions were unchanged. Thus, despite the likely synaptic inputs driven by both ears, the neurons are functionally monaural. The inputs driven by the ipsilateral ear may be subthreshold with little effect on the firing pattern to

contralateral stimulation. The role of these neurons in sound localization may be limited to the strength of their firing to highly lateralized stimuli.

The binaurally suppressed neurons showed the most obvious FRA changes. The bandwidth was narrower than with contralateral stimulation alone. Thus, binaural inhibition may be indirect and due to the suppression of excitatory inputs from the contralateral LSO and the concurrent direct inhibition from the glycinergic ipsilateral LSO. Since the LSO inputs from the two sides terminate on adjacent laminae in IC (Shneiderman and Henkel, 1987), this might produce the sideband inhibition observed with binaural two-tone stimulation. Since binaural suppression produced a sharpening of tuning, these neurons are the most likely to show an interaction of spectral and binaural processing. The narrower binaural tuning might enhance the sensitivity to spectral notches that move as they are created by the acoustics of the pinnae in response to a sounds in different locations in space. This effect might be more profound when changes in azimuth and elevation are considered together.

A small group of cells had a binaural FRA with a higher firing rate but showed little effect on the FRA shape. Despite the higher firing rate the spectral tuning is unchanged. This suggests that the inhibition and excitation co-vary in a balance manner. If such neurons correspond to center-referred neurons stimulated best by sound sources directly in front of the animal, the invariant tuning may facilitate the identification of acoustic objects.

If we compare the results of enhancement and suppression binaural effects, the enhancement of binaural stimulation only increases the firing rate but keeps the shape similar with contralateral one, however binaural suppression can change the shape of the

FRA to be sharper. This indicates the function of IC neurons as the integration center of the spectral information from lower nuclei. The frequency tuning gets either sharper or stays the same under binaural conditions but seldom gets broader, suggesting that the benefit of binaural hearing is helping the neurons tune sharper with more accurate information. This is consistent with the insight that the higher level of the auditory system, the neuron tuning becomes sharper (Katsuki et al., 1958; Suga, 1995).

Although 60% of the neurons show changes either in maximal firing rate, bandwidth or threshold, the CF, BF are very close for most neurons under two conditions. This indicates that the monaural hearing can preserve a large part of spectral information. When the analysis of frequency happens with the population of IC neurons, the perception of frequency will stay similar whether with one ear or two ears. When sound comes from different angles, the perception of frequency would not change either.

CHAPTER 3: DISCUSSION

The bulk of previous studies on frequency tuning of auditory neurons are based on monaural sound stimulation with disregard to how binaural stimulation may alter that seen to monaural frequency tuning. The goal of this study was to determine if binaural stimulation created changes in frequency tuning and if so, what are the mechanisms underlying such changes.

We found that for the majority of neurons (56%), binaural stimulation changes the frequency tuning in one or more metrics of frequency tuning: the firing rate, bandwidth, BF/CF ratio, or threshold. To binaural stimulation, more than two thirds of these neurons showed decreased firing rate, narrower tuning or shifted thresholds. The remaining cells had higher firing rates but the tuning shape remained unchanged. The diversity of the results shows that there is no universal rule for how spectral processing changes with binaural stimulation.

Our experimental setup enabled us to study the binaural effects on frequency tuning in the absence of pinnae cues. Since the majority of the neurons showed a change in frequency tuning to binaural stimulation compared with monaural, it suggests that spectral information might be used in sound localization. In addition to ITD, ILD and spectral pinna cues, the changes in the neural frequency responses may add to the information about the location of a sound source.

With the activation of binaural pathways, the frequency tuning in the IC can get sharper or stay the same, but does not get broader. This indicates a refining process in the

IC. As the integration center of the lower brainstem nuclei, the IC receive inputs from different locations carrying excitatory, inhibitory, monaural and binaural information. The spectral information coming from those locations vary in shape, CF and frequency ranges. Even with many inputs innervating the cells in the IC, they usually have a similar CF, and this makes the binaural CF similar to monaural CF. Our comparison of the inhibition and excitation with two tone experiments also showed that the ipsilateral inhibitory FRA usually has a CF similar to that of the contralateral excitatory FRA. In addition, sideband inhibition also exists for some neurons.

3.1 The output of IC—spectral processing in the thalamus and cortex

The IC integrates all the inputs from lower nuclei and sends this integrated information to the medial geniculate body (MGB) in the thalamus. The MGB, in turn, send this information to the auditory cortex (Aitkin and Webster, 1972) .

In the MGB, there are tonotopically and non-tonotopically organized regions (Aitkin, 1984). The ventral division of the MGB is the tonotopically organized as is the lateral part of the posterior group of thalamic nuclei. For neurons in those regions, many of them are narrowly tuned, but some are broadly tuned or display multi-peaked FRAs (Aitkin and Webster, 1972; Phillips and Irvine, 1979). Many MGB neurons have side band inhibitory responses (Aitkin and Dunlop, 1969; Purser and Whitfield, 1972). The neurons at non-tonotopically organized regions usually have broader tuning.

The auditory cortex is organized into multiple regions. Within the primary auditory cortex (A1), a large portion of the neurons are narrowly tuned, but some are more broadly tuned or multi-peaked (Evans and Whitfield, 1964; Evans et al., 1965; Sally and Kelly, 1988). The majority of cells in anterior auditory field (AAF) and posterior

auditory field (PAF) are also narrowly tuned. In the contrast, the neurons in the secondary auditory field (AII) appear more broadly tuned than AI neurons (Knight, 1977; Schreiner and Cynader, 1984).

3.2 Methodological considerations and limitations

3.2.1 Different ways to measure spectral processing. The method we used is the frequency response area that encompassed the parameters of frequency, intensity, and firing rate. Tuning curves are another way to display the threshold at different frequencies. In addition to those, spectro-temporal receptive field (STRF) measurements can be used to determine the spectral processing in the auditory system (Evans, 1972; Escabi and Schreiner, 2002). The STRF describes the stimulus-response function of an auditory neuron along both the spectral and temporal acoustic dimensions, to a rich stimulus ensemble, and makes no assumptions about independence of spectral and temporal response attributes (Aertsen et al., 1981; Escabi and Schreiner, 2002).

3.2.2 Anesthetized animals and awake animals comparison. Anesthesia was used in these experiments to maintain the mouse in a stable physiological state. Anesthesia can influence neural response properties (Kuwada et al., 1989; Joris, 1998; Anderson and Young, 2004; Populin, 2005). Anesthesia almost always reduces spontaneous activity, making it difficult to observe inhibitory effects. It can also enhance inhibition (Kuwada et al., 1989). One way to avoid the effects of anesthesia is to use a decerebrate preparation (Rhode and Smith, 1986; Ramachandran et al., 1999). Another way is to use an awake preparation (Kuwada and Batra, 1999; Portfors et al., 2011). Comparisons of our findings with the awake mouse showed that all frequency response types were present but class IV types were more frequently observed in the awake mouse (Portfors et al., 2011). This

difference could be due to anesthesia but it also could be due to different sampling methods. Most of our recorded neurons are from the central nucleus as verified histologically. The Portofors study likely included neural recordings from the dorsal cortex of IC.

3.2.3 Recording methods. We used glass micropipettes to record extracellular spikes because of their small size and ability to eject dyes for location verification. Metal electrodes can be of comparable size but dye injection is not possible. Functional magnetic resonance imaging (fMRI) has been used to study auditory information processing in rodents and humans (De Martino et al., 2012; Lau et al., 2013). However, compared to the single cell extracellular studies, the spatial resolution of fMRI is very limited. In addition, the loud noise produced by the fMRI machine requires the experimental sounds to be presented in the gaps between the machine evoked sounds (De Martino et al., 2013). Consequently, the time delay between the experimental sound and the fMRI response compromises the accuracy of the data.

One way to study the inhibitory inputs to the IC is to record intracellularly using the whole cell patch clamp method. Both the intrinsic properties and synaptic inputs of neurons can be recorded and analyzed with this technique.

Studies have been conducted in the auditory cortex and IC with in vivo whole cell recordings (Wehr and Zador, 2003; Sun et al., 2010; Xiong et al., 2013; Ono and Oliver, 2014). In the auditory cortex, the relation of the spectral tuning for excitatory and inhibitory inputs was described as a balanced response, which means they cover very similar frequency response area (Wehr and Zador, 2003). This indicates that although

excitatory and inhibitory inputs come from different sources, the spectral information they carry is similar when they innervate neurons in the auditory cortex.

Whole cell patch clamp experiments have been conducted in the IC as well (Xiong et al., 2013; Ono and Oliver, 2014). Subthreshold monaural and binaural inhibition was observed in almost every neuron. Those results agree with our results in that there is hidden subthreshold inhibition induced by monaural stimulation of the ipsilateral ear.

3.3 Future direction of the study

Most of the binaural stimulation used in our experiments employed pure tones with 0 ILD. Future studies measuring FRA's at different ILDs should prove to be informative. Future experiments should employ more complex sounds such as amplitude modulated sounds because this feature is present in all natural sounds including speech.

Our sounds were unnatural since they did not include the spectral alterations created by the pinna, head and body (head related transfer functions). Such alterations recorded in the ear canal can be reproduced using virtual sound technology. Future studies could examine how these pinna based cues affect the frequency tuning of IC cells virtual technology.

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