SOUND DURATION SELECTIVITY IN BAT MIDBRAIN INFERIOR COLLICULUS

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The undersigned, appointed by the Dean of the Graduate School, have examined the dissertation entitled

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ABSTRACT

Sound duration is an important acoustic parameter that contributes to the distinct spectral and temporal attributes of individual biological sounds and is therefore important for sound recognition in human speech, animal communication and bat echolocation. In the past, many studies have examined duration selectivity of auditory neurons in different animal species using single pulses as stimuli. These studies show that auditory neurons behave as band-, short-, long- and all-pass filters to sound duration. However, naturally occurring sound pulses often are in temporally patterned pulse trains rather than in temporal isolation and previous studies have shown that a neuron's response to a single pulse is often suppressed when the single pulse is positioned within a pulse train. Therefore, a neuron's duration selectivity to single pulses in temporal isolation may not predict well its response to real-world complex temporal sounds.

The main objective of this thesis is to examine the duration selectivity of neurons in the central nucleus of the midbrain inferior colliculus (IC) using bats as the mammalian model system under stimulation conditions of single pulses, temporally patterned pulse

trains and pulse-echo (P-E) pairs. Because GABA is one of the major inhibitory transmitters in the IC, this thesis also studies the role of GABAergic inhibition in shaping the duration selectivity of IC neurons using iontophoretic application of GABA or bicuculline, which is an antagonist for $GABA_A$ receptors.

The data obtained from these studies show the following. (1) Neurons at upper IC have sharper duration selectivity than neurons in the deeper IC. (2) GABAergic inhibition contributes to sharpening of duration selectivity of IC neurons to sound pulses in rapid sequences. (3) Duration selectivity of IC neurons progressively improves with the pulse repetition rate (PRR) of pulse trains. (4) Bicuculline application decreases and GABA application increases echo duration selectivity of IC neurons. The effect of bicuculline application on duration selectivity is more pronounced at high than at low PRR while the opposite is true during GABA application. (5) Echo duration selectivity of IC neurons is sharper when determined with echo pulses of P-E pairs than with single echo pulses. Echo duration selectivity also sharpens with shortening of pulse duration and P-E gap.

These data suggest that duration selectivity of IC neurons systematically varies with $GABA_A$ receptor distribution gradient within the IC. During echolocation, the improvement of duration selectivity of IC neurons by GABAergic inhibition with PRR facilitates echo recognition throughout the course of hunting.

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CHAPTER 1

GENERAL INTRODUCTION

The auditory selectivity to sound duration

Sound duration selectivity is one of the essential response properties of auditory neurons for sound recognition particularly in human speech, animal communication and bat echolocation (Covey and Casseday 1999). For example, bats emit ultrasonic vocalizations and listen to the reflected echoes to determine the distance and other properties of objects in their environment (Popper and Fay 1995; Shannon et al. 1995). The time between vocalization and echo varies with object distance. Most bats precisely adjust their signal duration to avoid temporal overlap between the outgoing call and the returning echo (Jen and Kamada 1982; Griffin 1958; Kalko and Schnitzler 1989). In humans, speech perception is based on recognition of segmental units of various durations (Goodman et al. 1994). For this reason, the auditory neurons must somehow represent auditory selectivity to sound duration. Past studies have shown that most neurons in the periphery and lower brainstem sustainedly respond to sound stimulation. As such, they do not show duration selectivity. The neurons that show duration selectivity are first found in the midbrain auditory nuclei, inferior collicular (IC) (Casseday et al. 1994; Ehrlich et al. 1997; Fuzessery 1994; Narins and Capranica 1980; Pinheiro et al. 1991).

Previous studies on duration selectivity of inferior collicular neurons

The fact that auditory neurons tuned for the sound duration have been found in the central auditory systems of a number of vertebrates, including frogs (Feng et al. 1990; Gooler and Feng 1992; Narins and Capranica 1980), mice (Brand et al. 2000; Xia et al. 2000), chinchillas (Chen 1998), cats (He et al. 1997), and various species of echolocating bats (Casseday et al. 1994, 2000; Covey et al. 1996; Ehrlich et al. 1997; Fuzessery 1994; Fuzessery and Hall 1999; Galazyuk and Feng 1997; Jen and Feng 1999; Jen and Schelegel 1982; Jen and Zhou 1999; Pinheiro et al. 1991; Zhou and Jen 2001). In the auditory midbrain of bats and other mammals, this representation is achieved by neurons tuned to sound duration, with different cells having different best durations (BDs) (Casseday et al. 1994; Ehrlich et al. 1997; Fuzessery and Hall 1999; Brand et al. 2000). The inferior collicular (IC) is a huge nucleus in the auditory midbrain, readily accessible near the dorsal surface of the skull of most echolocation bats (e.g., Pollak and Casseday 1989). The central nucleus of the IC receives and integrates excitatory and inhibitory inputs from many lower auditory nuclei (Adams 1979; Aitkin 1985; Covey and Casseday 1995; Pollak and Casseday 1995) and from the auditory cortex (Faye-Lund 1985; Games and Winer 1988; Herbert et al. 1991; Huffman and Henson 1990). In echolocation bats and other mammals, the IC marks an important transformation in the processing of temporal information. For this reason, the neural selectivity of the IC to sound duration has been studied

extensively (Brand et al. 2000; Casseday et al. 1994, 2000; Covey et al. 1996; Ehrlich et al. 1997; Fuzessery 1994; Fuzessery and Hall 1999; Galazyuk and Feng 1997; Jen and Feng 1999; Jen and Schelegel 1982; Jen and Zhou 1999; Pinheiro et al. 1991; Zhou and Jen 2001). These studies showed that many auditory neurons within the IC respond maximally to a specific duration or a range of short or long durations. Also these auditory neurons show different best durations (BDs) to a specific duration. In the big brown bat, more than 30% of neurons in IC are tuned to sound duration (Ehrlich et al. 1997; Fuzessery 1994; Pinheiro et al. 1991). The durations of the echolocation sounds used by this species of bat range from 1 to 10 ms or more and match the range of duration tuning of IC neurons (Casseday et al. 1994, 2000; Simmons 1989). These studies also showed that most IC neurons behave as band-, short- or long-pass filters to pulse duration such that they respond maximally to a specific duration or a range of short or long durations. We refer to these IC neurons as "duration selective neurons."

The underlying mechanisms for duration selectivity

The underlying mechanisms for duration selectivity have been investigated using neuropharmacological experiments (Covey et al. 1996) and intracellular recordings (Casseday et al. 1994; Ehrlich et al. 1997). Neuropharmacological experiments and intracellular recordings indicate that duration selectivity is created

in the IC (Casseday et al. 1994, 2000; Covey et al. 1996). A conceptual model has been proposed to show how duration selectivity could be formed through interaction of excitatory and inhibitory events offset in time. These studies proposed that short- and band-pass duration selective of IC neurons is the result of interactions between an early sustained inhibitory input and a delayed transient excitatory input that coincide with an offset depolarization due to rebound from inhibition or offset excitation (Casseday and Covey 1995; Casseday et al. 1994; Covey et al. 1996; Ehrlich et al. 1997).

One study proposed an anti-coincidence mechanism for creating short-pass duration selectivity (Fuzessery and Hall 1999). This study suggested that the arrival of inhibitory input before excitatory input produces a neuron's short-pass duration selectivity and the neuron's response is reduced or eliminated when the inhibitory and excitatory inputs coincide for longer durations. Moreover, other studies proposed that duration selectivity may be a consequence of a long recovery cycle (Galazyuk and Feng 1997) or it may simply be due to prolonged inhibition after excitation (Suga 1964).

More recently, the underlying mechanism for duration selectivity has also been investigated by using paired tone stimulation and extra-cellular recording in the IC of the big brown bat (Faure et al. 2003). They used a probe tone at the neuron's best frequency (BF) and the BD, and a masking tone of the same frequency but differing in duration. By manipulating the onset, overlap, and offset of probe

and masking tone, the time course of inhibition has been shown to shape the BD, duration selectivity, and first spike latency.

The role of GABAergic inhibition in shaping duration selectivity of IC neurons

In the ascending auditory pathway, the IC receives and integrates excitatory and inhibitory inputs from many lower auditory nuclei (Adams 1979; Casseday and Covey 1995; Oliver et al. 1994; Pollak and Casseday 1989; Shneiderman and Oliver 1989). Neurochemical experiments with bicuculline, a $GABA_A$ receptor antagonist, provide evidence that GABA is a major inhibitory neurotransmitter in the central nervous system (Bormann 1988; Cooper et al. 1982; Curtis et al. 1970a, b). Many studies have shown that the GABAergic inhibition contributes importantly to shape sound duration selectivity of IC neurons of the big brown bat (Casseday et al. 1994, 2000; Covey et al. 1996; Ehrlich et al. 1997; Jen et al. 1998; Jen and Zhang 1999). By application of bicuculline, the duration selectivity of bat IC neurons was abolished (Casseday et al. 1994, 2000; Covey et al. 1996; Ehrlich et al. 1997; Jen and Feng 1999). In addition to shaping the duration selectivity, GABAergic inhibition also plays an important role in shaping the auditory selectivity of IC neurons in other parameters (Cherubini et al. 2001; Curtis 1995; Fubara et al. 1996; Roberts and Ribak 1987). For examples, previous studies have shown that iontophoretical application of GABA decreases the number of impulses of bat neurons in response to sound stimuli. In

contrast, application of bicuculline increases the number of impulses, changes the discharge patterns, shortens the response latencies and broadens the excitatory but decreases the inhibitory frequency tuning curves (FTCs) of bat IC neurons (Faingold et al. 1989, 1991; Palombi and Caspary 1996; Park and Pollak 1993a, b; Vater et al. 1992; Yang et al. 1992). Moreover, application of bicuculline also shortens the recovery cycles (Lu et al. 1997) and increases the ability of auditory neurons to follow the pulse repetition rate (Lu et al. 1998).

Neuropharmacological experiments showed that auditory neurons in the IC of the big brown bat with $GABA_A$ receptors are mostly distributed in the dorsomedial region but are sparsely distributed in the ventrolateral region which is mostly distributed with neurons containing glycine receptors (Fubara et al. 1996). This uneven distribution of $GABA_A$ receptors suggests that neurons at upper IC would receive more GABAergic inhibitory inputs than neurons at the deeper IC. If this were true, the strength of GABAergic inhibition that shapes the duration selectivity of IC neurons would progressively decrease along the spatial distribution gradient of $GABA_A$ receptors within the IC. Then it follows that GABA-mediated duration selectivity of IC neurons would orderly vary with the spatial distribution gradient of GABA receptors within the IC. As such, the sharpness of GABA-mediated duration selectivity of IC neurons would progressively decrease along the dorsoventral axis of the IC.

The duration selectivity of IC neurons to temporally patterned sound pulse trains

Under natural listening conditions, many sounds including echolocation sound pulses occur in temporally patterned sound pulse trains. Natural sounds such as vocal communication sounds of many animal species typically occur as sequential sound pulses (Feng and Ratnam 2000). Therefore, the neural response and auditory selectivity to a sound pulse would be affected when the sound pulse is preceded and succeeded by other sound pulses (i.e., forward and backward masking). The neural response of IC neurons progressively decreased with sequentially presented sound pulses (Jen and Zhou 1999; Jen et al. 2001; Lu et al. 1997; Lu et al. 1998; Moriyama et al. 1994; Pinheiro et al. 1991; Wu and Jen 1995; Zhou and Jen 2002). Moreover, a recent study using paired tones stimulation showed that temporal masking affects the duration selectivity of IC neurons (Faure et al. 2003). Echolocation bat emit ultrasonic signals and listen to the returning echoes which enable them to resolve target range, shape, and location (Griffin 1958; Simmons et al. 1979). Since the echoes return with shortening duration and increasing pulse repetition rate (PRR) as bats search, approach, and finally intercept insects (Simmons et al. 1979), auditory neurons of echolocation bats must be able to extract information from echoes with duration and PRR characteristic of the various phases of hunting. Presumably, this systematic variation in pulse parameters allows bats to extract as much information as possible about the localized target from the returning echoes. Because all these

pulse parameters co-vary throughout the entire course of hunting, duration selectivity of auditory neurons is inevitably affected by other changing echo parameters. Previous study has showed that duration selectivity of bat IC neurons systematically sharpen with increasing pulse repetition rate (PRR) of temporally patterned pulse trains (Jen and Zhou 1999).

Aims of the thesis

As described previously, bat IC neurons with $GABA_A$ receptors are mostly distributed in the dorsomedial region but are sparsely distributed in the ventrolateral region which is mostly distributed with neurons containing glycine receptors (Fubara et al. 1996). Thus, we hypothesized that the organization of duration selectivity of IC neurons of the big brown bat, *Eptesicus fuscus*, in relation to graded spatial distribution of $GABA_A$ receptors, which are mostly distributed in the dorsomedial region of the IC but are sparsely distributed in the ventrolateral region. In chapter 2 of this thesis, we examined the duration selectivity of IC neurons in relation to spatial distribution gradient of $GABA_A$ receptors in the IC using iontophoretic application of GABA and bicuculline, which is an antagonist for $GABA_A$ receptor (Bormann 1988; Cooper et al. 1982).

Previous study showed that duration selectivity of bat IC neurons systematically sharpens with increasing pulse repetition rate (PRR) of temporally patterned pulse trains (Jen and Zhou 1999). Other studies showed that GABAergic

inhibition plays an important role in sharpening duration selectivity of IC neurons (Casseday et al. 2000; Jen and Feng 1999). Thus, we hypothesized that GABAergic inhibition should play an important role in sharpening duration selectivity in response to changes of temporal parameters (e.g., PPR and sound sequences). In chapters 3 and 4 of this thesis, we examined the effect of GABAergic inhibition on duration selectivity of bat auditory neurons in response to changes of temporal parameters (e.g., pulse repetition rates and sound sequences).

When insectivorous bats such as the big brown bat emit ultrasonic signals and analyze the returning echoes to hunt insects, duration selectivity of auditory neurons plays an important role in echo recognition. The success of prey capture indicates that they can effectively encode progressively shortened echo duration throughout the hunting process. Thus, we hypothesized that GABAergic inhibition should play an important role in sharpening duration selectivity in response to P–E pairs. In chapter 5 of this thesis, we examined the echo duration selectivity of bat IC neurons under stimulation conditions of single pulses and pulse–echo (P–E) pairs. This study also examines the role of GABAergic inhibition in shaping echo duration selectivity of IC neurons in response to changes of temporal parameters within P-E pairs (e.g., emitting sound duration and P-E gap). Biological relevance of these findings to bat echolocation is also discussed in each chapter.

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CHAPTER 2

DURATION SELECTIVITY ORGANIZATION IN THE INFERIOR COLLICULUS OF THE BIG BROWN BAT, *EPTESICUS FUSCUS*

Abstract

Duration selectivity of auditory neurons plays an important role in sound recognition. Previous studies show that GABA-mediated duration selectivity of neurons in the central nucleus of the inferior colliculus (IC) of many animal species behave as band-, short-, long- and all-pass filters to sound duration. The present study examines the organization of duration selectivity of IC neurons of the big brown bat, *Eptesicus fuscus*, in relation to graded spatial distribution of $GABA_A$ receptors, which are mostly distributed in the dorsomedial region of the IC but are sparsely distributed in the ventrolateral region. Duration selectivity of IC neuron is studied before and during iontophoretic application of GABA and its antagonist, bicuculline. Bicuculline application decreases and GABA application increases duration selectivity of IC neurons. Bicuculline application produces more pronounced broadening of the duration tuning curves of neurons at upper IC than at deeper IC but the opposite is observed during GABA application. The best duration of IC neurons progressively lengthens and duration selectivity decreases with recording depth both before and during drug application. As such, low best

frequency neurons at upper IC have shorter best duration and sharper duration selectivity than high best frequency neurons in the deeper IC have. These data suggest that duration selectivity of IC neurons systematically varies with $GABA_A$ receptor distribution gradient within the IC.

Introduction

Sound duration is an important feature that contributes to the distinct spectral and temporal attributes of individual biological sounds. Therefore, duration selectivity of auditory neurons plays an important role for sound recognition particularly in speech, animal communication and bat echolocation (Covey and Casseday 1999; Dai and Wright 1995; Dai and Wright 1999; Hafter et al. 1993; Popper and Fay 1995; Shannon et al. 1995; Wright and Dai 1994). Previous studies indicated that the central nucleus of the inferior colliculus (IC) is the first stage where neurons show selectivity to sound duration (Covey and Casseday 1999). The IC neurons behave as short-, band-, long-, or all-pass filter to sound duration based on their duration tuning curves (i.e., impulse-duration functions) (Brand et al. 2000; Casseday et al. 1994; Casseday et al. 2000; Covey et al. 1996; Ehrlich et al. 1997; Feng et al. 1990; Fuzessery and Hall 1999; Galazyuk and Feng 1997; Gooler and Feng 1992; Jen and Feng 1999; Jen and Schlegel 1982; Jen and Wu 2005; Jen and Zhou 1999; Pinheiro et al. 1991; Wu and Jen 2006; Wu and Jen 2006; Zhou and Jen 2001).

In the ascending auditory pathway, the IC receives and integrates excitatory and inhibitory inputs from many lower auditory nuclei (Adams 1979; Casseday and Covey 1995; Oliver et al. 1994; Pollak and Casseday 1989; Shneiderman and Oliver 1989). The inhibitory inputs to the IC are glycinergic, which originates extrinsically, and GABAergic, which originates extrinsically and intrinsically (Fubara et al. 1996; Oliver and Shneiderman 1991; Roberts and Ribak 1987). Previous studies showed that the interplay between excitation and GABAergic inhibition shaped multi-parametric selectivity (e.g., duration, frequency, amplitude, direction, etc.) of IC neurons (Casseday et al. 1994; Casseday et al. 2000; Fuzessery and Hall 1999; Jen and Feng 1999; Jen et al. 2002; Jen and Zhang 2000; Jen and Zhou 1999; Jen et al. 2001; Klug et al. 1995; Koch and Grothe 1998; LeBeau et al. 1996; LeBeau et al. 2001; Lu and Jen 2001; Wu and Jen 2006; Wu and Jen 2006). In duration selectivity, these studies showed that duration tuning curves were broadened during bicuculline application and were narrowed during GABA application.

In the IC of the insectivorous bat, *Eptesicus fuscus*, neurons with $GABA_A$ receptors are mostly distributed in the dorsomedial region but are sparsely distributed in the ventrolateral region which is mostly distributed with neurons containing glycine receptors (Fubara et al. 1996). This uneven distribution of $GABA_A$ receptors suggests that neurons at upper IC would receive more GABAergic inhibitory inputs than neurons at the deeper IC. If this were true, the strength of

GABAergic inhibition that shapes the duration selectivity of IC neurons would progressively decrease along the spatial distribution gradient of $GABA_A$ receptors within the IC. Then it follows that GABA-mediated duration selectivity of IC neurons would orderly vary with the spatial distribution gradient of GABA receptors within the IC. As such, the sharpness of GABA-mediated duration selectivity of IC neurons would progressively decrease along the dorsoventral axis of the IC.

However, a previous study in the same bat species indicated that duration selectivity of IC neurons in terms of the best duration (BD) did not appear to be systematically organized along the tonotopic axis of the IC (Pinheiro et al. 1991). Contrary to this finding, we recently observed that duration selectivity of IC neurons sequentially isolated within an orthogonally penetrated electrode progressively decreased with the recording depth (Wu and Jen 2006). These conflicting observations prompt us to re-examine the organization of duration selectivity of neurons in the IC of this bat species. Specifically, we examined the duration selectivity of IC neurons in relation to spatial distribution gradient of $GABA_A$ receptors in the IC using iontophoretic application of GABA and bicuculline, which is an antagonist for $GABA_A$ receptor (Bormann 1988; Cooper et al. 1982).

Materials and methods

Animals and surgery

Sixteen big brown bats (10 males and 6 females, 15–28 g body weight, b.w.) were used for this study. As described in previous studies (Jen et al. 1987; Jen et al. 1989), the flat head of a 1.8-cm nail was glued onto the exposed skull of each Nembutal anesthetized bat (45–59 mg/kg b.w.) with acrylic glue and dental cement 1 or 2 days before the recording session. Exposed tissue was treated with an antibiotic (Neosporin) to prevent inflammation. During recording, the bat was administered the neuroleptanalgesic, Innovar-Vet (Fentanyl 0.08 mg/kg b.w. Droperidol 4 mg/kg b.w.), and placed inside a bat holder (made of wire mesh) that was suspended in an elastic sling inside a double-wall sound-proof room (temperature 28–30 °C). The ceiling and inside walls of the room were covered with 3-in. convoluted polyurethane foam to reduce echoes. After fixing the bat's head with a set screw, small holes were made in the skull above the IC for insertion of 3 M KCl glass pipette electrodes (impedance: 5–10 MΩ). Additional doses of Innovar-Vet were administered during later phases of recording when bats showed signs of discomfort. A local anesthetic (Lidocaine) was applied to the open wound area. The recording depth was read from the scale of a microdrive (David Kopf). A common indifferent electrode (silver wire) was placed at the nearby temporal muscles. Each bat was used in one to five recording sessions on separate days and each recording

session typically lasted for 2–6 h. New small holes were made in the skull above the IC for insertion of 3 M KCl glass pipette electrodes in each recording session. The experiments were conducted according to NIH publication No. 85-23, "Principles of Laboratory Animal Care" and with the approval of the Institutional Animal Care and Use Committee of the University of Missouri–Columbia.

Acoustic stimulation

Sound pulses (4 ms with 0.5 ms rise-decay times at 2 pulses per second) were generated with an oscillator (KH model 1200) and a homemade electronic switch driven by a stimulator (Grass S88). These stimuli were then amplified after passing through a decade attenuator (HP 350D) before they were fed to a small condenser loudspeaker (AKG model CK 50, 1.5 cm diameter, 1.2 g) that was placed 23 cm away from the bat and 30° contralateral to the recording site. Calibration of the loudspeaker was performed with a 1/4-in. microphone (B&K 4135) placed at the position of the bat's head during recording using a measuring amplifier (B&K 2607). The output of the loudspeaker was expressed in dB SPL in reference to 20 μPa root mean square.

Upon isolation of an IC neuron with 4-ms sound pulses, its best frequency (BF) was determined by changing the frequency and intensity of sound stimuli. The minimum threshold (MT) at the BF was defined as the sound level that elicited 50% response probability from the neuron. The duration selectivity of the IC neuron was

then studied by plotting its duration tuning curve using the number of impulses in response to BF sound pulses that were varied in 8 durations (1, 1.5, 2, 4, 6, 8, 10 and 20 ms). Rise-decay times for these different durations were typically 0.5 ms but they were 0.25 ms for 1-ms pulse duration. The amplitude of BF sound pulses was set at 10 dB above the MT. The duration tuning curve was plotted before and during drug application.

Iontophoresis and recording

Iontophoretic application of bicuculline and GABA to recorded IC neurons has been described in previous studies (Lu et al. 1997, 1998). Briefly, a three-barrel or five-barrel electrode (tip: 10–15 μm) was piggybacked to a 3 M KCl single-barrel electrode (tip: less than 1 μm; impedance: 5–10 MΩ) whose tip was extended about 10 μm from the tip of the three-barrel electrode. The 3 M KCl single-barrel recording electrode was used to record neural responses. One of the barrels of the three-barrel electrode was filled with bicuculline methiodide (10 mM in 0.16 m NaCl, pH 3.0; Sigma) or gamma-aminobutyric acid (GABA, 500 mM in distilled water, pH 3.5; Sigma). However, when a five-barrel electrode was used, two barrels were filled with both drugs respectively such that both drugs could be applied to the recorded neuron. The bicuculline and GABA were prepared just prior to each experiment and the electrode filled immediately before use. The drug channel was connected via silver–silver chloride wire to a microiontophoresis constant current generator

(Medical Systems Neurophore BH-2) that was used to generate and monitor iontophoretic currents. During drug application, a 1-s pulse of positive 40 nA at 0.5 pps was applied for 1 min before data acquisition. Bicuculline application was considered to have blocked $GABA_A$ receptors maximally for each neuron when three consecutive responses did not vary by more than 10% even at higher application current of 60–70 nA. The application current was changed to 10 nA during data acquisition. The other two barrels were filled with 1 M NaCl (pH7.4), one of which was used as the ground and the other as the balanced barrel. The balance electrode was connected to a balance module. The retaining current was negative 8–10 nA.

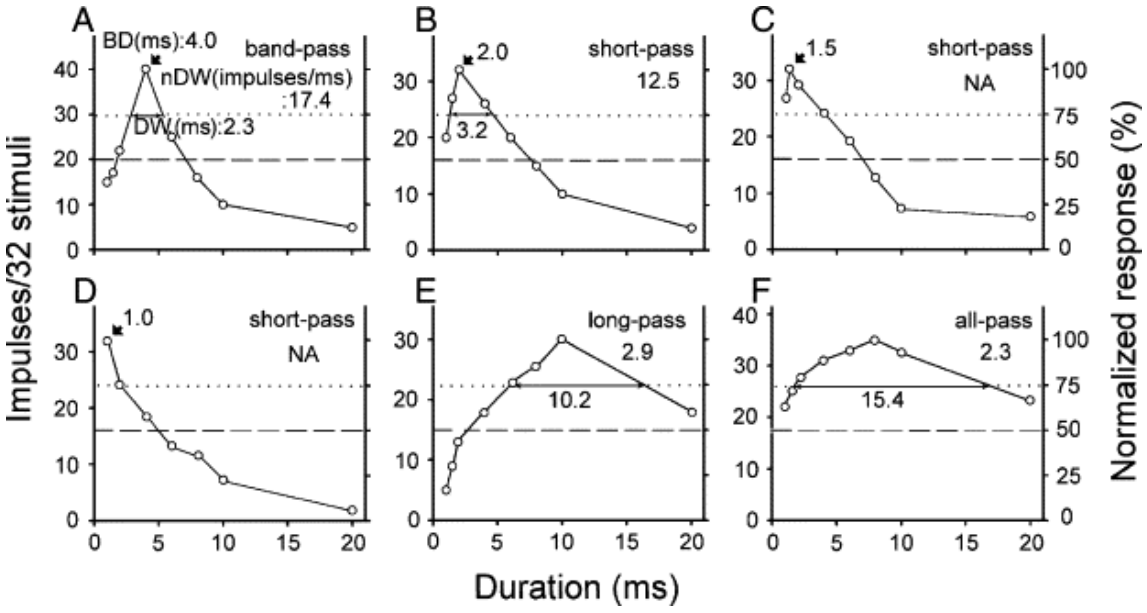
To determine any potential artifacts due to passing current or low pH values, the balanced barrel was filled with 1 M NaCl (pH 3.0) and the same amount of current used for bicuculline application was passed through the balanced barrel. Stimulus artifacts were considered negligible when the number of impulses of a neuron was affected by less than 10% before and after current application. Otherwise, the data were discarded, and a new electrode was used for the experiment. Data were also discarded when the impedance of the bicuculline-filled electrode varied more than 20 MΩ before and after the recording, the tip of the multi-barrel electrode broke when withdrawn from the recording site or both tips of the single and the multi-barrel electrode separated from each other.

Recorded action potentials were amplified, band-pass filtered (Krohn-Hite 3500), and then fed through a window discriminator (WPI 121) before being sent to an oscilloscope (Tektronix 5111) and an audio monitor (Grass AM6). They were then sent to a computer (Gateway 2000, 486) for acquisition of peri-stimulus time (PST) histograms (bin width: 500 μs, sampling period: 300 ms) to 32 presentations of stimuli.

Data analysis

In this study, echo duration selectivity of bat IC neurons was determined by plotting echo duration tuning curves with the number of impulses discharged to single pulses against durations of 1, 1.5, 2, 4, 6, 8, 10 and 20 ms. As in our previous study (Jen and Wu 2005), the tuning properties of an echo duration tuning curve were expressed with the best duration (BD) and normalized duration width (nDW) at 75% of the maximum. The BD is the duration of the maximum in band-, short- or long-pass duration tuning curves of IC neurons (indicated by a filled arrowed in ***Figure 2.1***, A, B, C, D). The nDW is obtained by dividing the maximum by the duration width of an echo duration tuning curve at 75% maximum. The BD and nDW of echo duration tuning curves of each IC neuron were compared statistically among these recording depths using repeated measures one-way or two-way ANOVA followed by a Student–Newman–Keuls multiple comparisons post-test with significance established at the $P<0.05$ level.

Figure 2.1 The duration tuning curves of 6 neurons in the central nucleus of the inferior colliculus (IC) of the big brown bat, *Eptesicus fuscus*. Left and right ordinates represent the number of impulses per 32 presentations of best frequency (BF) pulses and normalized response (%). The abscissa represents pulse duration (ms). Duration tuning properties of these neurons are (A) band-pass, (B–D) short-pass, (E) long-pass and (F) all-pass. The horizontal dotted and dashed lines indicate the 75% and 50% maximal response. Duration selectivity of each curve is expressed with a best duration (BD, filled arrowhead) and a normalized duration width (nDW, double arrowhead) which is obtained by dividing the maximum by the DW of a duration tuning curve at 75% maximum. The BF (kHz), latency (ms), MT (dB SPL) and recording depth (μm) of these neurons were 25.6, 11.5, 40, 416 (A); 22.4, 11.0, 37, 355 (B); 21.5, 11.5, 34, 338 (C); 19.5, 11.0, 35, 244 (D); 46.5, 13.5, 48, 1024 (E); 43.5, 13.0, 46, 946 (F).


A
BD(ms):4.0
band-pass
nDW(impulses/ms)
:17.4
DW(ms):2.3
B
2.0
short-pass
12.5
3.2
C
1.5
short-pass
NA
D
1.0
short-pass
NA
E
long-pass
2.9
10.2
F
all-pass
2.3
15.4
Impulses/32 stimuli
Normalized response (%)
Duration (ms)

Results

In this study, 216 IC neurons were isolated at depths between 125 and 1755 μm (610 ± 212 μm). Their BFs and MTs were 16.5–63.5 kHz (35.7 ± 7.4 kHz) and 28–58 dB SPL (44.5 ± 8.2 dB SPL). The first-spike latency determined with 4 ms BF sounds at 10 dB above the MT was between 8 and 19 ms (12.6 ± 1.3 ms).

Duration tuning curves of IC neurons

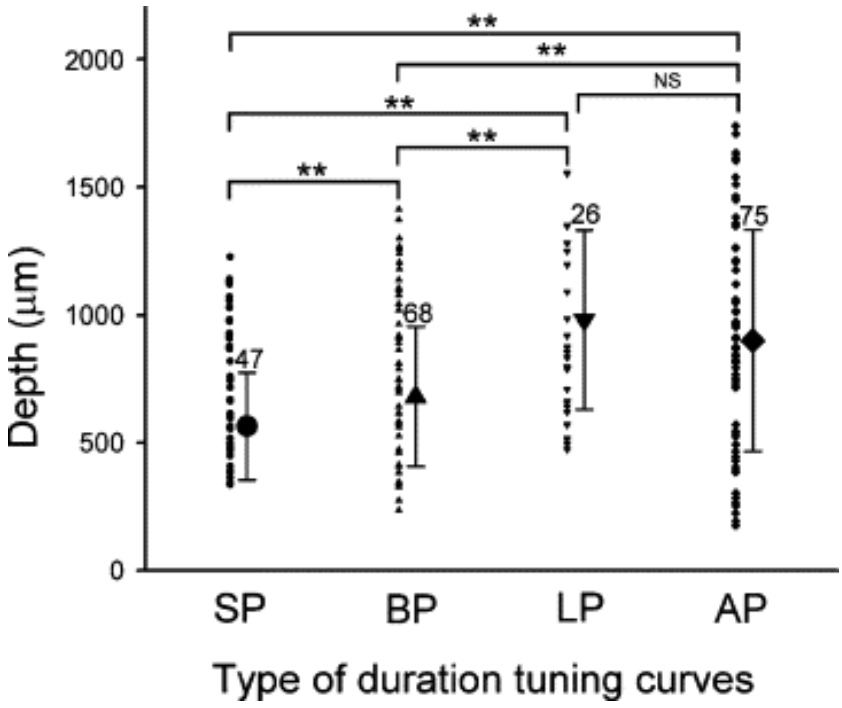
In total, 465 duration tuning curves were plotted for 216 neurons before and during drug application. Based on the variation in the number of impulses with pulse duration, these curves can be described as the following four types. (1) Neurons with *band-pass duration tuning curves* discharged a maximal number of impulses to a specific duration and the maximal number of impulses decreased at least 50% at both limbs (***Figure 2.1***, A, n = 68, 31%). (2) Neurons with *short-pass duration tuning curves* discharged a maximal number of impulses to a short duration. The maximal number of impulses of these neurons either decreased no more than 50% or ceased firing at a shorter duration but decreased more than 50% at a longer duration (***Figure 2.1***, B–D, n = 47, 22%). (3) Neurons with *long-pass duration tuning curves* discharged maximally to a long duration. The maximal number of impulses of these neurons either decreased no more than 50% or ceased firing at a longer duration but decreased more than 50% at a shorter duration (***Figure 2.1***, E, n = 26,

12%, other types are not shown). (4) The number of impulses of neurons with *all-pass duration tuning curves* often differed more than 25% but never more than 50% at all durations tested (***Figure 2.1***, F, n = 75, 35%).

To determine if these four types of neurons are spatially localized along the dorsoventral axis of the IC, we examined the distribution of the recording depth of these neurons. As shown in ***Figure 2.2***, the average recording was 565 ± 210 μm (range: 336–1229 μm) for the short-pass neurons, 681 ± 274 μm (range: 236–1415 μm) for the band-pass neurons, 982 ± 352 μm (range: 475–1556 μm) for the long-pass neurons, and 901 ± 431 μm (range: 176–1811 μm) for the all-pass neurons.

It is clear that the all-pass IC neurons were recorded over a wider range of depth than the other three types of IC neurons were. Repeated measures one-way ANOVA showed significant differences among these recording depths ($P < 0.05$). A Student–Newman–Keuls multiple comparisons post test indicated that excluding the average recording depth between long- and all-pass neurons ($P > 0.05$), the difference in the average recording depth between short- and band-pass neurons, short- and long-pass neurons, short- and all-pass neurons, band- and long-pass neurons, band- and all-pass neurons was significantly different ($P < 0.01$). We did not specifically examine if these neurons were spatially localized along the rostrocaudal or mediolateral axes of the IC, although we sampled the IC neurons with electrodes inserted into most area of the IC.

Figure 2.2 The range of recording depth (μm) of IC neurons with short-, band-, long- or all-pass (SP, BP, LP, AP) duration tuning curves. The sample size, mean and standard deviation of the recording depth for each type of IC neurons are shown by each distribution (see text for details).


**
**
NS
**
**
**
2000
1500
1000
500
0
Depth (µm)
47
68
26
75
SP
BP
LP
AP
Type of duration tuning curves

Duration selectivity of band- and short-pass IC neurons

Although we classified the filtering properties of 141 neurons as band-, short- and long-pass, we did not determine if the long-pass neurons obtained in this study were truly duration selective or simply due to a long integration time that they required to reach maximal response. For this reason, we mainly examined the organization of duration selectivity of band- and short-pass neurons in relation to spatial distribution gradient of $GABA_A$ receptors in the IC in this study. The short- and band-pass duration tuning is known to be shaped by GABAergic inhibition (Casseday et al. 1994; Casseday et al. 2000; Covey et al. 1996; Ehrlich et al. 1997).

We expressed the duration selectivity of band- and short-pass duration tuning curve with the best duration (BD) and duration width (DW). The BD is the duration of the maximal number of impulses in the duration tuning curves (BD, indicated by filled arrowhead in ***Figure 2.1***, A–D). Because bicuculline and GABA application respectively produced an increase and decrease in the number of impulses of IC neurons, the duration tuning curves varied in opposite ways. As such, the maximal number of impulses obtained from a neuron varied greatly before and during drug application. To take into account of the variation in the number of impulses during drug application, we used a normalized DW to express the sharpness of a duration tuning curve. The nDW of a duration selective neuron was obtained by dividing the maximum by the DW of a duration tuning curve at 75% of

the maximum (***Figure 2.1***, DW indicated by a double arrowhead). Thus, a neuron with a large nDW has a sharper duration tuning curve and greater duration selectivity than a neuron with a small nDW. By definition, short-pass duration tuning curves in which the maximal number of impulses did not decrease more than 25% do not have an nDW (***Figure 2.1***, C, D).

The BD of 47 band-pass duration tuned IC neurons ranged between 1.5 and 10 ms (m ± SD: 4.9 ± 2.4 ms) with 78% (53/68) shorter than 6.0 ms (***Figure 2.3*** unfilled bar). The BD of 68 short-pass duration tuned IC neurons ranged between 1.0 and 8.0 ms (m ± SD: 3.8 ± 2.0 ms) with most 91% (43/47) shorter than 6.0 ms (***Figure 2.3*** filled bar).

To study the correlation of the sharpness of duration tuning curves with the BD, we plot the distribution of the nDW of IC neurons against the BD (***Figure 2.4***, A). A linear regression analysis reveals that the nDW of IC neurons significantly decreases with the BD ($P < 0.01$). As shown in ***Figure 2.4*** B, the average nDW significantly decreases with the BD such that IC neurons with short BD have sharper duration selectivity than IC neurons with long BD (one-way ANOVA, $P < 0.01$).

Figure 2.3 Bar distribution of the BD of IC neurons with band- and short-pass duration tuning curves (see text for details).

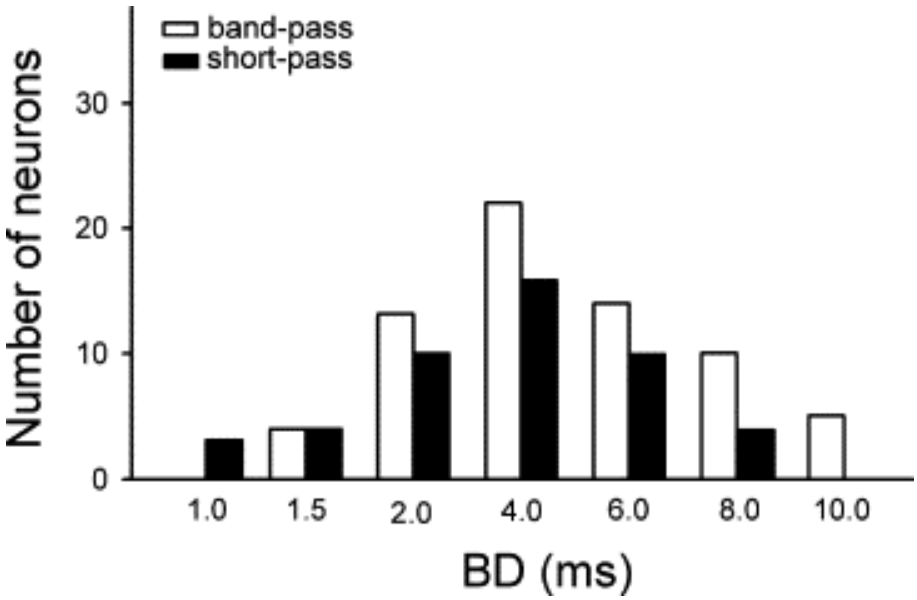
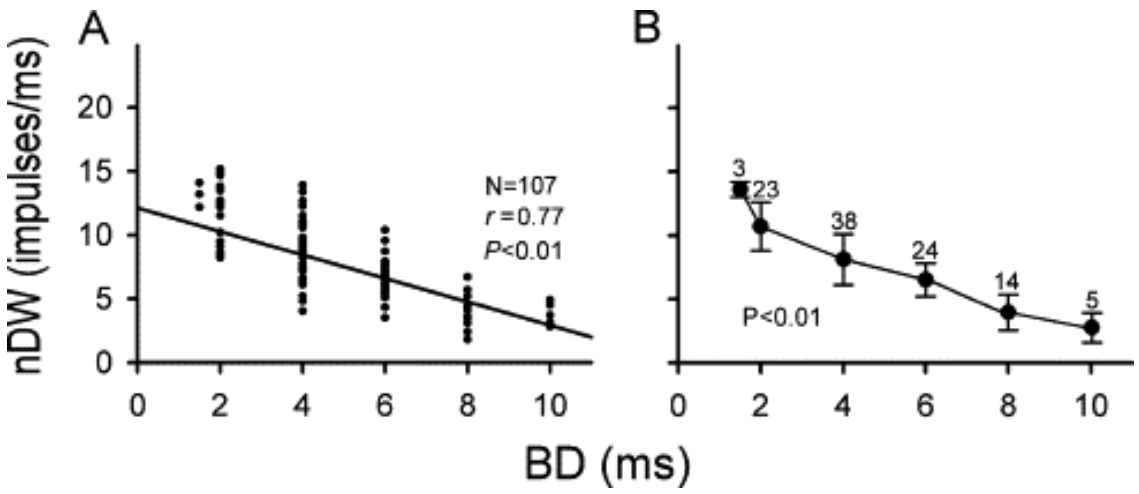

band-pass
short-pass
Number of neurons
30
20
10
0
1.0
1.5
2.0
4.0
6.0
8.0
10.0
BD (ms)

Figure 2.4 (A) A scatter plot showing the distribution of nDW of IC neurons against the best duration (BD). The solid line indicated the linear regression line. *r*: correlation coefficient. *P*: significance level. *N*: number of neurons. (B) A plot showing the average nDW of IC neurons in relation to BD. The number of neurons obtained at each BD is shown atop of the standard deviation bar. Repeated measures one-way ANOVA reveals significant decrease in nDW with BD ($P < 0.01$).


A
B
nDW (impulses/ms)
BD (ms)
N=107
r=0.77
P<0.01
P<0.01
3
23
38
24
14
5
0
5
10
15
20
0
2
4
6
8
10

Duration tuning curves plotted before and during drug application

Among the 115 band-pass and short-pass duration tuned IC neurons studied, 38 received bicuculline application and 36 received GABA application. In another 41 neurons, they received bicuculline application first. After recovery from the drug effect, they then received GABA application. Therefore, a total of 79 band (n = 32)- or short-pass (n = 47) neurons received bicuculline application and 77 band (n= 29)- or short-pass (n = 48) neurons received GABA application.

Consonant with previous studies (Casseday et al. 1994; Casseday et al. 2000; Jen and Feng 1999; Jen and Wu 2005; Wu and Jen 2006; Wu and Jen 2006), bicuculline application broadened and GABA application narrowed the duration tuning curves of IC neurons. ***Figure 2.5*** shows the duration tuning curves of two IC neurons plotted before, during and after drug application. In one neuron that received bicuculline application, the application produced greater increase in the number of impulses for non-BD durations than for near BD durations such that the neuron's band-pass duration tuning curve was broadened and changed into all-pass duration tuning curve with greatly increased DR and decreased nDW (***Figure 2.5***, Aa vs. Ab). Upon recovery from bicuculline application, the neuron's duration tuning curve is very similar to the pre-drug duration tuning curve (***Figure 2.5***, Aa vs. Ac).

In the other neuron that received GABA application, the application greatly decreased the number of impulses that was greater for non-BD durations than for

near BD durations. As a result, the neuron's short-pass duration tuning curve became narrower with increased duration selectivity as evident by greatly decreased DW and increased nDW (***Figure 2.5***, Ba vs. Bb). The neuron's duration tuning curve returned to the pre-drug one after recovery from GABA application (***Figure 2.5***, Ba vs. Bc).

Among 79 IC neurons that received bicuculline application, 34 neurons changed into long- or all-pass neurons (e.g., ***Figure 2.5***, Aa vs. Ab). As a result, only 45 IC neurons still had a BD in which the BD significantly increased in 16 neurons (increased from 2.7 ± 1.6 ms, range: 1.0–8.0 ms to 7.8 ± 2.4 ms, range: 2.0–10.0 ms, *t* test, $P < 0.01$) but did not change in the other 29 neurons. As a whole, the average pre-drug BD significantly increased from 4.1 ± 1.5 (range: 1.0–10 ms) to 5.7 ± 1.9 ms (range: 2.0–10 ms, ***Figure 2.6***, Aa Pre vs. Bic, t test, $P < 0.01$). Similarly, only 58 neurons still had an nDW during bicuculline application. The average pre-drug nDW decreased from 7.2 ± 2.5 (range: 1.8–14.1) to 3.8 ± 1.7 impulses/ms (range: 1.4–5.5, ***Figure 2.6***, Ab, Pre vs. Bic, *t* test, $P < 0.01$).

GABA application narrowed the duration tuning curves of all 77 IC neurons (***Figure 2.5***, Ba vs. Bb). The BD significantly decreased in 29 neurons (from 6.2 ± 2.1 ms, range: 2.0–10.0 ms to 2.4 ± 1.5 ms, range: 1.0–8.0 ms, *t* test, $P < 0.01$) but did not change in the other 48 neurons. As a whole, the average pre-drug BD significantly decreased from 4.3 ± 1.7 (range: 1.0–10 ms) to 2.4 ± 1.2 ms (range:

1.0–8.0 ms, ***Figure 2.6***, Ba Pre vs. GABA, *t* test, $P < 0.01$). In parallel to this observation, the average pre-drug nDW increased from 7.0 ± 2.5 (range: 1.1–14.7) to 8.6 ± 2.8 impulses/ms (range: 2.5–16.5, ***Figure 2.6***, Bb, Pre vs. GABA, *t* test, $P < 0.01$).

Figure 2.5 Duration tuning curves of two IC neurons plotted before (Aa, Ba), during (Ab, Bb) and after (Ac, Bc) recovery from drug application. Note that bicuculline application broadened the duration tuning curve (Aa vs. Ab). Conversely, GABA application narrowed the duration tuning curve (Ba vs. Bb). Furthermore, the pre- and post-drug duration tuning curves are very similar (see text for details).

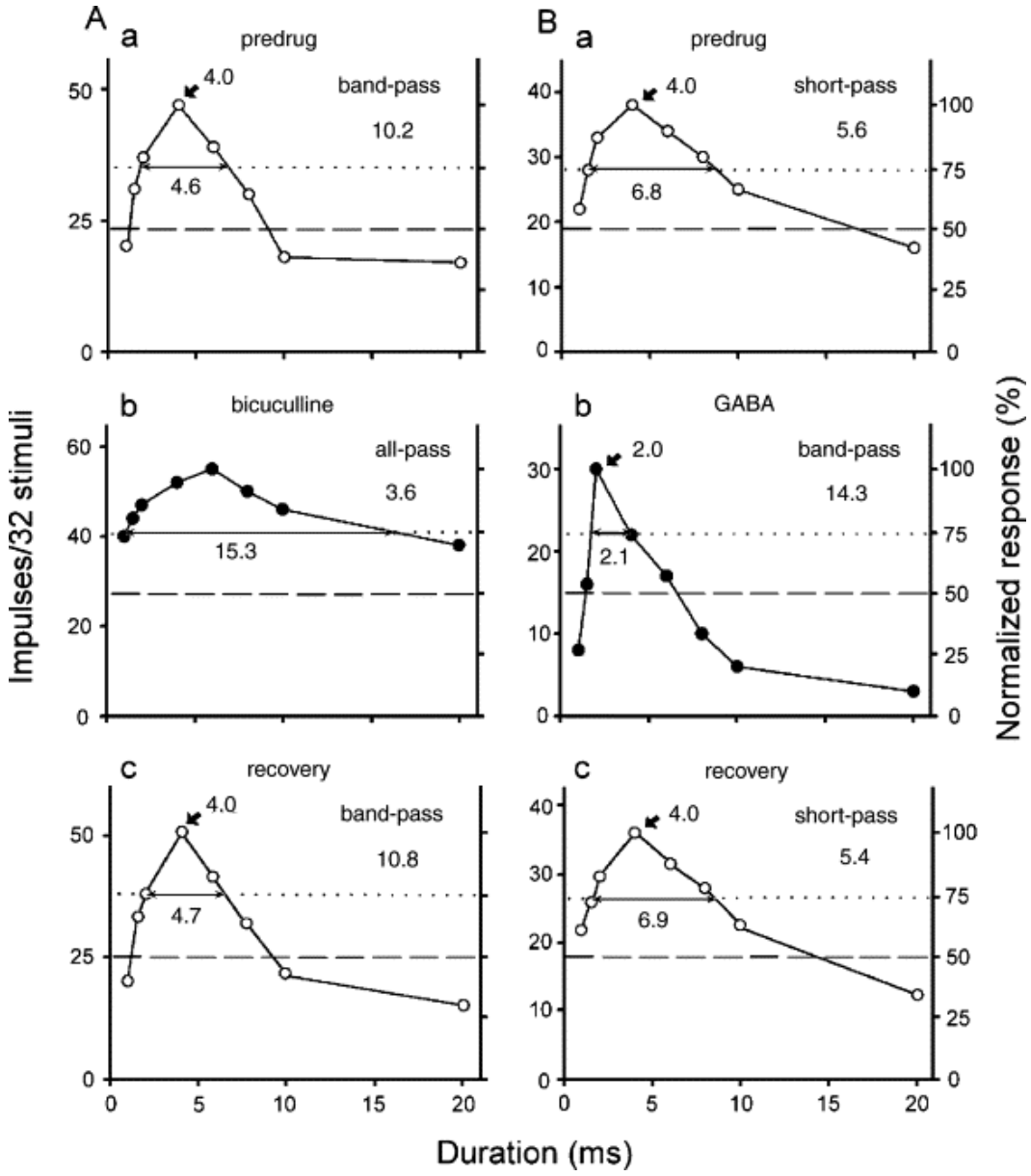
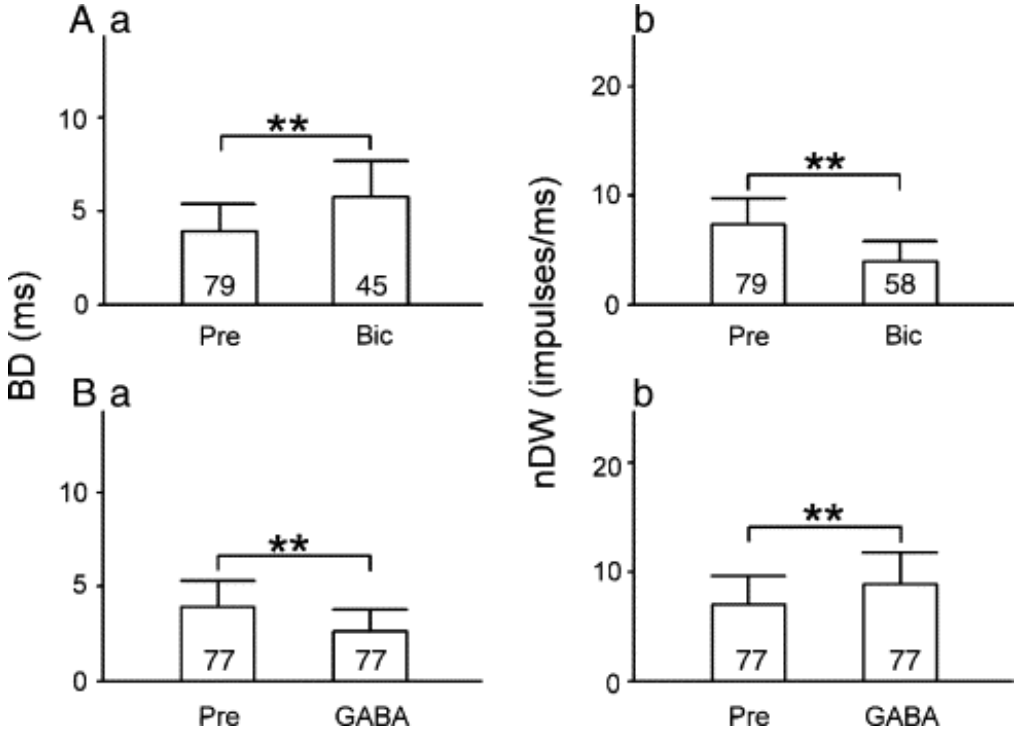

A
a
predrug
4.0
band-pass
10.2
4.6
B
a
predrug
4.0
short-pass
5.6
6.8
b
bicuculline
all-pass
3.6
15.3
b
GABA
2.0
band-pass
14.3
2.1
c
recovery
4.0
band-pass
10.8
4.7
c
recovery
4.0
short-pass
5.4
6.9
Impulses/32 stimuli
Normalized response (%)
Duration (ms)

Figure 2.6 Comparisons of the average BD (A) and nDW (B) of IC neurons obtained before and during drug application. Note that the average BD significantly increased and the average nDW decreased during bicuculline application (*t* test, $^{**}P < 0.01$). Conversely, the average BD significantly decreased and the average nDW significantly increased during GABA application (*t* test, $^{**}P < 0.01$).


A a
BD (ms)
10
5
0
**
79
45
Pre
Bic
b
nDW (impulses/ms)
20
10
0
**
79
58
Pre
Bic
B a
10
5
0
**
77
77
Pre
GABA
b
20
10
0
**
77
77
Pre
GABA

Organization of duration selectivity of IC neurons

Consonant with previous studies (Jen and Schlegel 1982; Pinheiro et al. 1991; Poon et al. 1990; Wu and Jen 1991), the 115 band- and short-pass duration tuned IC neurons were tonotopically organized along the dorsoventral axis of the IC such that the BF of IC neurons increased with recording depth (***Figure 2.7***, A, linear regression analysis, $P < 0.01$). Furthermore, linear regression analyses of the scatter plots of BD and nDW against the BF revealed that the BD and nDW of IC neurons significantly increased and decreased with the BF respectively (***Figure 2.7***, B, C).

To examine the organization of duration selectivity of these neurons in relation to spatial distribution gradient of $GABA_A$ receptors in the IC, we plotted the distribution of the BD and the nDW of these IC neurons against the recording depth (***Figures 2.8-2.9***, Aa, Ba). Although individual BDs and nDWs are distributed over a wide range of recording depth, linear regression analyses indicated that the BD is significantly correlated with recording depth such that neurons with long BD tend to be located at deeper IC than neurons with short BD both before and during drug application (***Figure 2.8***, Aa, Ba, Ca, $P < 0.01$). This observation is further evident with the fact that the average BD significantly increased with the recording depth (***Figure 2.8***, Ab, Bb, Cb, one-way ANOVA, $P < 0.01$).

In parallel to this observation, the distribution of nDW of IC neurons shifted leftward (i.e., decreased) during bicuculline application but shifted rightward (i.e.,

increased) during GABA application (***Figure 2.9***, Aa, Ba, filled vs. unfilled circles). Linear regression analyses revealed that the nDW significantly decreased with recording depth both before and during drug application (***Figure 2.9***, Aa, Ba, solid and dashed lines, $P < 0.01–0.05$).

We further examined the change in nDW of the duration tuning curves of these neurons during drug application in relation to recording depths by plotting the change in nDW against recording depth. Linear regression analysis revealed that the change in nDW is significantly larger for IC neurons at upper IC than at deeper IC during bicuculline application (***Figure 2.9***, Ab, $P < 0.01$). Conversely, the change in nDW of IC neurons is significantly smaller for IC neurons at upper IC than at deeper IC during GABA application (***Figure 2.9*** , Bb, $P < 0.01$).

Figure 2.7 Scatter plots showing the distribution of BF of IC neurons in relation to the recording depth (A), BD (B) and nDW (C). The linear regression line and correlation coefficient in each plot are shown by a solid line and *r* (see text for details).

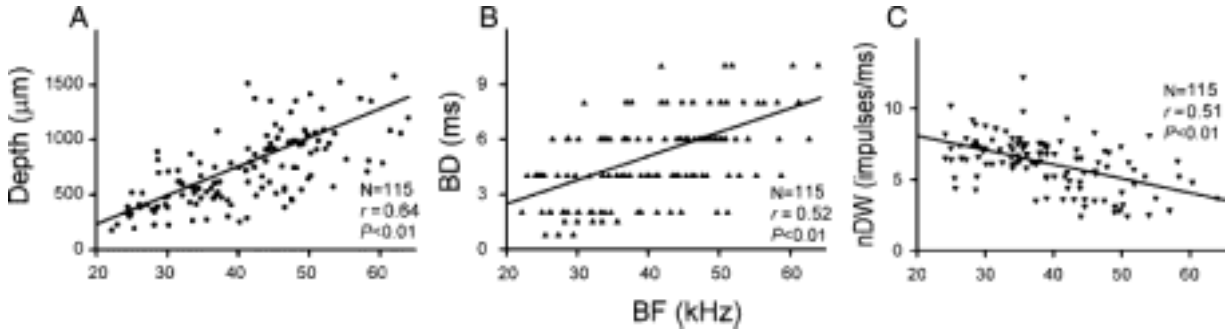

A
Depth (μm)
1500
1000
500
0
20
30
40
50
60
N=115
r = 0.64
P<0.01
B
BD (ms)
9
6
3
0
20
30
40
50
60
N=115
r = 0.52
P<0.01
BF (kHz)
C
nDW (impulses/ms)
10
5
0
20
30
40
50
60
N=115
r = 0.51
P<0.01

Figure 2.8 Aa, Ba, Ca: Scatter plots showing the distribution of BD in relation to recording depth that were obtained before (pre-drug) and during drug (bicuculline, GABA) application. The linear regression line and correlation coefficient for each plot are shown by a solid line and *r*. Ab, Bb, Cb: Scatter plots showing the average BD of IC neurons obtained before and during drug application in relation to recording depth. Repeated measures one-way ANOVA analysis showed that the BD significantly increased with recording depth (see text for details).

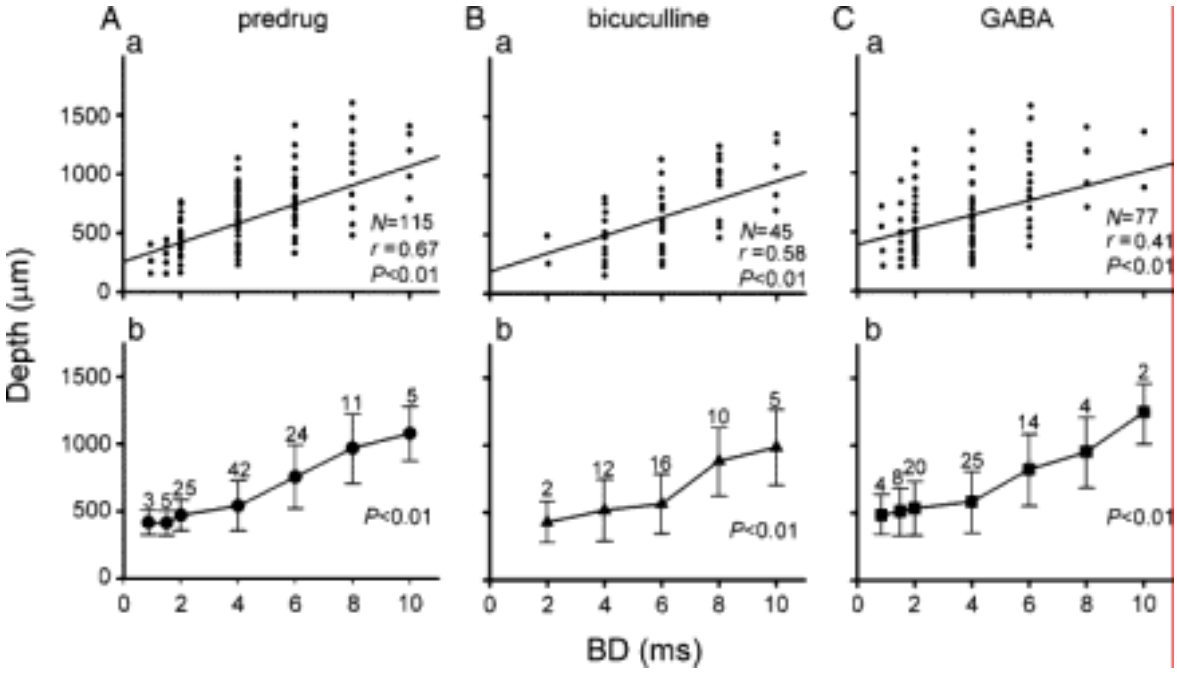
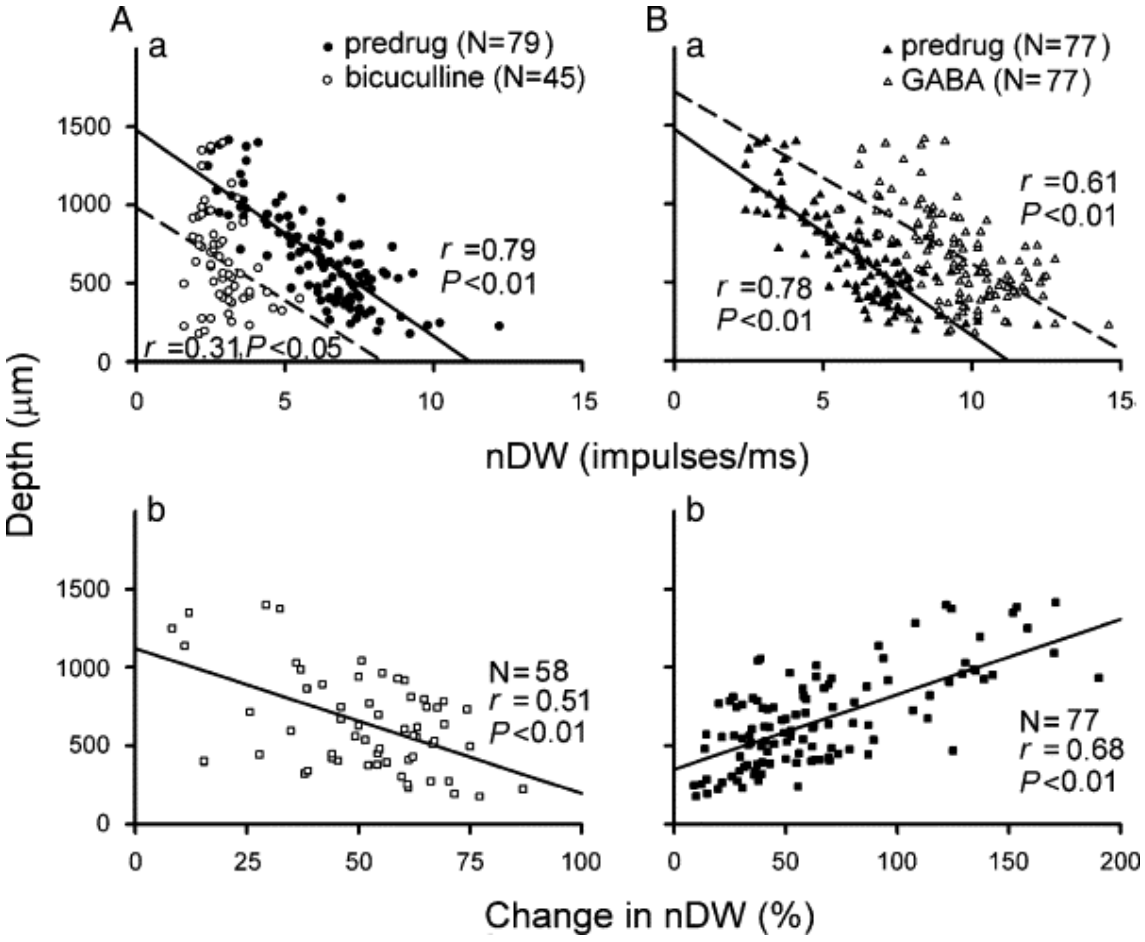

A
predrug
B
bicuculline
C
GABA
a
b
Depth (μm)
BD (ms)
N=115
r =0.67
P<0.01
N=45
r =0.58
P<0.01
N=77
r =0.41
P<0.01
P<0.01
P<0.01
P<0.01

Figure 2.9 Aa, Ba: Scatter plots showing the distribution of nDW obtained before (filled circles and triangles) and during (unfilled circles and triangles) bicuculline (Aa) and GABA (Ba) application in relation to the recording depth. Linear regression analyses indicate significant decrease in nDW with the recording depth both before and during drug application. Ab, Bb: Scatter plots showing the distribution of percent change in nDW in relation to the recording depth. Linear regression analyses indicate that the percent change in nDW significantly decrease with recording depth during bicuculline application while the opposite was observed during GABA application.


A
a
predrug (N=79)
bicuculline (N=45)
r =0.79
P<0.01
r =0.31 P<0.05
B
a
predrug (N=77)
GABA (N=77)
r =0.61
P<0.01
r =0.78
P<0.01
Depth (μm)
nDW (impulses/ms)
b
N=58
r =0.51
P<0.01
b
N=77
r =0.68
P<0.01
Change in nDW (%)

Discussion

Duration selectivity of IC neurons

We observed that IC neurons with short BD had large nDW indicating high sensitivity to change in pulse duration (***Figure 2.4***). This observation is consistent with a previous study showing that IC neurons of the same bat species with short BD have narrow 50% width of duration tuning curve (Ehrlich et al. 1997). We also observed that low BF IC neurons tend to have significantly shorter BD than high BF IC neurons had (***Figure 2.7***, B). However, Ehrlich et al. (1997) reported a wide range of distribution of the BD for neurons with BF at 20–30 kHz and a small range of distribution of the BD for neurons with BF at 30–50 kHz. The different observation may be due to the small sample size they studied (26 vs. 115, see ***Figure 2.7***, B) such that only a few neurons with BF higher than 40 kHz were sampled. As such, a meaningful comparison between their and our studies is not possible. Alternatively, the different observation between the two studies may be simply due to sampling bias or methodological differences.

The fact that GABAergic inhibition shapes duration selectivity of IC neurons is supported by the observations that bicuculline application broadened and GABA application narrowed the duration tuning curves (***Figure 2.5***). Consonant with previous studies (Casseday et al. 1994; Casseday et al. 2000; Jen and Feng 1999;

Wu and Jen 2006; Wu and Jen 2006), this GABA-mediated sharpening of duration tuning curves was achieved by a greater change in the responses to non-BD durations than to BD durations during drug application.

Organization of duration-selective neurons in the IC

As described in the Introduction, the main objective of the present study is to show that GABA-mediated duration selectivity of IC neurons of *E. fuscus* is highly correlated with the uneven distribution of $GABA_A$ receptors in the IC of this bat species (Fubara et al. 1996). The supporting data are as follow. (1) GABAergic inhibition shaped the duration selectivity of the band- and short-pass neurons obtained in this study (***Figures 2.5-2.6***). (2) The nDW of IC neurons significantly decreases with BD (***Figure 2.4***). Furthermore, both the BD and nDW of IC neurons are significantly correlated with the BF which is orderly organized along the tonotopic axis of the IC. As such, low BF IC neurons typically have shorter BD with large nDW than high BF IC neurons have (***Figure 2.7***). (3) The BD of IC neurons significantly increases and the nDW decreases with recording depth both before and during bicuculline and GABA application. (***Figures 2.8-2.9***).

These data indicate that the low BF neurons at upper IC receive more GABAergic inhibitory inputs than high BF neurons at the deeper IC. For this reason,

bicuculline application produces greater change and GABA application produces lesser change in the duration tuning curves of low BF neurons at upper IC than high BF neurons at the deeper IC.

A previous study has shown that glycine-mediated inhibition also shapes the duration selectivity of IC neurons (Ehrlich et al. 1997). Because neurons with glycine receptors are mostly distributed at the ventrolateral region of the IC but are sparsely distributed at dorsomedial region IC (Fubara et al. 1996), it is tempting to speculate that glycine-mediated duration selectivity of IC neurons may also be systematically organized along the dorsoventral axis of the IC. Future work is needed to determine whether GABA- and glycine-mediated duration selectivity of IC neurons is organized systematically but oppositely along the distribution gradients of $GABA_A$ and glycine receptors.

Comparisons with previous studies

We observed that band- and short-pass neurons at upper IC had shorter BD and sharper duration selectivity than neurons in the deeper IC (***Figures 2.8-2.9***, Aa, Ba). These duration tuned IC neurons were distributed widely across the entire dorsoventral extent of the IC although most short-pass neurons were recorded at upper IC than most band-pass neurons were (***Figure 2.2***). Although we did not

specifically examine if these neurons were spatially localized along the rostrocaudal or mediolateral axes of the IC, a previous study of the same bat species reported that duration-tuned neurons and most of non-duration-tuned neurons were located in the caudal half of the IC (Ehrlich et al. 1997). In the pallid bat, *Antrozous pallidus* (Fuzessery and Hall, 1999), the short- and long-pass neurons were not uniformly distributed across the IC. The short-pass neurons were equally common in the ventral high frequency region that serves echolocation and the lateral low-frequency region that serves passive listening. Conversely, the long-pass neurons were most common in the medial low-frequency region. However, both two studies did not examine the correlation of BD and recording site.

Galazyuk and Feng (1997) studied duration selectivity of neurons in the primary auditory cortex (AC) in the little brown bat, *Myotis lucifugus*. They reported that short- and long-pass AC neurons were narrowly distributed along the rostrocaudal tonotopical axis of the AC while band- and all-pass AC neurons were distributed more widely. Non-duration-tuned AC neurons were distributed more rostrally and ventrally to the region of duration-tuned neurons, but they did not find any correlation between a neuron's BD and its recording site.

Different from the present observation, our previous study did not find any correlation between the BD and BF or recording depth of tonotopically organized IC neurons (Pinheiro et al. 1991). In this previous study, 424 IC neurons were isolated at

depths between 40 and 2370 μm with BFs ranged between 12.1 and 87.6 kHz. The MTs were between 0 and 102 dB SPL while the latencies were between 3.4 and 28.4 ms. Since these basic response properties are comparable to those of 216 IC neurons obtained in the present study (see Results, ***Figure 2.7***, A), the different observation in the organization of duration selectivity of IC neurons between the present and previous studies is unlikely due to sampling bias. Then what might be the reason for this different observation?

In the present study, a neuron's BD was obtained from the duration tuning curve that was plotted with the number of impulses discharged to a BF pulse delivered at 2 pps and at 10 dB above the neuron's MT (***Figure 2.1***). In the previous study, a neuron's BD was obtained from a duration tuning curve that was plotted with the number of impulses discharged to a BF pulse delivered at a neuron's best intensity and best repetition rate (Pinheiro et al. 1991). It has been shown that the best intensity of IC neurons is typically 30–50 dB above the neuron's MT (Jen and Schlegel 1982; Pinheiro et al. 1991; Wu and Jen 1991) and the BD of IC neurons changes up to 2 ms with increasing sound intensity or even loses duration selectivity (Fremouw et al. 2005; Mora and Kössl 2004; Zhou and Jen 2001). Furthermore, the BD of IC neurons typically shortens with increasing pulse repetition rate (Wu and Jen, 2006). All these studies indicate that the BD of IC neurons varies with stimulus conditions.

Possible biological relevance of the present study

During hunting, insectivorous bats such as *E. fuscus* progressively increase pulse repetition rate, shorten the duration, decrease the amplitude and lower the frequency of emitted pulses as they search, approach and finally intercept the insects or avoid obstacles (Griffin et al. 1958; Jen and Kamada 1982; Simmons et al. 1979; Surlykke and Moss 2000). It has been suggested that shortening pulse duration avoids overlap between the pulse and echo, reducing pulse amplitude ensures optimal reception for echo amplitude and increasing pulse repetition rate increases extraction of information about localized prey (Hartley 1992a; Hartley 1992b; Jen and Kamada 1982; Kalko and Schnitzler 1989; Novick 1971). For example, we have previously shown that increasing pulse repetition rate sharpens frequency, intensity, direction and duration selectivity (Jen et al. 2002; Jen and Zhou 1999; Jen et al. 2001; Wu and Jen 1995,1996; Zhou and Jen 2002).

In the present study, the BD of IC neurons ranged between 1.0 and 10 ms covering the duration of pluses emitted by *E. fuscus* during different phases of hunting (***Figure 2.3***). These data suggest that as bats progressively shorten emitted pulses throughout the entire course of hunting, variation in the pulse and echo duration can be encoded by different populations of IC neurons. Because low BF neurons had shorter BD and sharper duration selectivity than high BF neurons had (***Figure 2.7***), the former would appear suitable for echo recognition during the

terminal phase of hunting when the highly repetitively emitted pulses are low in frequency and short in duration.

Conversely, the latter would be suitable for recognition of long duration echoes during search phase of hunting when the emitted pulses are low in repetition rate and high in frequency. Because duration selectivity of IC neurons is systematically organized along the tonotopic axis of the IC (***Figures 2.7-2.9***), it is tempting to speculate that echo recognition throughout different phases of hunting might be orderly processed by different populations of IC neurons across different frequency laminas.

Previous and present studies show that GABAergic inhibition shapes duration selectivity of IC neurons (e.g., ***Figure 2.5***) (Casseday et al. 1994; Casseday et al. 2000; Covey et al. 1996; Ehrlich et al. 1997; Jen and Feng 1999; Wu and Jen 2006; Wu and Jen 2006), and the massive descending corticofugal system modulates multi-parametric collicular auditory sensitivity via GABAergic inhibition (Jen et al. 1998; Ma and Suga 2001). Conceivably, a bat could utilize the corticofugal system to further improve its duration selectivity for echo recognition throughout the entire process of hunting to ensure prey capture.

On the other hand, many studies have shown that duration tuned auditory neurons have been found in non-echolocating animals including frogs (Narins and Capranica 1980), cats (He et al. 1997), chinchillas (Chen 1998), mice (Brand et al.

2000), and rats (Perez-Gonzalez et al. 2006). These studies show that the ranges of sound durations to which neurons are tuned correspond closely to the behaviorally relevant sounds in these animal species. In humans, it has been shown that when two sounds are presented in succession, auditory apparent motion is most often heard by the listeners when the duration of the lead and lag sounds are equivalent (Span 1999). Other studies reported that listeners can better detect an auditory sound when its duration or frequency is expected than unexpected (Dai and Wright 1995; Dai and Wright 1999; Hafter et al. 1993; Wright and Dai 1994). All these studies indicate that duration selectivity of auditory neurons plays an important role in sound recognition for echolocating bats and non-echolocating animals including humans.

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CHAPTER 3

THE ROLE OF GABAERGIC INHIBITION IN SHAPING DURATION SELECTIVITY OF BAT INFERIOR COLLICULAR NEURONS TO SOUND PULSES IN RAPID SEQUENCES

Abstract

Natural sounds, such as vocal communication sounds of many animal species typically occur as sequential sound pulses. Therefore, the response size of auditory neurons to a sound pulse would be inevitably affected when the sound pulse is preceded and succeeded by another sound pulse (i.e., forward and backward masking). The present study presents data to show that increasing strength of GABAergic inhibition relative to excitation contributes to decreasing response size and sharpening of duration selectivity of bat inferior collicular (IC) neurons to sound pulses in rapid sequences. The response size in number of impulses and duration selectivity of IC neurons were studied with a pulse train containing 9 sound pulses. A family of duration tuning curves was plotted for IC neurons using the number of impulses discharged to each presented sound pulse against pulse duration. Our data show that the response size of IC neurons progressively decreased and duration selectivity increased when determined with sequentially presented sound pulses. This variation in the response size and duration selectivity

of IC neurons with sequentially presented sound pulses was abolished or reduced during bicuculline and GABA application. Bicuculline application increased the response size and broadened the duration tuning curve of IC neurons while GABA application produced opposite results.

Introduction

In auditory physiology, the processing of auditory signals has traditionally been explained by excitatory and inhibitory interactions of divergent and convergent projections within the auditory system (Suga, 1997; Suga et al. 1998). For example, in the auditory pathway, the central nucleus of the inferior colliculus (IC) receives and integrates excitatory and inhibitory inputs from many lower auditory nuclei as well as from the auditory cortex (Adams 1979; Casseday and Covey 1995; Herbert et al. 1991; Huffman and Henson 1990; Oliver et al. 1994; Pollak and Casseday 1989; Saldana et al. 1996; Shneiderman and Oliver 1989; Winer et al. 1998). The inhibitory inputs to the IC are glycinergic, which originates extrinsically, and GABAergic, which originates extrinsically and intrinsically (Fubara et al. 1996; Oliver and Shneiderman, 1991; Roberts and Ribak 1987). Many studies have shown that the interplay between excitation and GABAergic and/or glycinergic inhibition shapes auditory response properties and multi-parametric selectivity of IC neurons (e.g., duration, frequency, amplitude, direction, etc.) (Casseday et al. 1994; Casseday et al. 2000; Fuzessery and Hall, 1996; Jen and Feng 1999; Jen and Zhang 2000; Jen and

Zhou 1999; Jen et al. 2001; Jen et al. 2002; Klug et al. 1995; Koch and Grothe 1998; LeBeau et al. 1996; LeBeau et al. 2001; Lu and Jen 2001). Furthermore, two recent studies have shown that inhibitory inputs with stronger intensity and longer duration are more effective in producing inhibition of auditory response of IC neurons than inhibitory inputs with weaker intensity and shorter duration (Lu and Jen 2002; Lu and Jen 2003).

In the real world, natural sounds such as vocal communication sounds of many animal species typically occur as sequential sound pulses. Therefore, the response size of auditory neurons to a sound pulse would be affected when the sound pulse is preceded and succeeded by other sound pulses (i.e., forward and backward masking). As evident in a previous study, the response size of IC neurons is often larger when stimulated with a single sound pulse than when stimulated with the same sound pulse presented in pulse trains (Moriyama et al. 1994). Furthermore, the response size of IC neurons progressively decreased with sequentially presented sound pulses (Jen and Zhou 1999; Jen et al. 2001; Lu et al. 1997; Lu et al. 1998; Moriyama et al. 1994; Pinheiro et al. 1991; Wu and Jen 1995 ; Zhou and Jen 2002). What might be the neural mechanisms underlying these observations?

During synaptic transmission, excitatory and inhibitory signals arrive repetitively at a neuron would produce temporal facilitation of opposite

postsynaptic potentials (IPSP vs EPSP). However, at higher repetition rates, temporal depression occurs due to depletion of neurotransmitters resulting in decreasing postsynaptic potentials (Wu and Betz 1998 ; Zucker 1989). A previous study on the rat pyramidal neurons showed that excitatory synaptic currents displayed stronger depression than inhibitory synaptic currents in response to sustained activation at high stimulus repetition rates (Galarreta and Hestrin 1998). This study indicates that the time course of temporal facilitation and depression may differ between the two opposing postsynaptic potentials.

In the present study, we hypothesize that increasing strength of inhibition over excitation contributes to variation in auditory sensitivity of IC neurons to rapidly presented sound pulses. To test this hypothesis, we studied the variation in the response size and duration selectivity of IC neurons in relation to increasing strength of GABAergic inhibition with sequentially presented sound pulses. Specifically, we obtained the number of impulses and duration tuning curves of IC neurons for each presented sound pulse before and during application of bicuculline (an antagonist for $GABA_A$ receptors) (Bormann 1988; Cooper et al. 1982) and GABA. Variation in the number of impulses and duration selectivity of IC neurons with sequentially presented sound pulses was then quantitatively determined and statistically compared.

Materials and methods

Animals and surgery

Eight big brown bats (4 males and 4 females, 18–25 g body weight, b.w.) were used for this study. As described in previous studies (Jen et al. 1987; Jen et al. 1989), the flat head of a 1.8 cm nail was glued onto the exposed skull of each Nembutal anesthetized bat (45–59 mg/kg b.w.) with acrylic glue and dental cement one or two days before the recording session. Exposed tissue was treated with an antibiotic (Neosporin) to prevent inflammation. During the day of recording, the bat was administered the neuroleptanalgesic, Innovar-Vet (Fentanyl 0.08 mg/kg b.w. Droperidol 4 mg/kg b.w.), and placed inside a bat holder (made of wire mesh) that was suspended in an elastic sling inside a double-wall sound-proof room (temperature 28–30 °C). The ceiling and inside walls of the room were covered with 3-in. convoluted polyurethane foam to reduce echoes. After fixing the bat's head with a set screw, small holes were made in the skull above the IC for insertion of 3 M KCl glass pipette electrodes (impedance: 5–10 MΩ). Additional doses of Innovar-Vet were administered during later phases of recording when bats showed signs of discomfort. A local anesthetic (Lidocaine) was applied to the open wound area. The recording depth was read from the scale of a microdrive (David Kopf). A common indifferent electrode (silver wire) was placed at the nearby temporal muscles. Each bat was used in one to five recording sessions on separate days and each recording

session typically last for 2–6 hours. The experiments were conducted according to NIH publication No. 85–23, "Principles of Laboratory Animal Care" and with the approval of the Institutional Animal Care and Use Committee of the University of Missouri-Columbia.

Acoustic stimulation

Acoustic stimuli (4 ms with 0.5 ms rise-decay times) were generated with an oscillator (KH model 1200) and a homemade electronic switch driven by a stimulator (Grass S88). These stimuli were then amplified after passing through a decade attenuator (HP 350D) before they were fed to a small condenser loudspeaker (AKG model CK 50, 1.5 cm diameter, 1.2 g) that was placed 23 cm away from the bat and 30° contralateral to the recording site. Calibration of the loudspeaker was performed with a 1/4-in. microphone (B & K 4135) placed at the position of the bat's head during recording using a measuring amplifier (B & K 2607). The output of the loudspeaker was expressed in dB SPL in reference to 20 µPa root mean square.

Upon isolation of an IC neuron with 4 ms pulses that delivered at 2 pps, its best frequency (BF) was determined by changing the frequency and intensity of sound stimuli. The minimum threshold (MT) at the BF was defined as the sound level that elicited 50% response probability from the neuron. The response size in number of impulses and duration selectivity of the IC neuron was studied with 32 presentations of a 300-ms pulse train containing 9 BF sound pulses delivered at 10

dB above the MT and at two trains per second. Thus there was always 200 ms silent period between pulse trains. The onset-to-onset inter-pulse interval was set at 33.3 ms which is equivalent to a pulse repetition rate of 30 pulses per second. Pulse durations of 1, 2, 4, 6, 8, 10 and 20 ms were used for this study. Rise-decay times were 0.5 ms but they were 0.25 ms for 1-ms pulse duration.

Iontophoresis and recording

Iontophoretic application of bicuculline and GABA to recorded IC neurons has been described in previous studies (Lu et al. 1997; Lu et al. 1998). Briefly, a three-barrel or five-barrel electrode (tip: 10–15 μm) was piggybacked to a 3 M KCl single-barrel electrode (tip: less than 1 μm; impedance: 5–10 MΩ) whose tip was extended about 10 μm from the tip of the three-barrel electrode. The 3 M KCl single-barrel recording electrode was used to record neural responses. One of the barrels of the three-barrel electrode was filled with bicuculline methiodide (10 mM in 0.16 m NaCl, pH 3.0; Sigma) or gamma-aminobutyric acid (GABA, 500 mM in distilled water, pH 3.5; Sigma). However, when a five-barrel electrode was used, two barrels were filled with both drugs, respectively, such that both drugs could be applied to the recorded neuron. The bicuculline and GABA were prepared just prior to each experiment and the electrode filled immediately before use. The drug channel was connected via silver–silver chloride wire to a microiontophoresis constant current generator (Medical Systems Neurophore BH-2) that was used to

generate and monitor iontophoretic currents. During drug application, a 1-s pulse of positive 40 nA at 0.5 pps was applied for 1 min before data acquisition. Bicuculline application was considered to have blocked GABA-A receptors maximally for each neuron when three consecutive responses did not vary by more than 10% even at higher application current of 60–70 nA. The application current was changed to 10 nA during data acquisition. The other two barrels were filled with 1 M NaCl (pH 7.4), one of which was used as the ground and the other as the balanced barrel. The balance electrode was connected to a balance module. The retaining current was negative 8–10 nA.

To determine any potential artifacts due to passing current or low pH values, the balanced barrel was filled with 1 M NaCl (pH 3.0) and the same amount of current used for bicuculline application was passed through the balanced barrel. Stimulus artifacts were considered negligible when the number of impulses of a neuron was affected by less than 10 % before and after current application (Ebert and Ostwald 1995). Otherwise, the data were discarded and a new electrode was used for the experiment. Data were also discarded when the impedance of the bicuculline-filled electrode varied more than 20 MΩ before and after the recording, the tip of the multi-barrel electrode broke when withdrawn from the recording site or both tips of the single and the multi-barrel electrode separated from each other.

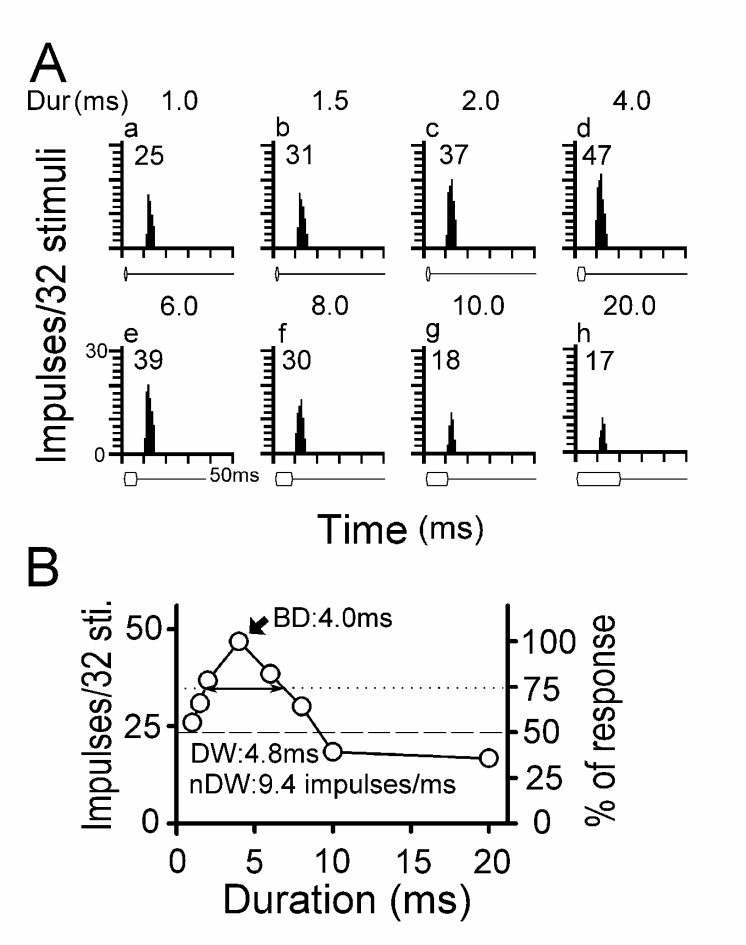
Recorded action potentials were amplified, band-pass filtered (Krohn-Hite

3500), and then fed through a window discriminator (WPI 121) before being sent to an oscilloscope (Tektronix 5111) and an audio monitor (Grass AM6). They were then sent to a computer (Gateway, 2000, p. 486) for acquisition of peri-stimulus-time (PST) histograms (bin width: 500 μs, sampling period: 300 ms) to 32 train presentations.

Data analysis

The number of impulses of each neuron discharged to each presented sound pulse was first obtained before and during drug application. As in previous studies (Jen and Feng 1999; Jen and Zhou 1999), a family of duration tuning curves was then plotted for the IC neuron before and during drug application using the number of impulses in response to each of 9 presented sound pulses against pulse duration. The pulse train of different pulse duration was presented randomly to avoid the potential effect of sequential presentation of pulse train on duration selectivity of IC neurons. The tuning selectivity of a duration tuning curve was expressed with the best duration (BD) and normalized duration width (nDW) at 75% of the maximum (i.e., ***Figure 3.1***). The BD and nDW of the duration tuning curves of IC neurons obtained for sequentially presented sound pulses before and during drug application. For comparison of multiple group means, repeated measures one- or two-way ANOVA were followed by a Student–Newman–Keuls multiple comparisons post-test, with significance established at the $P<0.05$ level.

Figure 3.1 Aa-h: Peri-stimulus-time (PST) histograms (bin width: 500 μs, sampling period: 100 ms) showing the discharge patterns of an inferior collicular (IC) neuron obtained with isolation pulses of different durations. Schematic sketches of acoustic stimuli are shown at bottom of each histogram. The pulse duration (Dur) and total number of impulses per 32 stimuli are shown above each histogram. B: The neuron's duration tuning curve plotted with the number of impulses per 32 pulse stimuli. Left and right ordinates represent the number of impulses per 32 pulse stimuli and normalized response. The abscissa represents pulse duration (ms). The horizontal dashed line indicates the 50 % maximal response and the horizontal dotted line indicates the 75 % maximal response. Duration selectivity of each curve is expressed with a best duration (BD) and a normalized duration width (nDW). The BD of duration tuning curves is indicated by an arrowhead. An nDW is obtained by dividing the maximum by the duration width (DW indicated with a double arrow-headed bar) of a duration tuning curve at 75% maximum. The BF (kHz), latency (ms), MT (dB SPL) and recording depth (μm) of this neuron were 28.5, 11.0, 42 and 356 (See text for details).


A
Dur(ms) 1.0
1.5
2.0
4.0
a
b
c
d
25
31
37
47
6.0
8.0
10.0
20.0
e
f
g
h
30
39
30
18
17
0
50ms
Impulses/32 stimuli
Time (ms)
B
BD:4.0ms
DW:4.8ms
nDW:9.4 impulses/ms
Impulses/32 sti.
% of response
50
25
0
100
75
50
25
0
0
5
10
15
20
Duration (ms)

Results

In this study, 122 IC neurons were isolated at depths between 125 and 2080 μm. Their BFs and MTs ranged 18.5–63.5 kHz (35.7 ± 7.4 kHz) and 20–58 dB SPL (44.5 ± 8.2 dB SPL). The latencies were between 8 and 18 ms (12.4 ± 1.5 ms). Consonant with previous studies (Jen and Schlegel 1982; Pinheiro et al. 1991; Poon et al. 1990; Wu and Jen 1991), the BF of these IC neurons progressively increased with recording depth indicating that they were tonotopically organized along the dorsoventral axis of the central nucleus of the IC. When stimulated with the pulse train, most (n = 98, 80%) neurons discharge impulses to all 9 pulses to varying degrees. The remaining (n = 24, 20%) discharge impulses to the initial few pulses or the first pulse only. These observations are in agreement with previous studies (Jen and Zhou 1999; Jen et al. 2001; Lu et al. 1997; Lu et al. 1998; Moriyama et al. 1994; Pinheiro et al. 1991; Wu and Jen, 1995; Zhou and Jen 2002).

For consistent comparison, we only study the response size and duration selectivity of those 98 neurons that discharged impulses to all presented pulses. Among them, 44 neurons received bicuculline application and 42 neurons received GABA application. The remaining 12 neurons received bicuculline application first. After recovery from the drug effect, they then received GABA application. Therefore, the response size and duration selectivity for sequentially presented sound pulses were studied before and during bicuculline application in 56 neurons and during GABA application in 54 neurons.

Discharge pattern of IC neurons determined with sequentially presented sound pulses before and during drug application

The discharge pattern and the number of impulses of a representative IC neuron in response to the pulse train before and during bicuculline application are shown in ***Figure 3.2***. The neuron's number of impulses to the first pulse was maximal which progressively decreased with sequentially presented sound pulses (***Figure 3.2***, Aa, Predrug). Bicuculline application produced varying degree of increase in the number of impulses of the IC neuron in response to all sound pulses (***Figure 3.2***, Ab, Bicuculline).

Figure 3.2 B shows the average number of impulses of 56 IC neurons in response to each presented sound pulse before and during bicuculline application. The maximal number of impulses in response to the first pulse significantly decreased with sequentially presented sound pulses (***Figure 3.2***, B, unfilled circles, two-way ANOVA, $P < 0.001$). Bicuculline application significantly increased the number of impulses of these IC neurons in response to each presented sound pulse (***Figure 3.2***, B, filled vs unfilled circles, two-way ANOVA, $P < 0.001$). However, the increase in the number of impulses during bicuculline application progressively increased with sequentially presented sound pulses (***Figure 3.2***, C, one-way ANOVA, $P < 0.01$). As a result, the trend of significant decrease of the response size with sequentially presented sound pulses was abolished during bicuculline application

(***Figure 3.2***, B, filled circles, two-way ANOVA, $P > 0.05$).

The discharge pattern and the number of impulses of another representative IC neuron in response to the pulse train before and during GABA application are shown in ***Figure 3.3***. Again, the maximal number of impulses discharged to the first pulse progressively decreased with sequentially presented sound pulses (***Figure 3.3***, Aa, Predrug). GABA application produced varying degree of decrease in the number of impulses of the IC neuron in response to all sound pulses (***Figure 3.3***, Ab, GABA).

The average number of impulses of 54 IC neurons significantly decreased with sequentially presented sound pulses before GABA application (***Figure 3.3***, B, unfilled circles, two-way ANOVA, $P < 0.001$). GABA application significantly decreased the number of impulses in response to each presented pulse (***Figure 3.3***, B, filled vs unfilled circles, two-way ANOVA, $P < 0.05$). The decrease in the number of impulses in response to the first pulse was maximal which progressively decreased with sequentially presented sound pulses (***Figure 3.3***, C, one-way ANOVA, $P < 0.001$). As a result, the trend of significant decrease in the number of impulses in response to sequentially presented sound pulses was also abolished (***Figure 3.3***, B, filled circles, two-way ANOVA, $P > 0.05$).

Figure 3.2 A: PST histograms (bin width: 500 μs, sampling period: 300 ms) showing the discharge pattern of an inferior collicular (IC) neuron of the big brown bat, *Eptesicus fuscus*, obtained with 300 ms pulse trains containing 9 best frequency (BF) sound pulses of 4 ms before (Aa) and during (Ab) bicuculline application. The position of sequentially presented pulses is shown at the bottom and the neuron's number of impulses in response to 32 presentations of each pulse is shown within each PST histogram. The neuron's BF (kHz), latency (ms), MT (dB SPL) and recording depth (μm) were 34.5, 11.0, 45.0 and 425. B: The average number of impulses discharged to sequentially presented sound pulses before (unfilled circles) and during (filled circles) bicuculline application. Bicuculline application produced significant increase in the number of impulses in response to each sound pulse (filled circles vs unfilled circles, two-way ANOVA followed by a Student–Newman–Keuls multiple comparisons post-test, ***$P < 0.001$ and **$P < 0.01$). Note that the average number of impulses significantly decreased with sequentially presented sound pulses only before (unfilled circles) but not during (filled circles) bicuculline application (two-way ANOVA, $P < 0.001$ vs >0.05). C: The average percent increase in the number of impulses in response to each sound pulse during bicuculline application. Note that the percent change progressively increased with sequentially presented sound pulses (one-way ANOVA, $P < 0.001$). In B and C, the solid line represents the linear regression line. The n, r, SL and P represent the number of IC neurons studied, correlation coefficients, slope and significance level for each linear regression line.

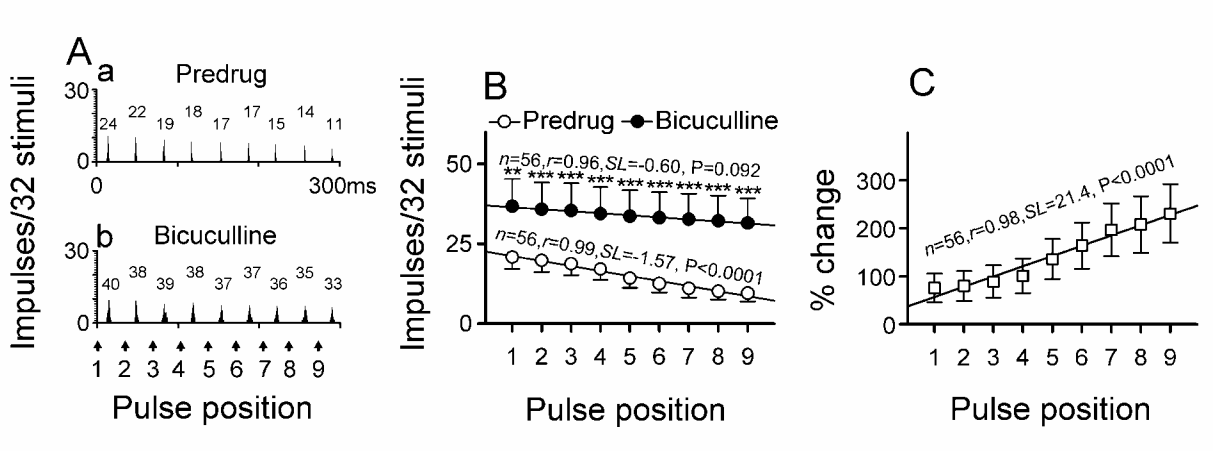
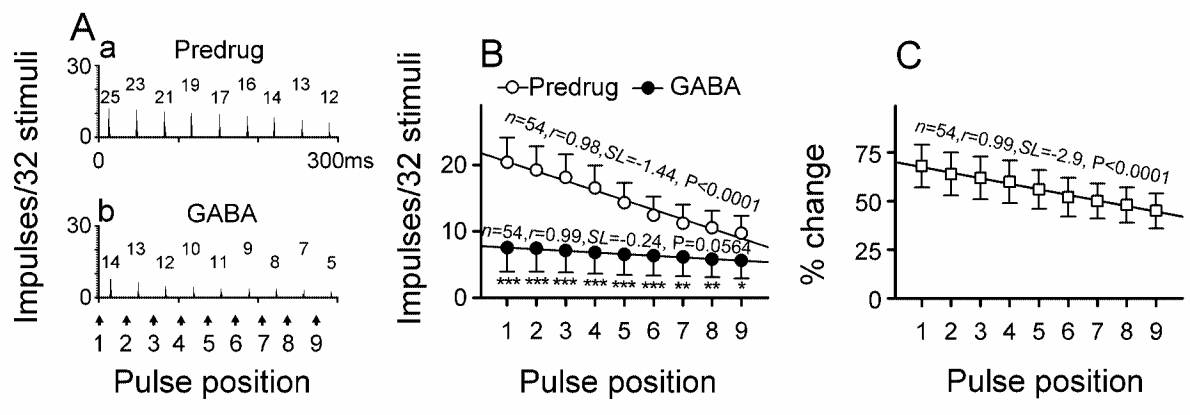

A
a
Predrug
30
0
24 22 19 18 17 17 15 14 11
0
300ms
b
Bicuculline
30
0
40 38 39 38 37 37 36 35 33
1 2 3 4 5 6 7 8 9
Pulse position
Impulses/32 stimuli
B
Predrug
Bicuculline
n=56,r=0.96,SL=-0.60, P=0.092
n=56,r=0.99,SL=-1.57, P<0.0001
50
25
0
1 2 3 4 5 6 7 8 9
Pulse position
Impulses/32 stimuli
C
n=56,r=0.98,SL=21.4, P<0.0001
300
200
100
0
% change
1 2 3 4 5 6 7 8 9
Pulse position

Figure 3.3 A: PST histograms showing the discharge pattern of another IC neuron obtained with 300 ms pulse trains before (Aa) and during (Ab) GABA application. The neuron's BF (kHz), latency (ms), MT (dB SPL) and recording depth (μm) were 28.5, 11.5, 43.0 and 385. B: The average number of impulses discharged to sequentially presented sound pulses before (unfilled circles) and during (filled circles) GABA application. GABA application significant decreased the number of impulses elicited by each sound pulse (filled circles vs unfilled circles, two-way ANOVA followed by a Student–Newman–Keuls multiple comparisons post-test, ***$P < 0.001$, **$P < 0.01$ and *$P < 0.05$). Note that the average number of impulses significantly decreased with sequentially presented sound pulses only before (unfilled circles) but not during (filled circles) GABA application (one-way ANOVA, $P < 0.001$ vs >0.05). C: The average percent decrease in the number of impulses elicited by each sound pulse during GABA application. Note that the percent change in the number of impulses progressively decreased with sequentially presented sound pulses (one-way ANOVA, $P < 0.001$, see ***Figure 3.2*** for legends).


A
a
Predrug
30
0
25 23 21 19 17 16 14 13 12
0
300ms
b
GABA
30
0
14 13 12 10 11 9 8 7 5
1 2 3 4 5 6 7 8 9
Pulse position
Impulses/32 stimuli
B
Predrug
GABA
n=54,r=0.98,SL=-1.44, P<0.0001
n=54,r=0.99,SL=-0.24, P=0.0564
*** *** *** *** *** *** ** ** *
20
10
0
1 2 3 4 5 6 7 8 9
Pulse position
Impulses/32 stimuli
C
n=54,r=0.99,SL=-2.9, P<0.0001
75
50
25
0
1 2 3 4 5 6 7 8 9
Pulse position
% change

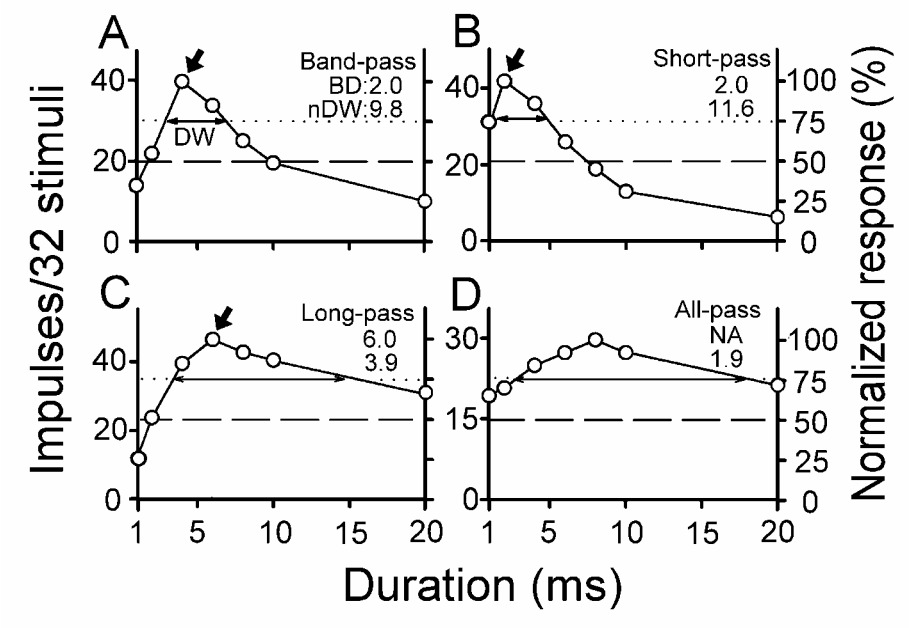
Duration tuning curves of IC neurons

In total, 1980 duration tuning curves were plotted using the number of impulses discharged to each presented sound pulse against the pulse duration before and during drug application. These curves can be described as the following four types using the same criteria adopted in previous studies (Jen and Feng 1999; Jen and Zhou 1999). (1) Band-pass (467, 24%): the maximal number of impulses at the most preferred pulse duration is at least 50% greater than the number of impulses at two minimal responses obtained at long and short durations (***Figure 3.4***, A); (2) Short-pass (491, 25%): the maximal number of impulses obtained at a short duration is 50% greater than at a long duration and 25% greater than at a short duration (***Figure 3.4***, B); (3) Long-pass (90, 5%): the maximal number of impulses obtained at a long duration is 50% greater than at a short duration and 25% greater at a long duration (***Figure 3.4***, C); and (4) All-pass (932, 47%): The number of impulses of all-pass duration tuning curves often differs by more than 25% but never by more than 50% at all durations tested (***Figure 3.4***, D). In this study, the duration of maximal response in the band-, short- and long-pass duration tuning curves is defined as the best duration (BD) (indicated by an arrow-head in ***Figure 3.4***, A-C).

The sharpness of a duration tuning curve is expressed with a normalized duration-width (abbreviated as nDW). The nDW is obtained by dividing the

maximum by the width of a duration tuning curve at 75% of the maximum. By obtaining the nDW, we exclude the possibility that a decrease in the duration width of duration tuning curves plotted for sequentially presented sound pulses might only reflect a decrease in response sensitivity rather than a true increase in duration selectivity. A neuron with a large nDW has a narrow duration tuning curve and sharp duration selectivity. Each band-, short- or long-pass duration tuning curve always has an nDW. An all-pass duration curve may have an nDW when the maximum decreases by more than 25% at the two limbs although the all-pass duration curve is duration non-selective according to our criterion (e.g., ***Figure 3.4***, D). As described below, this nDW becomes useful for comparison of the variation in duration selectivity of IC neurons when all-pass duration tuning curves change into other types during GABA application (e.g., ***Figure 3.6***, A2p1 vs B2p1).

Figure 3.4 A-D: Duration tuning curves of 4 representative IC neurons. Left and right ordinates represent the number of impulses per 32 stimuli and normalized response. The abscissa represents pulse duration (ms). Among these curves, the duration tuning curve in A is obtained from PST histograms of the IC neuron in A. Duration tuning properties of these neurons are band-pass (A), short-pass (B), long-pass (C) and all-pass (D). Each horizontal dashed line indicates the 50 % maximal response. Duration selectivity of each curve is expressed with a best duration (BD) and a normalized duration width (nDW). The BD of the band-, short- and long-pass duration tuning curves is indicated by filled arrowhead. The nDW is obtained by dividing the maximum by the duration width (DW indicated with a double arrow) of a duration tuning curve at 75% maximum. The BD and nDW of a duration tuning curve is shown in each plot. NA indicates that a BD is not available. The BF (kHz), latency (ms), MT (dB SPL) and recording depth (μm) of these neurons were 25.5, 10.5, 40.0, 355 (A); 37.4, 11.0, 41.0, 630 (B); 36.3, 13.5, 45.0, 824 (C); and 44.5, 15.0, 48.0, 1050 (D).


A
Band-pass
BD:2.0
nDW:9.8
DW
B
Short-pass
2.0
11.6
C
Long-pass
6.0
3.9
D
All-pass
NA
1.9
Impulses/32 stimuli
Normalized response (%)
Duration (ms)
0
20
40
15
30
25
50
75
100
1
5
10
15
20

Variation in response size and duration tuning curves of IC neurons before and during drug application

The discharge patterns and the family of duration tuning curves of two IC neurons plotted for each sound pulse before and during bicuculline and GABA application are, respectively, shown in ***Figures 3.5-3.6***. The number of impulses of both neurons not only varied with sequentially presented sound pulses but also with sound pulse duration. For example, the number of impulses of one neuron progressively decreased from 24 to 18 (−25%) when tested with 4 ms BF pulses but decreased from 16 to none (−100%) when tested with 20 ms BF pulses (***Figure 3.5***, A1, c vs g). The neuron's duration tuning curves plotted for sequentially presented sound pulses changed from all-pass to short-pass and then band-pass while the nDW progressively increased from 4.6 to 15.6 (***Figure 3.5***, A2, p1–p9). Bicuculline application increased the neuron's number of impulses in response to all sound pulses to varying degrees. The neuron's BD also changed from 4 to 6 ms (***Figure 3.5***, A1c vs B1d). During bicuculline application, the neuron had all-pass duration tuning curves when plotted for all sound pulses (***Figure 3.5***, B2).

Contrary to these observations, GABA application produced a decrease in the number of impulses of the other neuron and changed its BD from 4 to 2 ms (***Figure 3.6***, A1c vs B1b). Before GABA application, the neuron's duration tuning curves plotted for sequentially presented sound pulses progressively changed from all-pass

to short-pass and then to band-pass while the nDW increased from 4.2 to 12.2 (***Figure***, ***3.6*** A2). During GABA application, the neuron had band-pass duration tuning curves with increased DW (65–345%) when plotted for all presented sound pulses (***Figure 3.6***, B2).

To more clearly compare the variation in the number of impulses of these two IC neurons in response to all presented sound pulses at all pulse durations tested, we plotted the number of impulses against sequentially presented sound pulses before and during drug application (***Figures 3.7-3.8***, A, B). The number of impulses of both neurons decreased to varying degrees with sequentially presented pulses. The least degree of decrease in the number of impulses with sequentially presented sound pulses was obtained when stimulated with the 4 ms BD pulse (***Figures 3.7-3.8***, A, filled squares). Bicuculline and GABA application, respectively, shifted the entire family of impulse-pulse position curves upward and downward to varying degrees (***Figures 3.7-3.8***, B).

To further compare the change in the number of impulses of these two IC neurons with pulse duration before and during drug application, we plotted the percent decrease in the number of impulses against the pulse duration for all presented sound pulses (***Figures 3.7-3.8***, C, D). The percent decrease was calculated by dividing the decrease in the number of impulses by the number of impulses obtained for each pulse delivered at the BD. It is clear that the percent decrease in

the number of impulses became greater with shortening or lengthening of pulse duration away from the BD. It is also clear that this percent decrease in the number of impulses with pulse duration became progressively greater with sequentially presented sound pulses. As such, individual percent decrease-duration curves became progressively sharper when obtained with sequentially presented sound pulses (***Figures 3.7-3.8***, C, curves 1–9). Both limbs of all percent decrease-duration curves shifted upward during bicuculline application but shifted downward during GABA application (***Figures 3.7-3.8***, D). As a result, the sharpness of these curves decreased during bicuculline application and increased during GABA application.

Figures 3.9-3.10 show the variation in the type of duration tuning curves of IC neurons with sequentially presented sound pulses before and during drug application. Before drug application, the number of IC neurons with band- and short-pass duration tuning curves progressively increased with sequentially presented pulses (***Figures 3.9-3.10***, A, B, unfilled circles). The opposite was observed for IC neurons with all-pass duration tuning curves (***Figures 3.9-3.10***, D, unfilled circles). However, the number of IC neurons with long-pass duration tuning curves only decreased slightly with sequentially presented sound pulses (***Figures 3.9-3.10***, C, unfilled circles).

Bicuculline application increased the number of IC neurons with all-pass duration tuning curves and decreased the number of IC neurons with other three

types of duration tuning curves. The change in the number of IC neurons with band-, short- and all-pass curves was greater when determined with later than for initial pulses (***Figure 3.9***, A, B, D, dashed lines). Conversely, GABA application increased the number of IC neurons with band- and short-pass duration tuning curves and decreased the number of IC neurons with all- and long-pass duration tuning curves (***Figure 3.10***, A, B vs C, D, unfilled vs filled circles). The change in the number of IC neurons with band-, short- and all-pass duration tuning curves was greater when determined with initial than for later pulses (***Figure 3.10***, A, B and D, dashed lines).

Figure 3.5 A1, B1: PST histograms of an IC neuron obtained with 9 sequentially presented BF sound pulses at different durations (Dur, shown at far right) before (A1) and during (B1) bicuculline application. The number of impulses discharged to each sound pulse is shown within each PST histogram. N: the total number of impulses discharged to all 9 pulses. A2, B2: The family of duration tuning curves of the IC neuron plotted with the number of impulses in response to sequentially presented sound pulses (p1–p9) against pulse duration before (A2) and during (B2) bicuculline application. The type, BD and nDW of the duration tuning curve are shown within each panel. The neuron's BF (kHz), latency (ms), MT (dB SPL) and recording depth (μm) were 28.5, 10.5, 43.0 and 365.

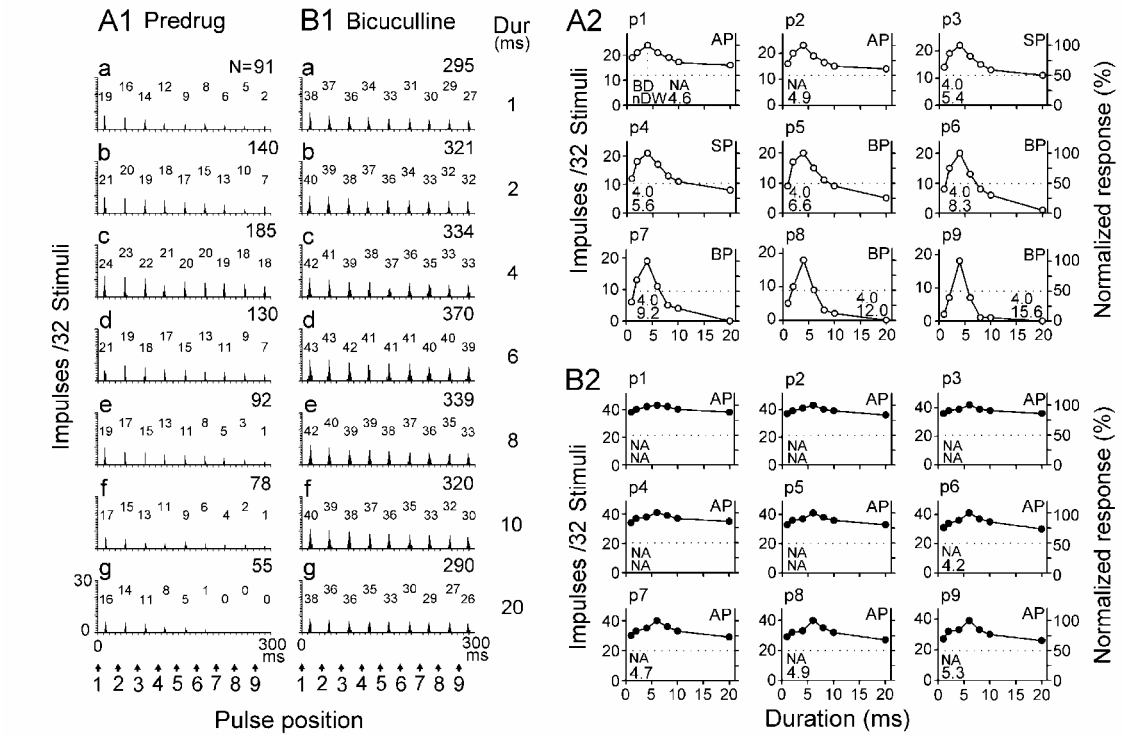

A1 Predrug
B1 Bicuculline
Dur (ms)
N=91
295
140
321
185
334
130
370
92
339
78
320
55
290
Impulses /32 Stimuli
0
300 ms
1 2 3 4 5 6 7 8 9
Pulse position
A2
B2
p1
p2
p3
p4
p5
p6
p7
p8
p9
AP
SP
BP
BD NA
nDW4.6
NA 4.9
4.0 5.4
4.0 5.6
4.0 6.6
4.0 8.3
4.0 9.2
4.0 12.0
4.0 15.6
NA NA
NA 4.2
NA 4.7
NA 4.9
NA 5.3
Normalized response (%)
Duration (ms)

Figure 3.6 A1, B1: PST histograms of another IC neuron obtained with 9 sequentially presented sound pulses at different durations before (A1) and during (B1) GABA application. A2, B2: The family of duration tuning curves of the IC neuron plotted with the number of impulses in response to sequentially presented sound pulses (p1–p9) against pulse durations before (A2) and during (B2) GABA application. The neuron's BF (kHz), latency (ms), MT (dB SPL) and recording depth (μm) were 36.4, 11.5, 40.0 and 655 (see ***Figure 3.5*** for legends).

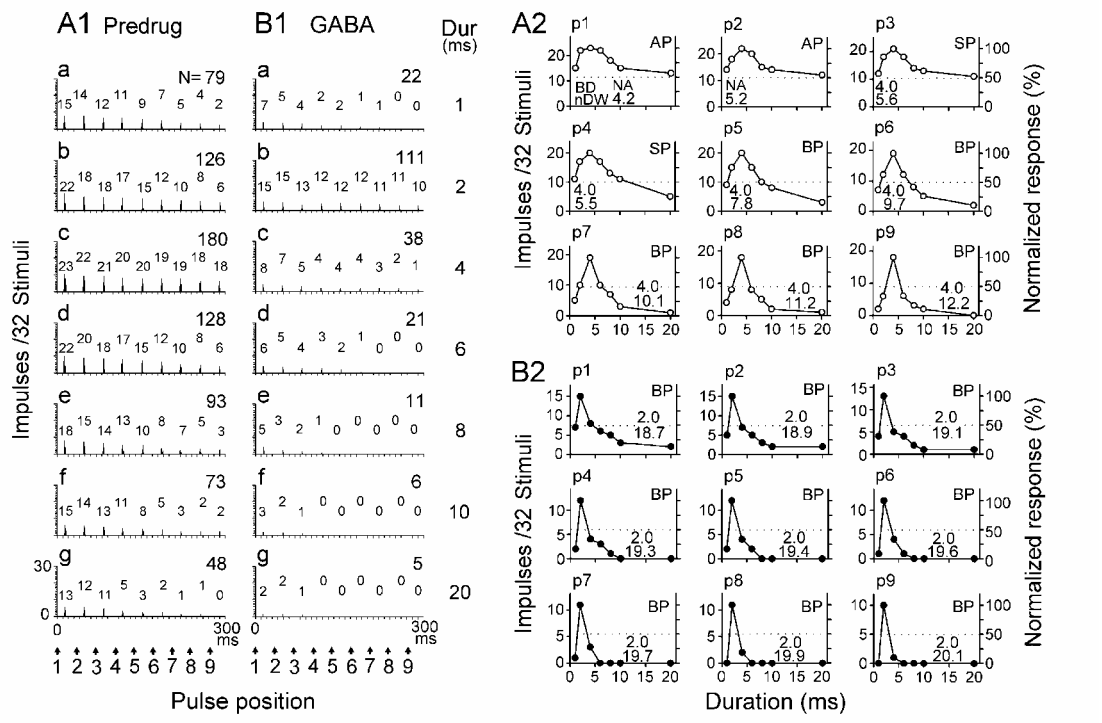

A1 Predrug
B1 GABA
Dur (ms)
N= 79
22
126
111
180
38
128
21
93
11
73
6
48
5
1
2
4
6
8
10
20
Impulses /32 Stimuli
0
300
ms
1 2 3 4 5 6 7 8 9
Pulse position
A2
B2
p1
p2
p3
p4
p5
p6
p7
p8
p9
AP
SP
BP
BD
NA
nDW 4.2
5.2
5.6
5.5
7.8
9.7
10.1
11.2
12.2
18.7
18.9
19.1
19.3
19.4
19.6
19.7
19.9
20.1
2.0
4.0
Normalized response (%)
Duration (ms)

Figure 3.7 A, B: A family of impulse-pulse position curves plotted with the number of impulses of the IC neuron of ***Figure 3.5*** in response to sequentially presented sound pulses at different pulse durations (shown at the far right of B) before (A) and during (B) bicuculline application. C, D: A family of percent decrease-duration curves showing % decrease in the number of impulses obtained from different pulse durations relative to the maximal number of impulses obtained with BD pulses for each presented sound pulse (shown at the far right of D) before and during drug application (see text for details).

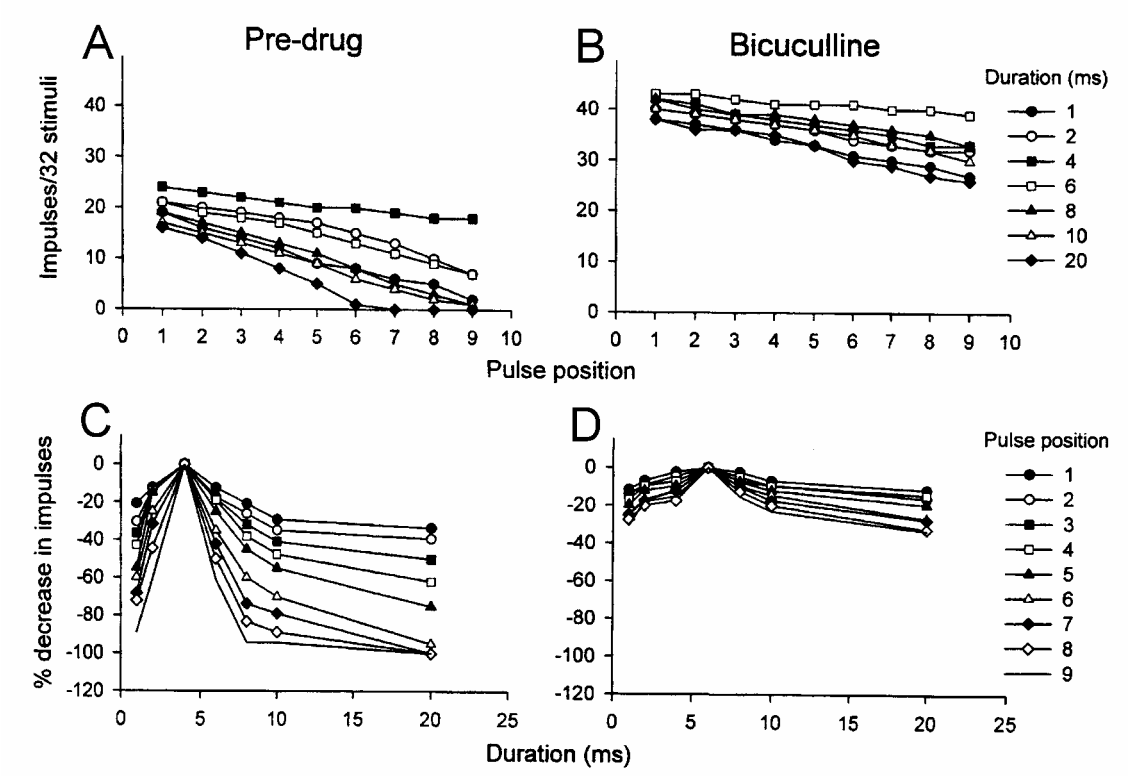

A
Pre-drug
B
Bicuculline
Impulses/32 stimuli
Duration (ms)
1
2
4
6
8
10
20
Pulse position
C
D
% decrease in impulses
Pulse position
1
2
3
4
5
6
7
8
9
Duration (ms)

Figure 3.8 A-D: A family of impulse-pulse position curves (A, B) and percent decrease-duration curves (C, D) of the IC neuron of ***Figure 3.6*** obtained before and during GABA application (see ***Figure 3.7*** for legends).

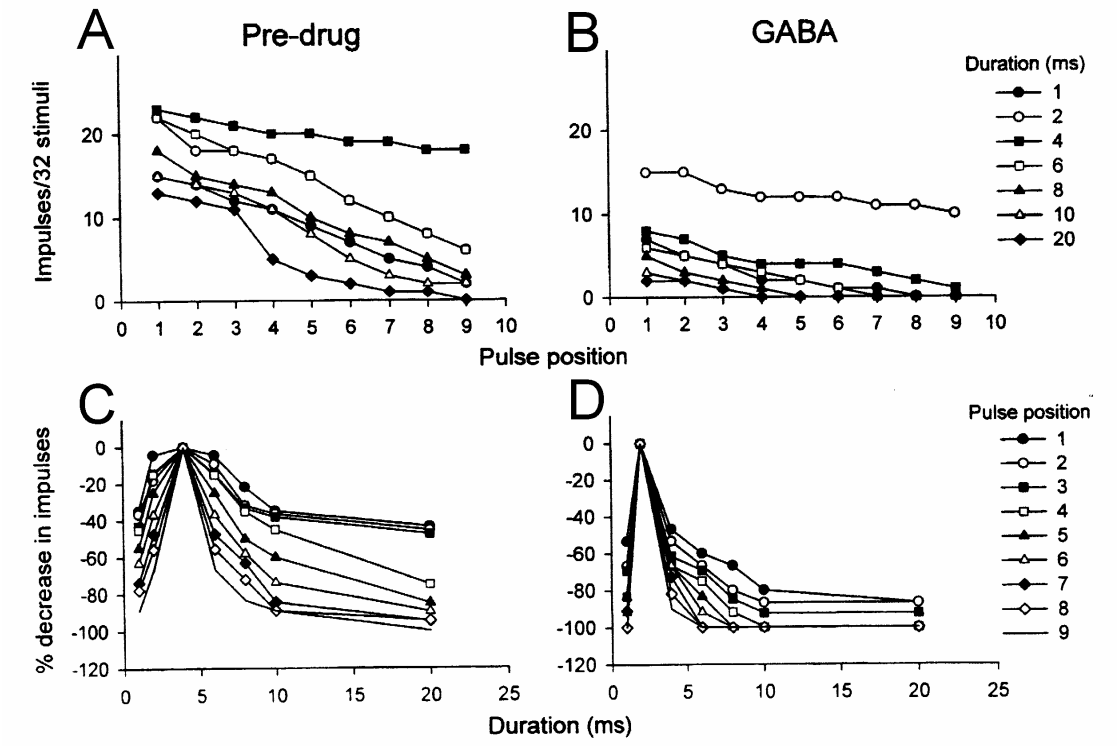

A
Pre-drug
B
GABA
Duration (ms)
1
2
4
6
8
10
20
Impulses/32 stimuli
Pulse position
C
D
Pulse position
1
2
3
4
5
6
7
8
9
% decrease in impulses
Duration (ms)

Figure 3.9 A-D: Variation in the number of each type of duration tuning neurons with sequentially presented sound pulses before (unfilled circles) and during (filled circles) bicuculline application. The dashed line indicates the difference in the number of duration tuning neurons determined before and during bicuculline application (see text for details).

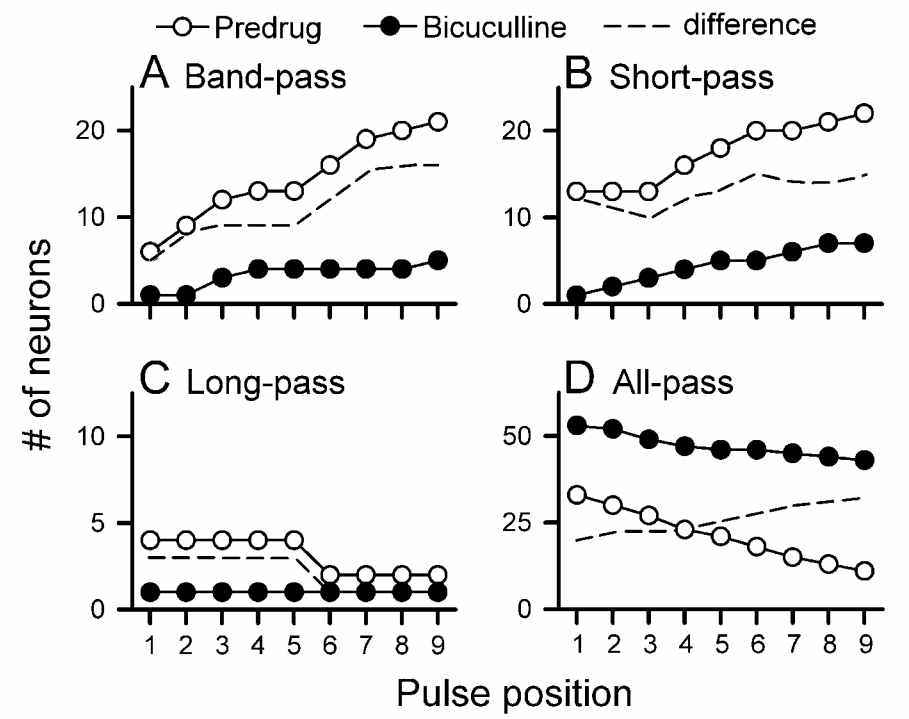
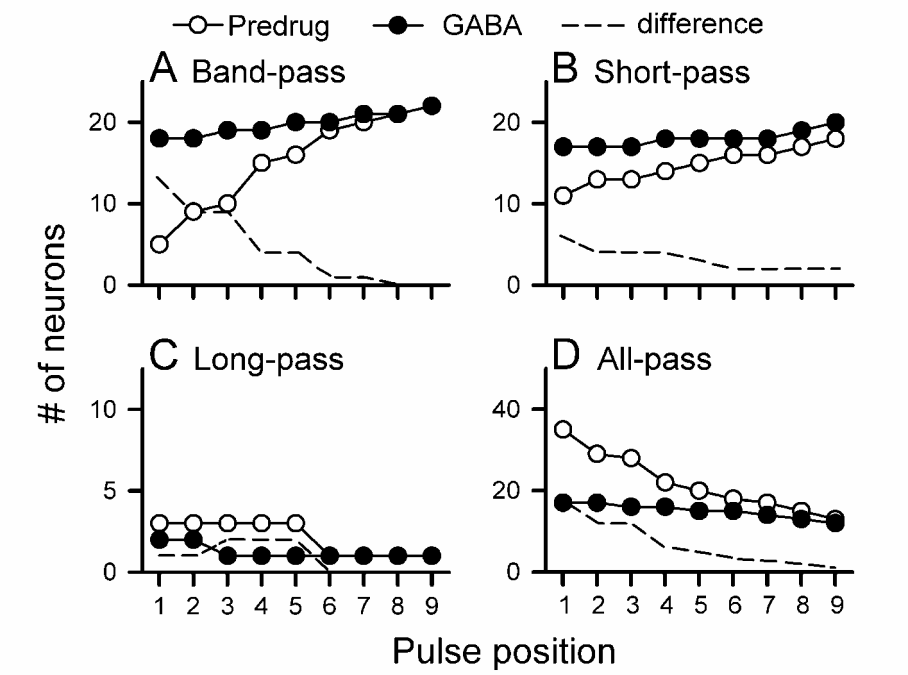

Predrug
Bicuculline
difference
A Band-pass
B Short-pass
C Long-pass
D All-pass
# of neurons
Pulse position
0
10
20
5
25
50
1 2 3 4 5 6 7 8 9

Figure 3.10 A-D: Variation in the number of each type of duration tuning neurons with sequentially presented sound pulses before (unfilled circles) and during (filled circles) GABA application (see ***Figure 3.9*** for legends and text for details).


Predrug
GABA
difference
A Band-pass
B Short-pass
C Long-pass
D All-pass
# of neurons
Pulse position
0
10
20
5
40
1 2 3 4 5 6 7 8 9

Variation in nDW and BD of duration tuning curves of IC neurons before and during drug application

We examined the variation in duration selectivity of IC neurons with sequentially presented sound pulses by comparing the BD and nDW of IC neurons obtained before and during drug application. Before drug application, the BD of duration tuning curves of IC neurons significantly decreased and the nDW increased with sequentially presented sound pulses (***Figures 3.11-3.12***, Aa, Ba, unfilled circles, two-way ANOVA, $P < 0.01$). Bicuculline application significantly increased the BD and decreased the nDW of IC neurons to varying degrees (***Figure 3.11***, filled vs unfilled, two-way ANOVA, $P < 0.05$). The increase in the BD and decrease in the nDW were small for the duration tuning curve plotted for the first pulse but became progressively larger for the duration tuning curves plotted for the subsequent pulses (***Figure 3.11***, Ab, Bb, one-way ANOVA, $P < 0.01$). As a result, the trend of significant decrease in the BD and increase in the nDW of duration tuning curves with sequentially presented sound pulses was abolished during bicuculline application (***Figure 3.11***, Aa, Ba filled circles, two-way ANOVA, $P > 0.05$). Opposite to these observations, GABA application significantly decreased the BD and increased the nDW of IC neurons to varying degrees (***Figure 3.12***, Aa, Ba, filled vs unfilled, two-way ANOVA, $P < 0.05$). The change in the BD and nDW was large for the duration tuning curve plotted for the first pulse but became progressively smaller for duration tuning curves plotted for the subsequent pulses (***Figure 3.12***, Ab,

Bb, one-way ANOVA, $P < 0.01$). As a result, the trend of significant decrease in the BD and increase in the nDW of duration tuning curves with sequentially presented sound pulses was also abolished during GABA application (***Figure 3.11***, Aa, Ba, filled circles, two-way ANOVA, $P > 0.05$).

Figure 3.11 Aa, Ba: Variation in the average BD (Aa) and nDW (Ba) of IC neurons obtained from duration tuning curves plotted for sequentially presented sound pulses before (unfilled circles) and during (filled circles) bicuculline application. The number of neurons and half a standard deviation are shown atop of each data point. Bicuculline application produced a significant increase in the BD (Aa) and decrease in the nDW (Ba) to varying degrees (unfilled circles vs filled circles, two-way ANOVA followed by a Student–Newman–Keuls multiple comparisons post-test, *** $P < 0.001$, ** $P < 0.01$ and *$P < 0.05$). As a result, the BD progressively decreased and the nDW increased with sequentially presented sound pulses only before but not during bicuclline application (unfilled circles vs filled circles, two-way ANOVA, $P < 0.01$ vs >0.05). Ab, Bb: Variation of percent change in the BD (Ab) and nDW (Bb) of duration tuning curves plotted for sequentially presented sound pulses during bicuculline application. Note that percent change in the BD and nDW significantly increased with sequentially presented pulses (one-way ANOVA, $P < 0.01$, see ***Figure 3.2*** for legends).

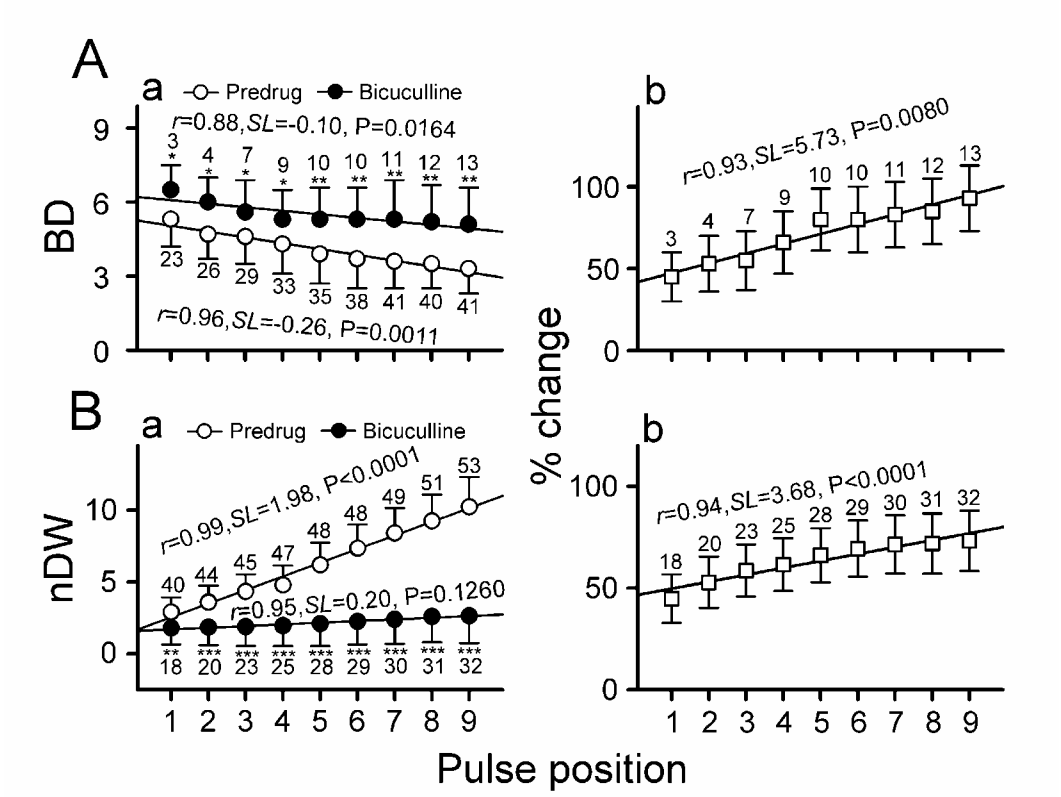
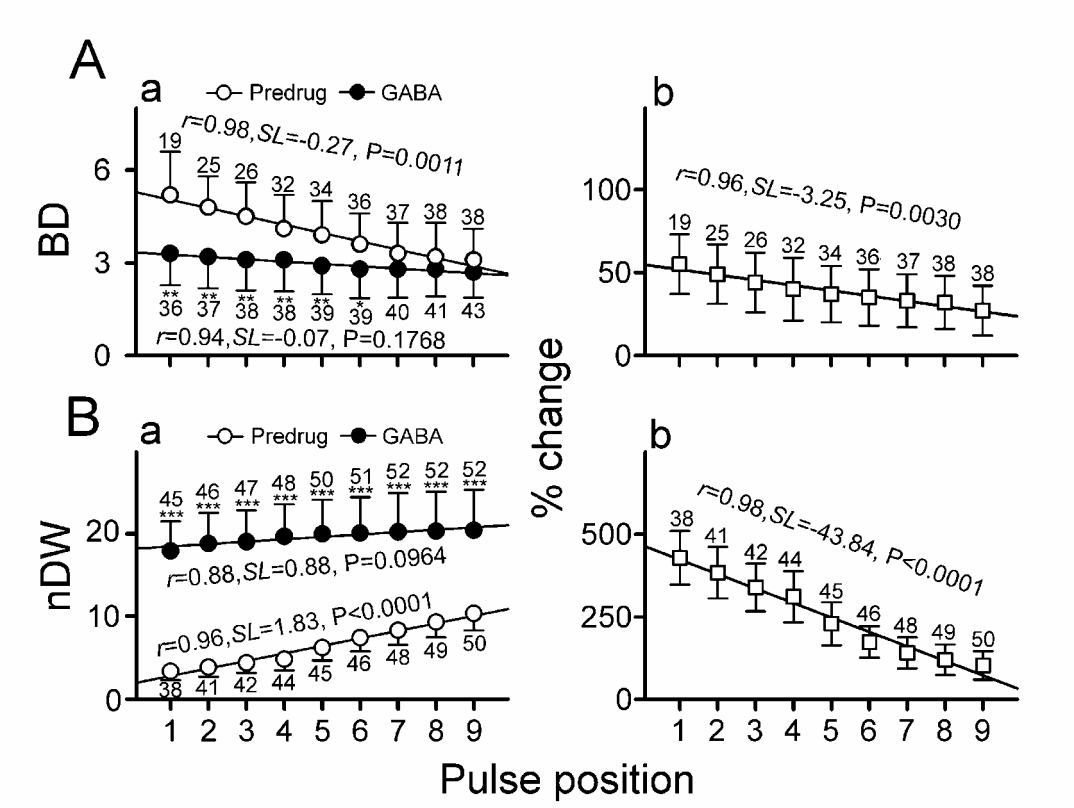

A
a
Predrug
Bicuculline
r=0.88,SL=-0.10, P=0.0164
r=0.96,SL=-0.26, P=0.0011
BD
b
r=0.93,SL=5.73, P=0.0080
% change
B
a
Predrug
Bicuculline
r=0.99,SL=1.98, P<0.0001
r=0.95,SL=0.20, P=0.1260
nDW
b
r=0.94,SL=3.68, P<0.0001
Pulse position

Figure 3.12 Aa, Ba: Variation in the average BD (Aa) and nDW (Ba) of IC neurons obtained from duration tuning curves plotted for sequentially presented sound pulses before (unfilled circles) and during (filled circles) GABA application. GABA application produced a significant decrease in the BD (Aa) and increase in the nDW (Ba) to varying degrees (unfilled circles vs filled circles, two-way ANOVA followed by a Student–Newman–Keuls multiple comparisons post-test, ***$P < 0.001$, **$P < 0.01$ and *$P < 0.05$). As a result, the BD progressively decreased and the nDW increased with sequentially presented sound pulses only before but not during GABA application (unfilled circles vs filled circles, two-way ANOVA, $P < 0.01$ vs >0.05). Ab, Bb: Variation of percent change in the BD (Ab) and nDW (Bb) of duration tuning curves plotted for sequentially presented sound pulses during GABA application. Note that percent change in the BD and nDW significantly decreased with sequentially presented pulses (one-way ANOVA, $P < 0.01$, See ***Figure 3.2*** for legends).


A
a
Predrug
GABA
r=0.98,SL=-0.27, P=0.0011
BD
r=0.94,SL=-0.07, P=0.1768
b
r=0.96,SL=-3.25, P=0.0030
% change
B
a
Predrug
GABA
nDW
r=0.88,SL=0.88, P=0.0964
r=0.96,SL=1.83, P<0.0001
b
r=0.98,SL=-43.84, P<0.0001
Pulse position

Discussion

The response size and duration selectivity of IC neurons determined with sequentially presented sound pulses

It has been reported in previous studies that IC neurons have different abilities for following rapidly presented sound pulses (Jen and Zhou 1999; Jen et al. 2001; Lu et al. 1997; Lu et al. 1998; Pinheiro et al. 1991; Wu and Jen 1995; Zhou and Jen 2002). These studies show that IC neurons with long recovery cycles had poor pulse following ability for sequentially presented pulses because they could not recover from preceding pulse stimulation (Lu et al. 1997; Lu et al. 1998). These studies also show that GABAergic inhibition shaped the recovery cycles of IC neurons because removal of GABAergic inhibition by bicuculline application shortened the recovery cycles and increased pulse-following ability of IC neurons.

In the present study, all 98 IC neurons discharged impulses to every sequentially presented pulses suggesting that they had short recovery cycles (***Figures 3.2-3.3, 3.5-3.6,*** A1). The response size of these neurons progressively decreased while the duration selectivity increased with sequentially presented sound pulses (***Figures 3.2-3.3,*** B, ***3.5-3.6***, ***3.11-3.12***). We expressed the sharpness of a duration tuning curve with an nDW which was based both on the variation in the response size and DW of the duration tuning curve of IC neurons obtained for each sound pulse (***Figure 3.4***). Thus, the finding of progressive increase in the nDW of

duration tuning curves plotted with sequentially presented sound pulses indicates a true increase in duration selectivity rather than a decrease in response sensitivity.

The role of GABAergic inhibition in shaping the response size and duration selectivity of IC neurons

Previous studies in cats have shown that the response size of auditory neurons to a sound pulse is decreased if the sound pulse is presented shortly after or before another one (i.e., forward and backward temporal masking, Brosch and Schreiner 1997; Calford and Semple 1995; Hocherman and Gilat 1981; Phillips et al. 1989). Recent studies showed that temporal forward and backward masking also affects the response size and duration selectivity of bat IC neurons (Faure et al. 2003; Galazyuk et al. 2000; Lu and Jen 2002). Conceivably, neural inhibition is the underlying mechanism for temporal forward and backward masking of auditory response.

In this study, we hypothesize that increasing strength of GABAergic inhibition over excitation contributes to variation in auditory sensitivity of IC neurons to sequentially presented sound pulses. Our hypothesis is substantiated by the following findings. One, significant decrease in the response size with sequentially presented pulses was abolished during bicuculline and GABA application (***Figures 3.2-3.3***, B). Two, significant increase of duration selectivity of IC

neurons with sequentially presented pulses was abolished during bicuculline and GABA application (***Figures 3.11-3.12***, Aa, Ba). Three, the percent change in the response size and duration selectivity of IC neurons with sequentially presented pulses significantly increased during bicuculline application but decreased during GABA application (***Figures 3.2*** vs ***3.3***, C; ***Figures 3.11*** vs ***3.12***, Ab, Bb). Four, all impulse-pulse position curves progressively decreased to varying degrees with sequentially presented sound pulses (***Figures 3.7-8***, A). Five, the sharpness of individual percent decrease-duration curves progressively increased with sequentially presented sound pulses (***Figures 3.7-8***, C). Six, the change in the number of band-, short- and all-pass duration tuning curves increased and decreased, respectively, with sequentially presented sound pulses during bicuculline and GABA application (***Figures 3.9-10***, dashed lines).

What might be the possible mechanisms underlying increasing strength of GABAeric inhibition with sequentially presented pulses? In this study, the inter-pulse interval of 9 presented pulses was 33.3 ms that is equivalent to a pulse repetition rate of 30 pps. It is conceivable that at a stimulation rate of 30 pps, the release of GABA was facilitated at a faster rate than the release of excitatory transmitters. Alternatively, the release of GABA was depressed at a slower rate than the release of excitatory transmitters. In either case, the relative ratio of GABAergic inhibition over excitation would become progressively larger with sequentially

presented sound pulses. For this reason, the effect of bicuculline application on the response size and duration selectivity of IC neurons became progressively effective with sequentially presented sound pulses while the opposite effect was observed during GABA application (***Figures 3.2-3.3***, ***3.11-3.12***, A(b), B(b)).

Comparisons with previous studies

We show that increasing strength of GABAergic inhibition relative to excitation contributed to decreasing response size with sound pulses sequentially presented (***Figures 3.2-3.3***). The fact that different strength of GABAergic inhibition affects the response properties has been reported in previous studies (LeBeau et al. 1996; Pollk and Park 1993). These studies show that the response size of IC neurons decreased and the rate-amplitude function lowered with increasing strength of GABAergic inhibition produced by increasing iontophoretic current.

Previous studies have shown that bicuculline application not only increased the response size of IC neurons to sound stimulation but also changed the discharge pattern of some phasic responders to tonic firing (Jen and Feng 1999; LeBeau et al. 1996; Yang et al. 1992). In the present study, however, bicuculline application produced an increase in the response size but did not change the discharge pattern of IC neurons to sequentially presented sound pulses (***Figures 3.2***, ***3.5***). This finding

suggests that these IC neurons receive a very specific and limited set of GABAergic inputs that shape their phasic discharge pattern to sound stimulation. It has been suggested that the GABAergic inputs to IC neurons with phasic discharge pattern either originate intrinsically or from the ventral nucleus of the lateral lemniscus (LeBeau et al. 1996).

A recent study reported that directional selectivity of IC neurons also progressively increased with sound pulses presented in rapid sequences (Zhou and Jen 2004). This study indicated that increasing directional selectivity with sequentially presented sound pulses was due to variation in recovery cycle of IC neurons with azimuthal angle. Because GABAergic inhibition shapes recovery cycles of IC neurons with azimuthal angle (Zhou and Jen 2003), we believe that increasing strength of GABAergic inhibition with sequentially presented sound pulses observed in the present study may also contribute to increasing directional selectivity of IC neurons with sequentially presented sound pulses. In parallel to our present finding, some recent studies also showed that increasing strength of GABAergic inhibition contributes to improving multi-parametric selectivity of IC neurons with increasing pulse repetition rate (Jen et al. 2001, Jen et al. 2002; Zhou and Jen 2002).

In this study, we only examined the role of GABAergic inhibition in shaping the response size and duration selectivity of IC neurons to sound pulses with rapid

sequences. As indicated earlier, the IC also receives glycinergic inputs from the superior olivary complex and ventral complex of the lateral lemniscus (Glendenning et al. 1992; Malmeierca et al. 1998; Saint Marie et al. 1989). Previous studies have shown that glycinergic inhibition also contributes to the temporal response properties, frequency tuning and binaural processing of IC neurons (Klug et al. 1995; Koch and Grothe, 1998; Lu and Jen 2001; LeBeau et al. 1996; LeBeau et al. 2001). It is therefore conceivable that increasing strength of glycinergic inhibition may also contribute to variation in auditory sensitivity of IC neurons with sequentially presented pulses. Future works need to be conducted to confirm this speculation.

Possible biological relevance of the present study

During hunting, insectivorous bats such as *Eptesicus fuscus* progressively increase pulse repetition rate as they search, approach and finally intercept the insects (Griffin 1958; Simmons et al. 1979). It has been suggested that this increase in pulse repetition rate not only enables the bat to obtain as much information as possible about the localized prey but also sharpens echo selectivity in multi-parametric domains for accurate prey capture (Jen and Zhou 1999; Jen et al. 2001; Jen et al. 2002; Zhou and Jen 2002; Wu and Jen 1996). In the present study, we studied the role of GABAergic inhibition in shaping the response size and duration selectivity of bat IC neurons using a pulse train of 30 pps that is comparable to that

occurring during the approach phase of hunting. Although the pulse train used in the present study is not exactly the same as the naturally occurring ones, our data suggest that increasing strength of GABAergic inhibition could potentially improve echo duration selectivity of IC neurons. Improving echo duration selectivity would undoubtedly facilitate echo recognition by bats during hunting.

Previous studies have shown that the auditory system of bats is fundamentally similar to that of other mammals and the interplay of inhibition and excitation that shapes many response properties of IC neuron is similar across many mammals (Casseday and Covey 1995; Covey and Casseday 1995; Fuzessery and Hall 1996; Jen and Feng 1999; Jen and Zhang 2000; Jen and Zhou 1999; Jen et al. 2001; Jen et al. 2002; Klug et al. 1995; Koch and Grothe 1998; LeBeau et al. 1996; LeBeau et al. 2001; Lu and Jen, 2001; Pollak and Casseday 1989). Therefore, our present findings are likely to be applicable to other mammalian species as well. Conceivably, increasing strength of GABAergic inhibition with sequentially presented sound pulses shown in the present study might be the neural mechanism underlying the psychophysical phenomena of temporal masking. It is also possible that increasing GABAergic inhibition with sounds in rapid sequence may serve as a neural basis underlying facilitation of speech processing in humans.

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CHAPTER 4

THE ROLE OF GABAERGIC INHIBITION IN SHAPING DURATION SELECTIVITY OF BAT INFERIOR COLLICULAR NEURONS DETERMINED WITH TEMPORALLY PATTERNED SOUND TRAINS

Abstract

A previous study has shown that duration selectivity of neurons in the inferior colliculus (IC) of the big brown bat, *Eptesicus fuscus* becomes sharper with increasing pulse repetition rate (PRR). The present study examines the role of GABAergic inhibition in improving duration selectivity of bat IC neurons with PRR by means of iontophoretic application of GABA as well as its antagonist, bicuculline. Duration selectivity of IC neurons is studied by plotting the duration tuning curves with the number of impulses per pulse against the pulse duration. Duration tuning curves of IC neurons are described as band-, short-, long- and all-pass in terms of filtering properties to sound duration. Bicuculline application produces more pronounced broadening of duration tuning curves at high than at low PRR. Conversely, GABA application produces more pronounced narrowing of duration tuning curves at low than at high PRR. In either case, sharpening of duration selectivity of IC neurons with increasing PRR is abolished during drug application. The duration tuning curves of IC neurons progressively broadens with recording

depth. Broadening of duration tuning curves during bicuculline application is more pronounced for neurons at upper than at deep IC. This progressive decrease in duration selectivity with recording depth is discussed in relation to spatial distribution gradient of $GABA_A$ receptors in the IC. Possible biological significance of these findings relevant to bat echolocation is discussed.

Introduction

Sound duration is an important feature that contributes to the distinct spectral and temporal attributes of individual biological sounds. Previous studies of selectivity of auditory neurons to sound duration has been conducted in many animals including frogs (Feng et al. 1990; Gooler and Feng 1992; Narins and Capranica 1980), bats (Casseday et al. 1994, 2000; Ehrlich et al. 1997; Fuzessery and Hall 1999; Galazyuk and Feng 1997; Jen and Feng 1999; Jen and Schlegel 1982; Jen and Zhou 1999;Pinheiro et al. 1991; Zhou and Jen 2001), cats (He et al. 1997), chinchillas (Chen 1998), mice (Brand et al. 2000) and rats (Perez-Gonzalez et al. 2006). These studies show that most auditory neurons behave as band-, short- or long-pass filters to pulse duration such that they respond maximally to a specific duration or a range of durations.

In the mammalian auditory pathway, the central nucleus of the inferior

colliculus (IC) receives and integrates excitatory and inhibitory inputs from many lower auditory nuclei (Adams 1979; Casseday and Covey 1995; Oliver et al. 1994; Shneiderman and Oliver 1989). Neurotransmitters that mediate the inhibitory inputs are γ-aminobutyric acid (GABA) or glycine (Fubara et al. 1996; Oliver et al. 1994; Roberts and Ribak 1987). Previous studies show that the interplay between these two opposing inputs contributes to auditory temporal processing and shapes multi-parametric selectivity (e.g., duration, frequency, amplitude, direction, etc.) of IC neurons using single repetitive sound pulses or temporally patterned trains of sound pulses (Casseday and Covey 1995; Casseday et al. 1994, 2000; Jen and Feng 1999; Jen and Zhang 2000; Jen et al. 2002; Klug et al. 1995; Koch and Grothe 1998; LeBeau et al. 1996, 2001; Lu and Jen 2001; Lu et al. 1997, 1998; Zhou and Jen 2002).

When studied with temporally patterned trains of sound pulses at different pulse repetition rates (PRR), the response selectivity of IC neurons in bats has been shown to improve with increasing PRR in multi-parametric domains (Galazyuk et al. 2000; Jen and Zhou 1999; Jen et al. 2001, 2002; Smalling et al. 2001; Wu and Jen 1995a, b, 1996; Zhou and Jen 2002). In our laboratory, we have previously studied the role of GABAergic inhibition in shaping the frequency and directional selectivity of bat IC neurons with PRR (Jen et al. 2002; Zhou and Jen 2002). We showed that increasing GABAergic inhibition with the PRR contributes to improving frequency and directional selectivity of IC neurons. As an extension of these studies, the main

objective of the present study is to show that increasing GABAergic inhibition with the PRR also contributes to improving duration selectivity of IC neurons. To achieve this objective, we studied the duration selectivity of IC neurons with pulse trains of three PRRs before and during application of GABA or bicuculline which is an antagonist for $GABA_A$ receptor (Bormann 1988; Cooper et al. 1982). By means of application of both drugs that produced opposite effect on duration selectivity of IC neurons, we were able to double confirm the role of GABAergic inhibition in improving duration selectivity of IC neurons with PRR.

Materials and methods

Animals and surgery

Eight *Eptesicus fuscus* (five males, three females, 18–22 g, body weight, b.w.) were used for this study. As in previous studies (Jen et al. 1987), one or two days before the recording session, a 1.8 cm nail was glued onto the exposed skull of Nembutal-anesthetized (45–50 mg/kg b.w.) bat with acrylic glue and dental cement. Exposed tissue was treated with an antibiotic (Neosporin) to prevent infection. During the day of recording, the bat was administered the neuroleptanalgesic Innovar-Vet (Fentanyl 0.08 mg/kg b.w. Droperidol 4 mg/kg b.w.). The bat was then placed inside a bat holder (made of wire mesh) that was suspended in an elastic

sling inside a double-wall soundproof room (Industrial Acoustics Company, Inc., temperature 28–30 °C). The ceiling and inside walls of the room were covered with 3-in. convoluted polyurethane foam to reduce echoes. After fixing the bat's head with a set screw, small holes (about 25–30 μm in diameter) were bored in the skull above the IC for insertion of piggyback multibarrel electrodes to record auditory responses of IC neurons and for iontophoretic application of bicuculline or GABA. Additional doses of Innovar-Vet were administered during later phases of recording if the bat showed signs of discomfort. Recording depth was read from the scale of a microdrive (David-Kopf). An indifferent electrode (silver wire) was placed at the nearby temporal muscles. Each bat was used for one to five recording sessions on separate days and each recording session typically last for 2–6 h. These procedures were conducted in compliance with NIH Publication No. 85–23, "Principles of Laboratory Animal Care" and with the approval of the Institutional Animal Care and Use Committee (#1438) of the University of Missouri-Columbia.

Acoustic stimulation

Sound pulses were generated with an oscillator (KH model 1200) and a homemade electronic switch driven by a stimulator (Grass S88). These stimuli were then amplified after passing through a decade attenuator (HP 350D) before they were fed to a small condenser loudspeaker (AKG model CK 50, 1.5 cm diameter, 1.2 g) that was placed 23 cm away from the bat and 40° contralateral to the recording

site. Calibration of the loudspeaker was performed with a 1/4 in. microphone (B&K 4135) placed at the position where the bat's head would be during recording. The output of the loudspeaker was expressed in dB SPL in reference to 20 μPa root mean square. The maximum output was flat between 50 between 80 kHz (100 ± 5 dB SPL). The output then fell with a slope of about 15 dB/octave at the lower and higher frequency range.

Upon isolation of an IC neuron with 4 ms sound pulses, its best frequency (BF) was determined by changing the frequency and intensity of sound stimuli. The minimum threshold (MT) at the BF was defined as the sound level that elicited 50% response probability from the neuron. As described previously (Jen and Zhou 1999), duration selectivity of IC neurons was studied with 300-ms pulse trains with pulse durations of 1, 2, 4, 6, 8, 10 and 20 ms of BF sound pulses set at 10 dB above the MT. These pulse trains were delivered at 2 trains/s with PRR of 10, 30 and 90 pps by setting the inter-pulse interval within pulse trains at 100, 33.3 and 11.1 ms (i.e., the number of pulses was 3, 9 and 27 within these pulse train). The 20 ms pulse was not used in pulse trains of 90 pps because of overlap between pulses. Rise-decay times were 0.5 ms but they were 0.25 ms for 1 ms pulse duration. These three PRRs are comparable to the PRRs occurring during the search, approach and terminal phases of hunting by the big brown bat (Griffin 1958; Simmons et al. 1979; Surlykke and Moss 2000).

Iontophoresis and recording

Iontophoretic application of bicuculline or GABA to recorded neurons has been described in our previous studies (Lu et al. 1997, 1998). Briefly, a three-barrel electrode (tip: 10–15 μm) was piggybacked to a 3 M KCl single-barrel electrode (tip: less than 1 μm; impedance: 5–10 MΩ) whose tip was extended about 10 μm from the tip of the three-barrel electrode. The 3 M KCl single-barrel recording electrode was used to record neural responses. One of the barrels of the triple barrel electrode was filled with bicuculline methiodide (10 mM in 0.16 M NaCl, pH 3.0, Sigma) or GABA (500 mM in distilled water, pH 3.5, Sigma) that could be injected to recording site. The bicuculline or GABA was prepared just prior to each experiment and the electrode filled immediately before use. This drug channel was connected via silver–silver chloride wire to a microiontophoresis constant current generator (Medical Systems Neurophore BH-2) that was used to generate and monitor iontophoretic currents. During bicuculline or GABA application, 1 s pulse of 40 namp at 0.5 pps was applied for 1 min before data acquisition. Electrical current was then changed to 10 namp during data acquisition. The other two barrels were filled with 1 M NaCl (pH 7.4), one of which was used as the ground and the other as the balanced barrel. The balance electrode was connected to balance module. The retaining current was negative 8–10 namp.

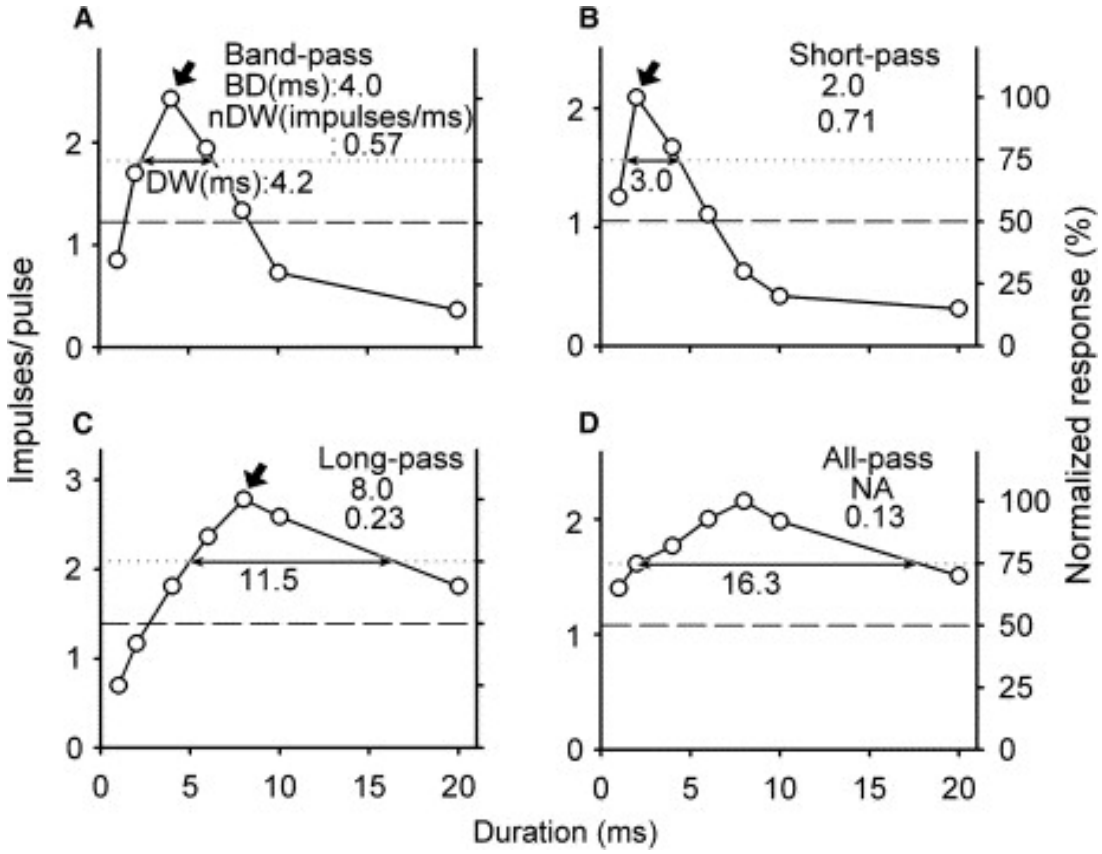
Recorded action potentials were amplified, band-pass filtered (Krohn-Hite

3500), and fed through a window discriminator (WPI 121) before being sent to an oscilloscope (Tektronix 5111) and an audio monitor (Grass AM6). They were then sent to a computer (Gateway 2000, 486) for acquisition of peri-stimulus-time (PST) histograms (bin width: 500 μs, sampling period: 300 ms) to 32 train presentations.

Data analysis

Duration selectivity of IC neurons was studied by plotting their duration tuning curves under different stimulation conditions. Because the number of pulses varied in the three pulse trains, we plotted the duration tuning curves of IC neurons with the average number of impulses per pulse in each pulse train against pulse duration. The tuning properties of duration tuning curves of IC neurons were expressed with a best duration (BD) and normalized duration width (nDW) (see ***Figure 4.1***). These two tuning properties obtained at different PRRs were then quantitatively studied and statistically compared before and during drug application using repeated measures one-way or two-way ANOVA followed with a Student–Newman–Keuls multiple comparisons post-test at $P < 0.05$.

Figure 4.1 Duration tuning curves of four representative inferior collicular (IC) neurons in the big brown bat, *Eptesicus fuscus*. Left and right ordinates represent the number of impulses per pulse and normalized response. The abscissa represents pulse duration (ms). Duration tuning properties of these neurons are (A) band-pass, (B) short-pass, (C) long-pass and (D) all-pass. Each horizontal dashed line indicates the 50% maximal response. Duration selectivity of each curve is expressed with a best duration (BD) and a normalized duration width (nDW). The BD of the band-, short- and long-pass duration tuning curves is indicated by an arrow head at the duration of maximal response. An nDW is obtained by dividing the maximum by the duration width (DW indicated with a double-head arrow) of a duration tuning curve at 75% maximum. The BF (kHz), latency (ms), MT (dB SPL) and recording depth (μm) of these neurons were 25.6, 11.0, 40, 316 (A); 42.4, 12.0, 45, 715 (B); 48.2, 12.5, 44, 938 (C); 54.5, 13.0, 48, 1044 (D) (see text for details).


A
Band-pass
BD(ms):4.0
nDW(impulses/ms)
:0.57
DW(ms):4.2
B
Short-pass
2.0
0.71
3.0
C
Long-pass
8.0
0.23
11.5
D
All-pass
NA
0.13
16.3
Impulses/pulse
Normalized response (%)
Duration (ms)

Results

Duration tuning properties of IC neurons determined with pulse trains

As in our previous studies (Jen and Zhou 1999; Jen et al. 2001; Zhou and Jen 2002), IC neurons differed in the number of impulses discharged to each pulse and in the ability in response to each pulse of a pulse train. Among 178 neurons studied, 134 (75%) neurons discharged impulses to each pulse of all three presented pulse trains. The remaining 44 (25%) neurons only discharged impulses to each pulse of pulse trains of 10 and 30 pps (26, 15%) or to each pulse of pulse train of 10 pps only (18, 10%). For consistence of comparison of duration tuning properties, we only examined the duration selectivity of those 134 neurons that responded to each pulse of three pulse trains. Among them, 78 received bicuculline application and 56 received GABA application.

In total, 1608 duration tuning curves were plotted before and during drug application. These curves can be described as four types using the same criterion adopted in our previous studies (Jen and Feng 1999; Jen and Zhou 1999; Wu and Jen 1995a). The band-pass duration tuning curves showed a maximum to a specific duration and the maximum decreased at least 50% at both limbs (***Figure 4.1*** A, n = 407, 25%). The short-pass duration tuning curves showed a maximum to a short duration which decreased more than 25% at a shorter duration and more than 50%

at a longer duration (***Figure 4.1*** B, *n* = 354, 22%). Conversely, the long-pass duration tuning curves showed a maximum to a long duration which decreased more than 25% at a longer duration and more than 50% at a shorter duration (***Figure 4.1*** C, *n* = 149, 10%). IC neurons with any of these three types of duration tuning curves are called duration selective neurons. The number of impulses of all-pass duration tuning curves often differed by more than 25% but never by more than 50% at all durations tested (***Figure 4.1*** D, *n* = 698, 43%). IC neurons with all-pass duration tuning curves are called duration non-selective neurons.

To express the duration tuning properties of a duration tuning curve, the duration of the maximum in the band-, short and long-pass duration curves is defined as the best duration (BD, indicated by filled arrows in ***Figure 4.1*** A–C). Because bicuculline application increased and GABA application decreased the number of impulses of IC neurons, the duration tuning curves varied in opposite ways. As such, the maximal number of impulses obtained from a neuron varied greatly before and during drug application. For this reason, we used a normalized duration-width (nDW) to express the sharpness of a duration tuning curve by taking into account the variation in the number of impulses before and during drug application. An nDW was obtained by dividing the maximal number of impulses by the width of a duration tuning curve at 75% of the maximum (***Figure 4.1***, DW indicated by a double-head arrow). Thus, a neuron with a large nDW has a narrow

duration tuning curve and sharp duration selectivity. Every band-, short- and long-pass duration tuning curve has an nDW. Although an all-pass duration tuning curve is duration non-selective according to our criterion, an all-pass duration curve may have an nDW when the maximum decreases by more than 25% at both limbs (e.g. ***Figure 4.1*** D). This nDW becomes useful to compare the change of duration selectivity of IC neurons when the all-pass duration tuning curves changed into other types during GABA application.

Discharge patterns and duration selectivity of IC neurons obtained before and during bicuculline application

The discharge patterns and duration tuning curves of two representative IC neurons obtained with three pulse trains before and during bicuculline application are shown in Figures 4.2 and 4.3. The response of these two neurons showed varied degree of adaptation to sequentially presented pulses at each pulse train (***Figures 4.2-4.3***, A–C). When the duration tuning curve was plotted with the average number of impulses per pulse against pulse duration, one neuron always had a band-pass duration tuning curve regardless of PRR (***Figure 4.2***, Da, Ea, Fa). However, the neuron's BD shortened from 6 ms at 10 pps to 4 ms at 30 and 90 pps. Conversely, the neuron's nDW progressively increased from 0.24 at 10 pps to 0.36 at 30 pps and to 0.51 at 90 pps. These two observations indicate that the neuron's duration selectivity

progressively increased with PRR.

The other neuron had an all-pass duration tuning curve when plotted at 10 pps (***Figure 4.3,*** Da).The all-pass duration tuning curve changed into short-pass and then into band-pass when the PRR increased from 10 to 30 pps and then to 90 pps (***Figure 4.3,*** Ea, Fa). This neuron did not have a BD when tested at 10 pps but had a BD of 4 ms when tested at 30 and 90 pps. The neuron's nDW also progressively increased from 0.23 to 0.33 and then to 0.49 with PRR indicating increasing duration selectivity.

Bicuculline application produced an increase in the number of impulses of both neurons in response to each pulse. However, the increase in the number of impulses during bicuculline application was greater for non-BD durations than for the BD and near-BD durations. As a result, the duration tuning curves of both neurons were broadened and changed into all-pass duration tuning curves when tested at 10 and 30 pps (***Figures 4.2-4.3***, Da, Ea vs. Db, Eb). When tested at 90 pps, bicuculline application changed the band-pass duration tuning curve of both neurons into a short-pass duration tuning curve (***Figures 4.2-4.3***, Fa vs. Fb). Bicuculline application abolished the BD of both neurons at 30 pps and lengthened the 4 ms BD to 6 ms at 90 pps (***Figures 4.2-4.3***, Ea, Fa vs. Eb, Fb). Bicuculline application also decreased the sharpness of both duration tuning curves as evident by decreasing nDW at all three PRRs. However, the degree of decrease in nDW was

small at low PRR but large at high PRR. For example, the nDW of one neuron decreased 46% at 10 pps, 58% at 30 pps and 69% at 90 pps (***Figure 4.2***, D–F). Similarly, the nDW of the other neuron decreased 39% at 10 pps, 51% at 30 pps and 57% at 90 pps (***Figure 4.3***, D–F).

To quantitatively study the effect of bicuculline application on the duration selectivity of IC neurons, we compared the average BD and nDW of duration tuning curves of 78 IC neurons obtained before and during bicuculline application at three PRRs. We observed that the average BD of IC neurons significantly shortened with PRR only before but not during bicuculline application (***Figure 4.4***, Aa, Two-way ANOVA; unfilled bars, $P < 0.001$; filled bars, $P > 0.1$). A Student–Newman–Keuls multiple comparisons post-test showed significant differences between each set of BDs (**$P < 0.01$, *$P < 0.05$). Since bicuculline application often lengthened the BD of IC neurons, we compared the percent change in the BD of IC neurons at three PRRs. The percent change in the BD during bicuculline application was small at 10 pps but became significantly large at 30 and 90 pps (***Figure 4.4***, Ab, One-way ANOVA, $P < 0.001$). A Student–Newman–Keuls multiple comparisons post-test showed significant differences between each set of the percent changes in the BD (*$P < 0.05$).

In parallel to this observation, the average nDW of duration tuning curves of these 78 neurons significantly increased with PRR only before but not during bicuculline application (***Figure 4.4***, Ba, Two-way ANOVA, unfilled bars, $P < 0.001$;

filled bars, $P > 0.1$). A Student–Newman–Keuls multiple comparisons post-test showed significant differences between each set of nDWs ($^{**}P < 0.01$; $^{*}P < 0.05$). The percent decrease in nDW of IC neurons during bicuculline application was also small at 10 pps but became significantly large at 30 and 90 pps (***Figure 4.4***, Bb, One way ANOVA, $P < 0.001$). A Student–Newman– Keuls multiple comparisons post-test showed significant differences between each set of the percent change in the nDW ($^{**}P < 0.01$).

Table 4.1 shows the type of duration tuning curves of 78 neurons plotted before and during bicuculline application. It is clear that the number of band-pass duration tuning curve progressively increases (from 19% to 29% then to 37%) while the number of all-pass duration tuning curves decreases (from 37% to 25% then to 18%) with increasing PRR before bicuculline application. However, the number of short-pass and long-pass duration tuning curves is hardly affected by PRR. Bicuculline application changed 50–63% of band-, short and long-pass duration tuning curves into all-pass duration tuning curves. As such, 79–87% of IC neurons have all-pass duration tuning curves during bicuculline application. The percent increase in the all-pass duration tuning curves is 50% at 10 pps, 60% at 30 pps and 61% at 90 pps.

Figure 4.2 A, B, C: Peri-stimulus-time (PST) histograms showing the discharge patterns of an IC neuron obtained with three pulse trains containing pulses of different durations (shown at far right) before (predrug) and during bicuculline application. D, E, F: The neuron's duration tuning curves plotted with the average number of impulses per pulse before (unfilled circles) and during (filled circles) bicuculline application at three pulse repetition rates (PRRs). The type, BD and nDW of the duration tuning curve are shown within each plot. NA indicates that a BD is not available. Dur: pulse duration. The BF (kHz), latency (ms), MT (dB SPL) and recording depth (μm) of this neuron were 28.5, 11.0, 42, 356 (see ***Figure 4.1*** for legends).

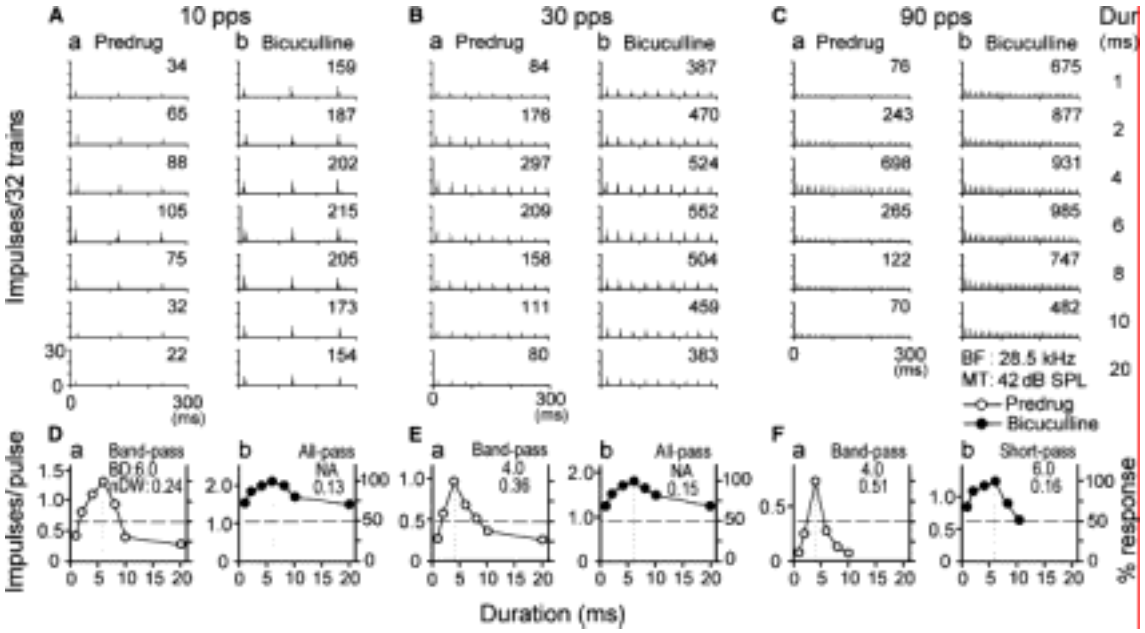

A
10 pps
B
30 pps
C
90 pps
Dur
(ms)
a Predrug
b Bicuculline
Impulses/32 trains
34
65
88
105
75
32
30
22
0
300
(ms)
159
187
202
215
205
173
154
84
176
297
209
158
111
80
387
470
524
552
504
459
383
76
243
698
265
122
70
675
877
931
985
747
482
1
2
4
6
8
10
20
BF : 28.5 kHz
MT: 42 dB SPL
Predrug
Bicuculline
D
E
F
Band-pass
BD:6.0
nDW: 0.24
All-pass
NA
0.13
4.0
0.36
0.15
0.51
Short-pass
6.0
0.16
Impulses/pulse
% response
Duration (ms)

Figure 4.3 PST histograms and duration tuning curves of another IC neuron obtained with three pulse trains before (predrug) and during bicuculline application. The BF (kHz), latency (ms), MT (dB SPL) and recording depth (μm) of this neuron were 33.7, 12.0, 45 and 412 (see ***Figure 4.2*** for legends).

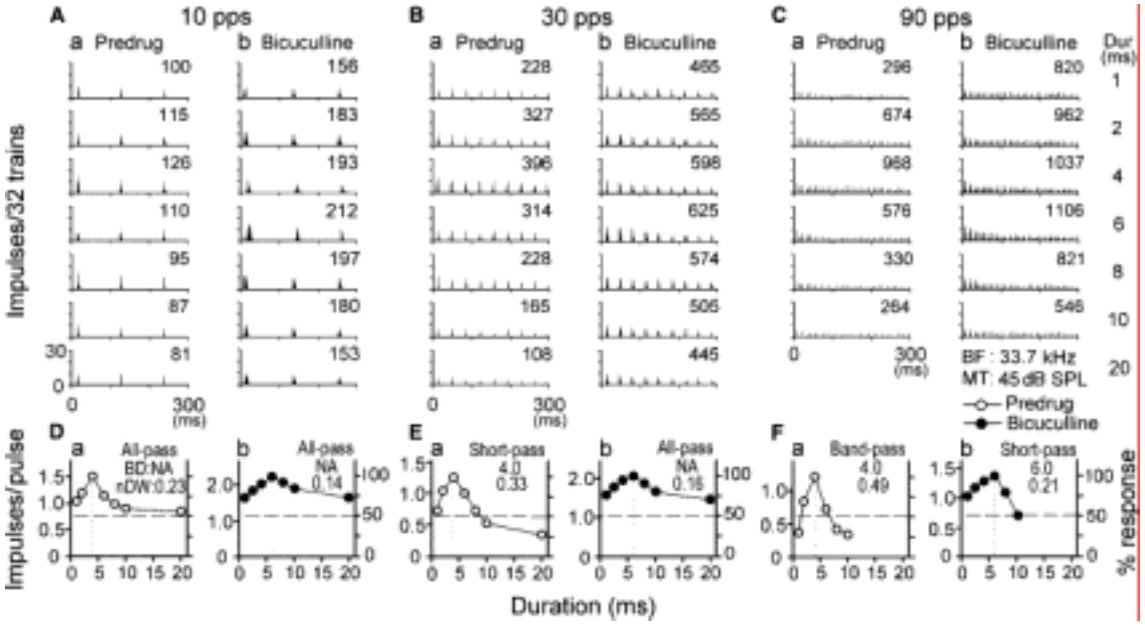

A
10 pps
a Predrug
b Bicuculline
100
156
115
183
126
193
110
212
95
197
87
180
81
153
30
0
0
300
(ms)
B
30 pps
a Predrug
b Bicuculline
228
455
327
565
396
598
314
625
228
574
185
506
108
445
0
300
(ms)
C
90 pps
a Predrug
b Bicuculline
Dur (ms)
296
820
1
674
962
2
988
1037
4
576
1106
6
330
821
8
284
548
10
20
0
300
(ms)
BF : 33.7 kHz
MT: 45 dB SPL
Predrug
Bicuculline
Impulses/32 trains
D
a All-pass
BD:NA
rDW:0.23
b All-pass
NA
0.14
E
a Short-pass
4.0
0.33
b All-pass
NA
0.16
F
a Band-pass
4.0
0.49
b Short-pass
6.0
0.21
Impulses/pulse
% response
Duration (ms)

Figure 4.4 Bar histograms showing the average BD and nDW obtained from the duration tuning curves of IC neurons plotted before (unfilled bars) and during (filled bars) bicuculline application (Aa, Ba). Note that the number of BD and nDW shown in each bar is different because duration tuning curve of IC neurons often change from one type to another with PRR. Note also that the average BD significantly decreased and the average nDW increased with PRR only before but not during bicuculline application (Repeated measures two-way ANOVA; unfilled bars, $P < 0.001$; filled bars, $P > 0.1$). Ab, Bb: percent change in the average BD and nDW of IC neurons during bicuculline application at three PRRs. The average percent change of BD and nDW was significantly greater at high than at low PRR (repeated measures one-way ANOVA; $P < 0.001$) (see text for details).

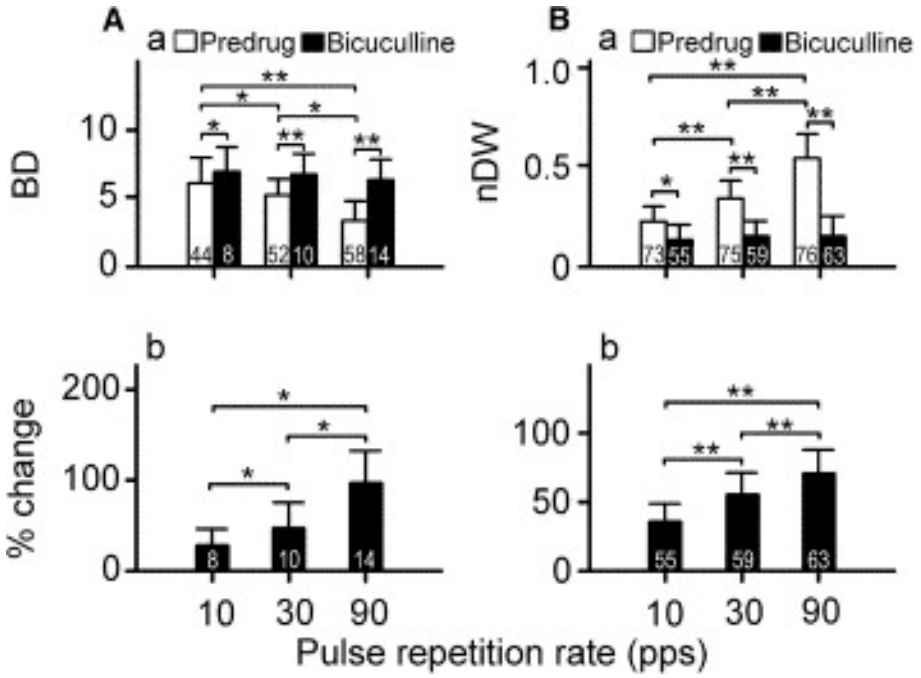

A
a
Predrug
Bicuculline
BD
10
5
0
44
8
52
10
58
14
B
a
Predrug
Bicuculline
nDW
1.0
0.5
0
73
55
75
59
76
63
b
% change
200
100
0
8
10
14
b
100
50
0
55
59
63
10
30
90
10
30
90
Pulse repetition rate (pps)

Table 4.1 The effect of bicuculline application on duration tuning curves of 78 IC neurons

PRR	Tuning	Predrug	Bicuculline			
(pps)	properties		BP	SP	LP	AP
10	BP	15 (19 %)	3	3		9
	SP	24 (31 %)		2		22
	LP	10 (13 %)			2	8
	AP	29 (37 %)				29
	Total	78	3 (4 %)	5 (6 %)	2 (3 %)	68 (87 %)
30	BP	22 (29 %)	4	3		15
	SP	25 (32 %)		3		22
	LP	11 (14 %)			2	9
	AP	20 (25 %)				20
	Total	78	4 (5 %)	6 (7 %)	2 (3 %)	66 (85 %)
90	BP	28 (37 %)	4	4		20
	SP	25 (32 %)		5		20
	LP	11 (13 %)			3	8
	AP	14 (18 %)				14
	Total	78	4 (5 %)	9 (12 %)	3 (4 %)	62 (79 %)

BP: Band-pass; SP: Short-pass; LP: Long-pass; AP: All-pass.

Discharge patterns and duration selectivity of IC neurons determined before and during GABA application

Figure 4.5 shows the discharge patterns and duration tuning curves of a representative IC neuron determined with three pulse trains before and during GABA application. Like the other two neurons in ***Figures 4.2 and 4.3***, the response of this neuron also showed adaptation to sequentially presented pulses within each pulse train. When studied at three PPRs, the neuron's all-pass duration tuning curve plotted at 10 pps changed into a short-pass one at 30 pps and a band-pass one at 90 pps (***Figure 4.5***, Da, Ea, Fa). This neuron did not have a BD when tested at 10 pps but had a 4 ms BD at 30 pps and a 2 ms BD at 90 pps (***Figure 4.5***, Da, Ea, Fa). The neuron's nDW also progressively increased from 0.15 to 0.21 and then to 0.26 with PRR indicating increasing duration selectivity.

GABA application produced a decrease in the number of impulses of this neuron in response to each pulse (***Figure 4.5***, Ab, Bb, Cb, GABA). However, the decrease in the number of impulses during GABA application was greater for non-BD durations than for the BD and near-BD durations. As a result, the neuron's all-pass and short-pass duration tuning curves plotted at 10 and 30 pps greatly sharpened and changed into band-pass duration tuning curve (***Figure 4.5***, Da, Ea vs. Db, Eb). During GABA application, this neuron had a 4 ms BD at 10 pps and its 4 ms BD obtained at 30 pps shortened to 2 ms (***Figure 4.5*** , Da vs. Db, Ea vs. Eb). At 90 pps,

GABA application sharpened the neuron's band-pass duration tuning curve (as evident by increased nDW from 0.26 to 0.37) but did not change its 2 ms BD (***Figure 4.5***, Fa vs. Fb). Sharpening of duration tuning curve during GABA application was greater at low than at high PRR. For example, the percent increase in the neuron's nDW was 113% at 10 pps, 67% at 30 pps and 42.3% at 90 pps (***Figure 4.5***, D–F).

We quantitatively compared the average BD and nDW of duration tuning curves of 56 IC neurons obtained before and during GABA application at three PRRs. Similar to what was observed during bicuculline application (i.e., ***Figure 4.4***), the average BD of IC neurons significantly shortened with PRR only before but not during GABA application (***Figure 4.6***, Aa, Two-way ANOVA; unfilled bars, $P < 0.001$; filled bars, $P > 0.1$). A Student–Newman–Keuls multiple comparisons post-test showed significant differences between each set of BDs ($^{**}P < 0.01$, $^{*}P < 0.05$). However, opposite to what was observed during bicuculline application, shortening of BD during GABA application was large at 10 pps but became significantly small at 30 and 90 pps (***Figure 4.6***, Ab, one-way ANOVA, $P < 0.001$). A Student–Newman–Keuls multiple comparisons post-test showed significant differences between each set of the percent changes in the BD ($^{**}P < 0.01$).

In parallel to this observation, the average nDW of duration tuning curves significantly increased with PRR only before but not during GABA application (***Figure 4.6***, Ba, Two-way ANOVA, unfilled bars, $P < 0.001$; filled bar, $P > 0.1$). A

Student–Newman–Keuls multiple comparisons post-test showed significant differences between each set of the nDWs before GABA application ($^{**}P < 0.01$, $^{*}P < 0.05$). The percent increase in nDW of IC neurons during GABA application was also large at 10 pps but became significantly small at 30 and 90 pps (***Figure 4.6***, Bb, One way ANOVA, $P < 0.001$). A Student–Newman–Keuls multiple comparisons post-test showed significant differences between each set of the percent change in the nDW ($^{**}P < 0.01$).

Table 4.2 shows the type of duration tuning curves of 56 neurons plotted before and during GABA application. Similar to what is shown in ***Table 4.1***, the number of band-pass duration tuning neurons progressively increases (from 18% to 25% and then to 34%) while the number of all-pass duration tuning neurons decreases (from 45% to 36% and then to 23%) with PRR before GABA application. The number of short-pass and long-pass duration tuning curves is hardly affected by PRR. Opposite to the effect of bicuculline application, GABA application changes more than half of short- and long-pass duration tuning curves into band-pass ones. The application also changes 62–76% of all-pass duration tuning curves into band- and short-pass duration tuning curves. As such, 46–66% of IC neurons have band-pass duration tuning curves during GABA application. The percent increase in the band-pass duration tuning curves is 28% at 10 pps and 32% at both 30 and 90 pps.

Figure 4.5 PST histograms and duration tuning curves of an IC neuron obtained with three pulse trains before (predrug) and during GABA application. The BF (kHz), latency (ms), MT (dB SPL) and recording depth (μm) of this neuron were 25.5, 11.0, 40, 316 (see ***Figure 4.2*** for legends).

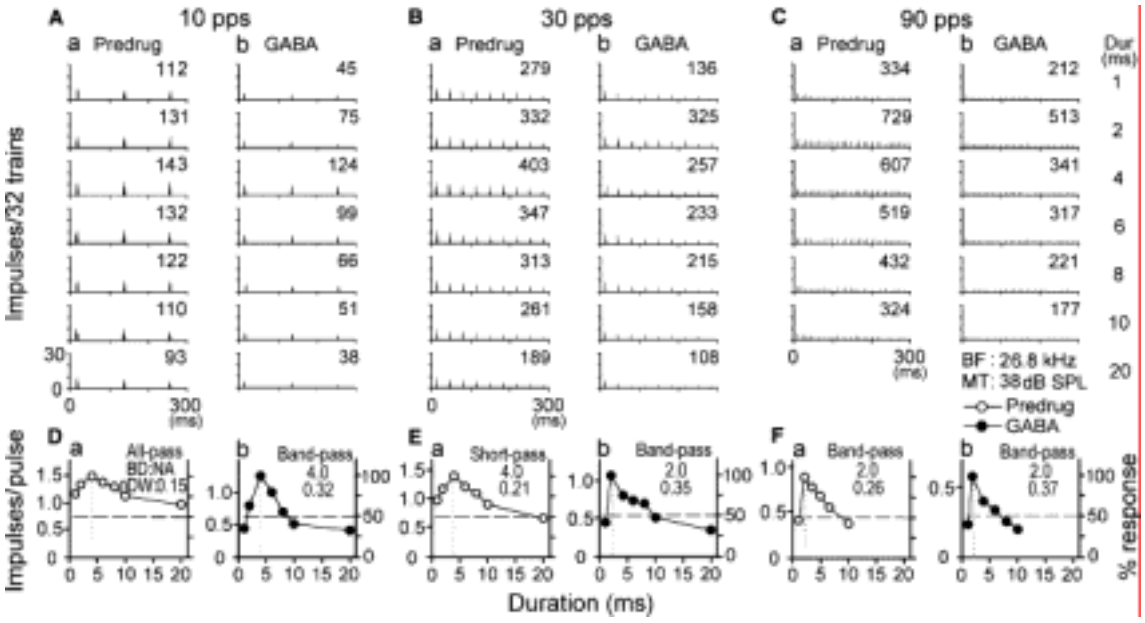

A
10 pps
B
30 pps
C
90 pps
a Predrug
b GABA
Dur (ms)
Impulses/32 trains
112
45
131
75
143
124
132
99
122
66
110
51
93
38
279
136
332
325
403
257
347
233
313
215
281
158
189
108
334
212
729
513
607
341
519
317
432
221
324
177
1
2
4
6
8
10
20
0
300
(ms)
BF : 26.8 kHz
MT: 38 dB SPL
Predrug
GABA
D
E
F
All-pass
BD:NA
DW:0.15
Band-pass
4.0
0.32
Short-pass
4.0
0.21
2.0
0.35
2.0
0.26
2.0
0.37
Impulses/pulse
% response
Duration (ms)

Figure 4.6 Bar histograms showing the average BD and nDW obtained from the duration tuning curves of IC neurons plotted before (unfilled bars) and during (filled bars) GABA application (Aa, Ba). Note that the number of BD and nDW shown in each bar is different because duration tuning curve of IC neurons often change from one type to another with PRR. Note also that the average BD significantly decreased and the nDW increased with increasing PRR only before but not during GABA application (Repeated measures two-way ANOVA; unfilled bars, $P < 0.001$; filled bars, $P > 0.05$.). Ab, Bb: percent change in the average BD and nDW during GABA application. The average percent change of BD and nDW was significantly greater at low than at high PRR (Repeated measures one-way ANOVA; $P < 0.001$) (see text for details).

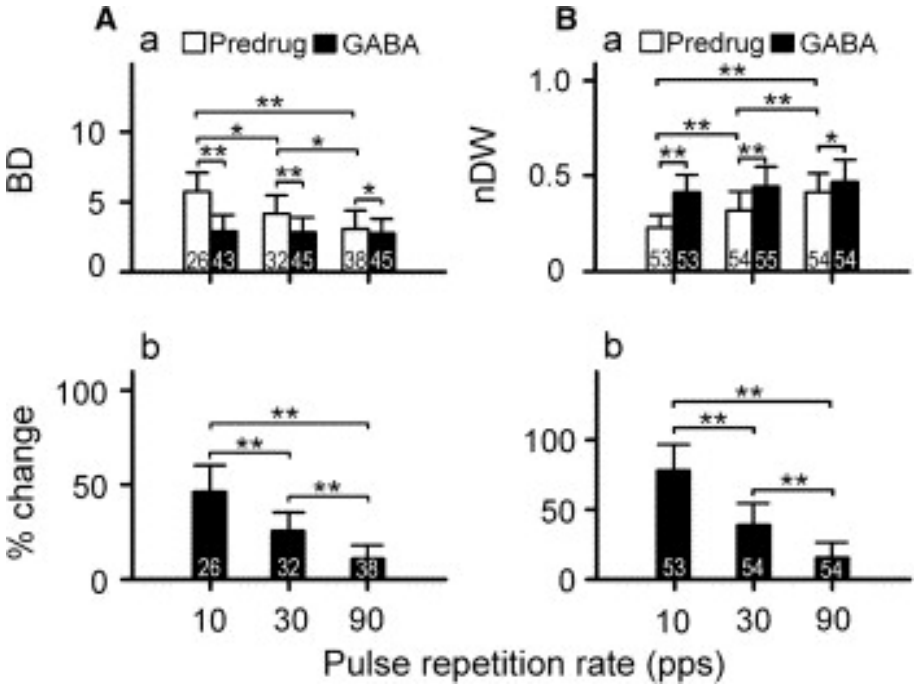

A
a
Predrug
GABA
BD
10
5
0
26 43
32 45
38 45
b
% change
100
50
0
26
32
38
10
30
90
B
a
Predrug
GABA
nDW
1.0
0.5
0
53 53
54 55
54 54
b
100
50
0
53
54
54
10
30
90
Pulse repetition rate (pps)

Table 4.2 The effect of GABA application on duration tuning curves of 56 IC neurons

PRR	Tuning	Predrug	GABA			
(pps)	properties		BP	SP	LP	AP
10	BP	10 (18%)	10			
	SP	16 (29%)	12	4		
	LP	5 (9%)	2		3	
	AP	25 (45%)	2	13	4	6
	Total	56	26 (46%)	17 (30%)	7 (13%)	6 (11%)
30	BP	14 (25%)	14			
	SP	16 (29%)	12	4		
	LP	6 (11%)	3		3	
	AP	20 (36%)	3	9	3	5
	Total	56	32 (57%)	13 (23%)	6 (11%)	5 (9%)
90	BP	19 (34%)	19			
	SP	17 (30%)	12	5		
	LP	7 (13%)	4		3	
	AP	13 (23%)	2	3	3	5
	Total	56	37 (66%)	8 (14%)	6 (11%)	5 (9%)

See ***Table 4.1*** for legends.

Duration selectivity vs recording depth and BF

Throughout the course of study, we typically examined the duration selectivity of IC neurons sequentially isolated within an orthogonally penetrated electrode. This sampling method provided us an opportunity to observe the variation of duration selectivity of sequentially isolated IC neurons. ***Figure 4.7*** shows the duration tuning curves of three representative IC neurons sequentially isolated within an orthogonally penetrated electrode plotted before and during bicuculline application obtained at three PRRs. As described before, the duration selectivity of each neuron increased with PRR as evident by increasing nDW with PRR (***Figure 4.7***, Aa vs. Ab vs. Ac; Ba vs. Bb vs. Be; Ca vs. Cb vs. Cc).

Consonant with previous studies (Jen and Schlegel 1982; Pinheiro et al. 1991; Poon et al. 1990; Wu and Jen 1991), the BF of sequentially isolated neurons increased with recording depth (shown within each panel of ***Figure 4.7***). However, the duration selectivity of sequentially isolated neurons decreased with recording depth as evident by decreasing nDW and lengthening of BD (***Figure 4.7***, Aa vs. Ba vs. Ca; Ab vs. Bb vs. Cb; Ac vs. Be vs. Cc). The duration tuning curves of all three neurons were broadened during bicuculline application such that the nDW decreased. The percent decrease in the nDW became progressively small with recording depth (% decrease shown within each panel of ***Figure 4.7***). This observation indicates that broadening of duration tuning curves of IC neurons during bicuculline application

was greater for neurons at upper than at deep IC.

To quantitatively show this point, we analyzed the scatter plots of the percent change in the nDW of IC neurons during bicuculline application in relation to the recording depth and BF. As shown in ***Figure 4.8***, most neurons with low BF at upper IC had larger percent change in the nDW than most neurons with high BF at deeper IC had. Linear regression analyses showed that the percent decrease in nDW during bicuculline application significantly decreased with the recording depth and BF regardless of the PRR ($P < 0.01–0.05$). However, the change in the nDW was greater when studied at high than at low PRR as shown by the overall higher distribution of data points obtained at high than at low PRR (***Figure 4.8***, Aa vs. Ba vs. Ca; Ab vs. Bb vs. Cb).

Figure 4.7 Duration tuning curves of three IC neurons sequentially isolated within an orthogonally penetrated electrode plotted before (solid circles) and during (unfilled circles) bicuculline application at three PRRs. The BF, MT and nDW of each neuron are shown. Parenthesized percent indicates percent change in nDW during bicuculline application. Right: A sketch showing the coronal section of the midbrain and the recording sites of three IC neurons. A: aqueduct, PVG: paraventricular gray, M: medial, L: lateral, D: dorsal, V: ventral.

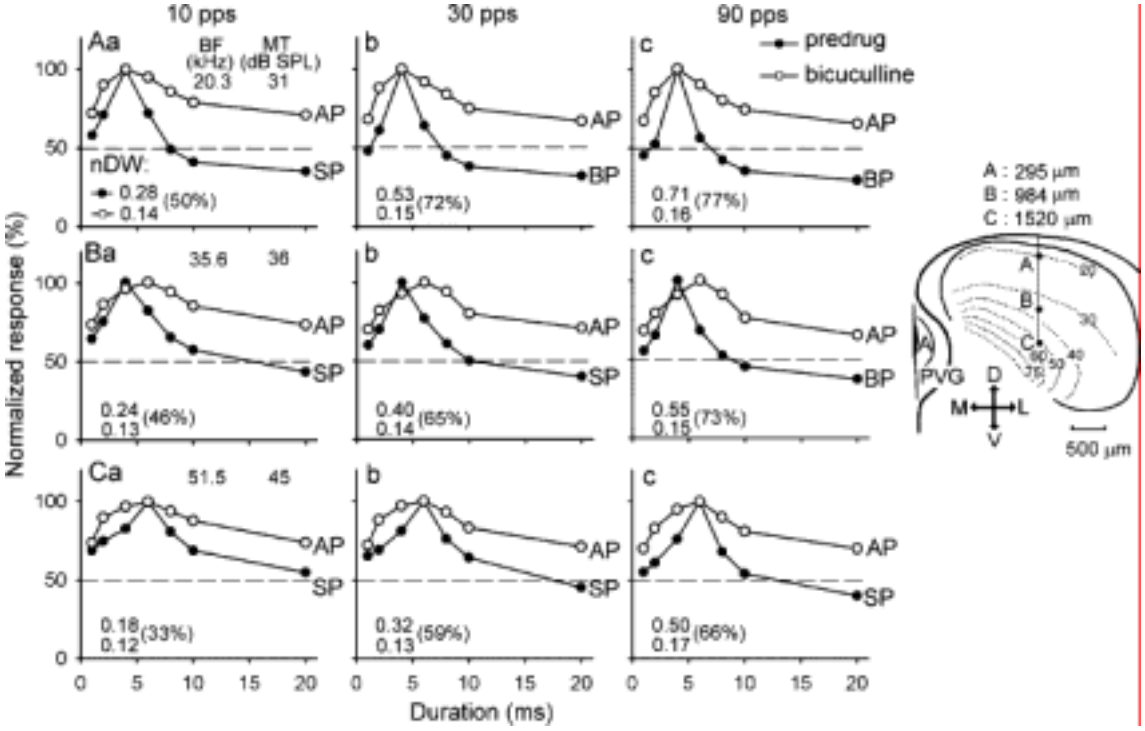
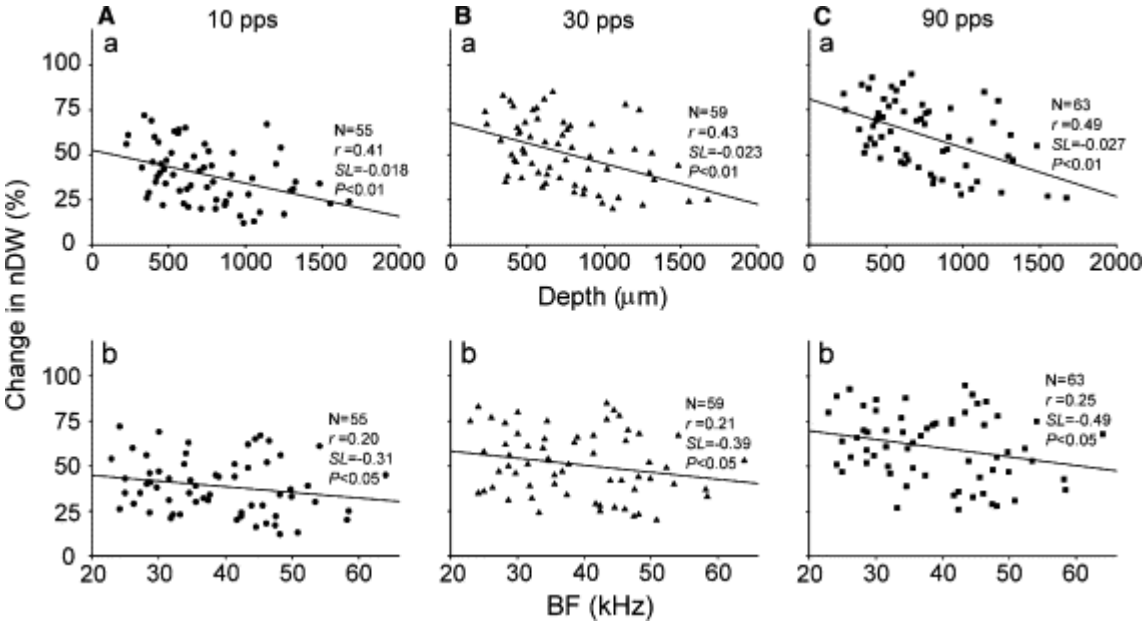

10 pps
30 pps
90 pps
predrug
bicuculline
Aa
BF (kHz) 20.3
MT (dB SPL) 31
nDW:
0.28
0.14 (50%)
0.53
0.15 (72%)
0.71
0.16 (77%)
Ba
35.6
36
0.24
0.13 (46%)
0.40
0.14 (65%)
0.55
0.15 (73%)
Ca
51.5
45
0.18
0.12 (33%)
0.32
0.13 (59%)
0.50
0.17 (66%)
AP
BP
SP
Normalized response (%)
Duration (ms)
A : 295 μm
B : 984 μm
C : 1520 μm
PVG
D
M
L
V
500 μm

Figure 4.8 Scatter plots showing distribution of percent change in the nDW of duration curves of IC neurons in relation to recording depth (Aa, Ba, Ca) and BF (Ab, Bb, Cb) during bicuculline application at three PRRs. The linear regression line and correlation coefficient for each distribution are shown with a solid line and r. N: number of neurons, SL: slope of the regression line, P, significance level (see text for details).


A
10 pps
a
B
30 pps
a
C
90 pps
a
N=55
r=0.41
SL=-0.018
P<0.01
N=59
r=0.43
SL=-0.023
P<0.01
N=63
r=0.49
SL=-0.027
P<0.01
Change in nDW (%)
Depth (μm)
b
b
b
N=55
r=0.20
SL=-0.31
P<0.05
N=59
r=0.21
SL=-0.39
P<0.05
N=63
r=0.25
SL=-0.49
P<0.05
BF (kHz)

Discussion

The role of GABAergic inhibition in improving duration selectivity of IC neurons with PRR

The opposite effect of bicuculline and GABA application on duration tuning curves of IC neurons observed in the present study (i.e., ***Figures 4.2–4.7***) clearly supports the role of GABAergic inhibition in shaping the duration selectivity of IC neurons as reported earlier (Casseday et al. 1994, 2000; Jen and Feng 1999). Sharpening of duration tuning curve by GABAergic inhibition is a result of varying degree of inhibition with pulse duration. This is evident by the observation that the increase in the number of impulses during bicuculline application is greater for non-BD durations than for the BD and near-BD durations (***Figures 4.2-4.3***). Conversely, the decrease in the number of impulses during GABA application is greater for non-BD durations than for the BD and near-BD durations (***Figure 4.5***).

In agreement with our previous study (Jen and Zhou 1999), our data showed that duration selectivity of IC neurons improves with increasing PRR. As an extension of our earlier studies in frequency and directional domains (Jen et al. 2002; Zhou and Jen 2002), we have shown that increasing strength of GABAergic inhibition with PRR contributes to improving duration selectivity. Supporting data include the following.

One, bicuculline application produced more pronounced broadening of duration tuning curves and significantly larger increase in the BD and decrease in the nDW of IC neurons at high than at low PRR regardless of recording depth (***Figures 4.2–4.4*** Ab, Bb and ***4.8***). Two, GABA application produced more pronounced narrowing of duration tuning curves and significantly larger decrease in the BD and increase in the nDW of IC neurons at low than at high PRR (***Figures 4.5*** and ***4.6***, Ab, Bb). Three, significant improvement of duration selectivity with PRR was abolished during both drug applications (***Figure 4.4***, Aa, Ba; ***Figure 4.6***, Aa, Ba).

Possible neural mechanisms underlying increasing GABAergic inhibition with PRR have been proposed in previous studies (Jen et al. 2002; Zhou and Jen 2002). These studies speculate that GABAergic inhibition is either facilitated at a faster rate or depresses at a slower rate than excitation with increasing PRR. As such, the strength of GABAergic inhibition relative to excitation would become progressively stronger with PRR. Because GABAergic inhibition shapes the duration selectivity of IC neurons (Casseday et al. 1994, 2000; Jen and Feng 1999), increasing GABAergic inhibition with PRR would result in sharper duration selectivity at high than at low PRR. If this speculation were true, modulation of duration tuning curves would be larger during bicuculline application but smaller during GABA application at high than at low PRR because of relatively strong GABAergic inhibition at high PRR (***Figure 4.4***, Ab, Bb; ***Figure 4.6***, Ab, Bb). Conversely,

modulation of duration tuning curves would be smaller during bicuculline application but larger during GABA application at low than at high PRR because of relatively weak GABAergic inhibition at low PRR (***Figure 4.4***, Ab, Bb; ***Figure 4.6***, Ab, Bb).

In this study, we only examined the role of GABAergic inhibition in improving the duration selectivity of IC neurons with PRR. Because the extrinsically originated glycinergic inhibition also contributes to multi-parametric response properties of IC neurons (Casseday et al. 2000; Klug et al. 1995; Koch and Grothe 1998; Lu and Jen 2001; LeBeau et al. 1996, 2001), it is conceivable that glycinergic inhibition may also play a role in PRR-dependent duration selectivity. Future works need to be conducted to confirm this speculation.

Duration selectivity vs. recording depth and BF

We observed that the duration tuning curves of tonotopically organized IC neurons progressively broadened with recording depth at all three PRRs (***Figure 4.7***). Furthermore, the degree of broadening of duration tuning during bicuculline application was more pronounced for neurons at upper than at deep IC (***Figures 4.7-4.8***). These observations suggest that neurons at upper IC with low BFs had sharper duration selectivity than neurons in the deeper IC with high BFs. It has been

indicated that the tonotopical organization of IC neurons along the dorso-ventral axis of the IC is based on the topographic projections originated from the cochlea and sequentially ascended through the lower order auditory nuclei (Gelfand 1990). What might be the neural basis underlying the decreasing duration selectivity of IC neurons with recording depth?

A previous study has shown that neurons with $GABA_A$ receptors are mostly distributed in the dorso-medial region of the IC but are sparsely distributed in the ventro-lateral region, which contains mostly glycinergic terminals (Fubara et al. 1996). For this reason, high BF neurons at deep IC would receive less GABAergic inhibition than low BF neurons at upper IC. It is therefore conceivable that bicuculline application would produce a greater degree of change in nDW of duration tuning curves of low BF neurons at upper IC than that of high BF neurons at deeper IC. In sum, our data show that duration selectivity of IC neurons shaped by GABAergic inhibition appears to be systematically organized along the dorso-ventral axis of the IC.

Possible biological relevance of PRR-dependent duration selectivity

During hunting, *Eptesicus fuscus* progressively increase PRR of emitted pulses throughout different hunting phases (Griffin 1958; Simmons et al. 1979; Surlykke

and Moss 2000). We have previously suggested that increasing PRR not only enables the bat to obtain as much information as possible about the localized prey from analysis the echoes but also sharpens multi-parametric echo selectivity for accurate prey capture (Jen and Zhou 1999; Jen et al. 2001, 2002; Zhou and Jen 2002; Wu and Jen 1996). In the present study, we show that duration selectivity of IC neurons improves and the BD shortens with increasing PRR (***Figures 4.4*** and **4.6**). These observations suggest that increasing PRR and shortening duration of emitted pulses by the bat during hunting would facilitate echo duration recognition. This dynamic aspect of echo processing has also been shown in frequency, amplitude and direction domains (Jen and Zhou 1999; Jen et al. 2001; Smalling et al. 2001; Galazyuk et al. 2000; Wu and Jen 1996; Zhou and Jen 2002).

In summary, we have shown that the duration selectivity of most IC neurons improves with increasing PRR. Bicuculline application produces more pronounced decrease in duration selectivity of IC neurons at high than at low PRR. Conversely, GABA application produces more pronounced increase in duration selectivity of IC neurons at low than at high PRR. This differential GABAergic inhibition abolishes PRR-dependent duration selectivity of IC neurons. This finding is in parallel with our previous studies on PRR-dependent directional and frequency selectivity of IC neurons in the same bat species (Jen et al. 2002; Zhou and Jen 2002).

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CHAPTER 5

GABA-MEDIATED ECHO DURATION SELECTIVITY OF BAT INFERIOR COLLICULAR NEURONS DETERMINED WITH SINGLE PULSES AND PULSE–ECHO PAIRS

Abstract

When insectivorous bats such as *Eptesicus fuscus* emit ultrasonic signals and analyze the returning echoes to hunt insects, duration selectivity of auditory neurons plays an important role in echo recognition. The success of prey capture indicates that they can effectively encode progressively shortened echo duration throughout the hunting process. The present study examines the echo duration selectivity of neurons in the central nucleus of the bat inferior colliculus (IC) under stimulation conditions of single pulses and pulse–echo (P–E) pairs. This study also examines the role of gamma-aminobutyric acid (GABA)ergic inhibition in shaping echo duration selectivity of IC neurons. The data obtained show that the echo duration selectivity of IC neurons is sharper when determined with P–E pairs than with single pulses. Echo duration selectivity also sharpens with shortening of pulse duration and P–E gap. Bicuculline application decreases and GABA application increases echo duration selectivity of IC neurons. The degree of change in echo duration selectivity progressively increases with shortening of pulse duration and P–E gap during bicuculline application while the opposite is observed during the

GABA application. These data indicate that the GABAergic inhibition contributes to sharpening of echo duration selectivity of IC neurons and facilitates echo recognition by bats throughout different phases of hunting.

Introduction

In the central auditory pathway, the central nucleus of the inferior colliculus (IC) receives and integrates excitatory and inhibitory inputs from many lower auditory nuclei and from the auditory cortex (Casseday and Covey 1995; Herbert et al. 1991; Huffman and Henson 1990; Saldana et al. 1996; Winer et al. 1998). During signal processing, temporal and spectral interaction of these two opposing inputs shapes response properties of IC neurons. Presumably, inhibition only occurs when inhibitory inputs arrive prior to the excitatory inputs at IC neurons within a certain temporal window. Furthermore, inhibitory inputs with stronger intensity and longer duration should be more effective in inhibition of auditory response of IC neurons than inhibitory inputs with weaker intensity and shorter duration (Lu and Jen 2002, 2003).

One of the major inhibitory inputs in the IC is mediated by GABA (Fubara et al. 1996; Roberts and Ribak 1987). By means of application of GABA or bicuculline, which is an antagonist for $GABA_A$ receptors (Bormann 1988; Cooper et al. 1982),

many studies have shown that interaction of excitation and GABAergic inhibition contributes to auditory temporal processing and shapes multi-parametric selectivity (e.g., duration, frequency, amplitude, direction, etc.) of IC neurons using single repetitive sound pulses or temporally patterned trains of sound pulses (Casseday et al. 1994, 2000; Faingold et al. 1991; Jen and Feng 1999; Jen and Zhang 2000; Jen et al. 2002; Klug et al. 1995; Koch and Grothe 1998; LeBeau et al. 2001; Lu et al. 1997, 1998; Lu and Jen 2001; Park and Pollak 1993; Yang et al. 1992; Vater et al. 1992).

Among the multi-parametric selectivity, duration selectivity of auditory neurons plays an important role for sound recognition particularly in human speech, animal communication and bat echolocation (Covey and Casseday 1999; Popper and Fay 1995; Shannon et al. 1995). For example, the big brown bat, *Eptesicus fuscus*, must effectively analyze the changing echo features including echo duration throughout the entire course of hunting for successful prey capture. The neural basis underlying the duration selectivity of bats has been supported by many studies which show that bat's IC neurons behave as band-, short-, long- and all-pass filters to sound duration (Casseday et al. 1994, 2000; Ehrlich et al. 1997; Faure et al. 2003; Fremouw et al. 2005; Fuzessery and Hall 1999; Galazyuk and Feng 1997; Jen and Feng 1999; Jen and Zhou 1999; Pinheiro et al. 1991; Zhou and Jen 2001).

However, these studies examined duration selectivity of IC neurons using single sound pulses or temporally patterned pulse trains that consist of sound pulses

with equal amplitude and inter-pulse gap. Yet, in the real world the bat emits intense pulses and listens to greatly attenuated returning echoes in which the gap between the pulse and echo (abbreviated as P–E gap) is always shorter than the gap between two succeeding P–E pairs. Therefore, in reality the bat analyzes the echo from a series of P–E pairs of unequal amplitude with progressively shortening P–E gap during hunting. For this reason, a study of variation of echo duration selectivity of the bat auditory neurons with shortening of P–E gap is necessary for better understanding of echo recognition by bats during different phases of hunting.

The main objective of the present study is to examine the echo duration selectivity of IC neurons of the big brown bat to show the following. (1) Echo duration selectivity of bat IC neurons is sharper when determined with the echo pulses of P–E pairs than with temporally isolated single pulses. (2) Echo duration selectivity of IC neurons progressively improves throughout a target approaching sequence. (3) GABAergic inhibition shapes the echo duration selectivity of IC neurons.

Materials and methods

Animals and surgery

Ten *E. fuscus* (6 males, 4 females, 10–27 g, body weight, b.w.) were used for

this study. As described in previous studies (Jen et al. 1987), the flat head of a 1.8 cm nail was glued onto the exposed skull of each Nembutal anesthetized bat (45–59 mg/kg b.w.) with acrylic glue and dental cement 1 or 2 days before the recording session. Exposed tissue was treated with an antibiotic (Neosporin) to prevent inflammation. During recording, the bat was administered the neuroleptanalgesic, Innovar-Vet (Fentanyl 0.08 mg/kg b.w. Droperidol 4 mg/kg b.w.), and placed inside a bat holder (made of wire mesh) that was suspended in an elastic sling inside a double-wall sound-proof room (temperature 28–30°C). The ceiling and inside walls of the room were covered with 3-in. convoluted polyurethane foam to reduce echoes. After fixing the bat's head with a set screw, small holes were made in the skull above the IC for insertion of 3 M KCl glass pipette electrodes (impedance: 5–10 MΩ). Additional doses of Innovar-Vet were administered during later phases of recording when bats showed signs of discomfort. A local anesthetic (Lidocaine) was applied to the open wound area. The recording depth was read from the scale of a microdrive (David Kopf). A common indifferent electrode (silver wire) was placed at the nearby temporal muscles. Each bat was used in one to five recording sessions on separate days and each recording session typically last for 2–6 h. The experiments were conducted according to NIH publication no. 85–23, "Principles of Laboratory Animal Care" and with the approval of the Institutional Animal Care and Use Committee of the University of Missouri-Columbia.

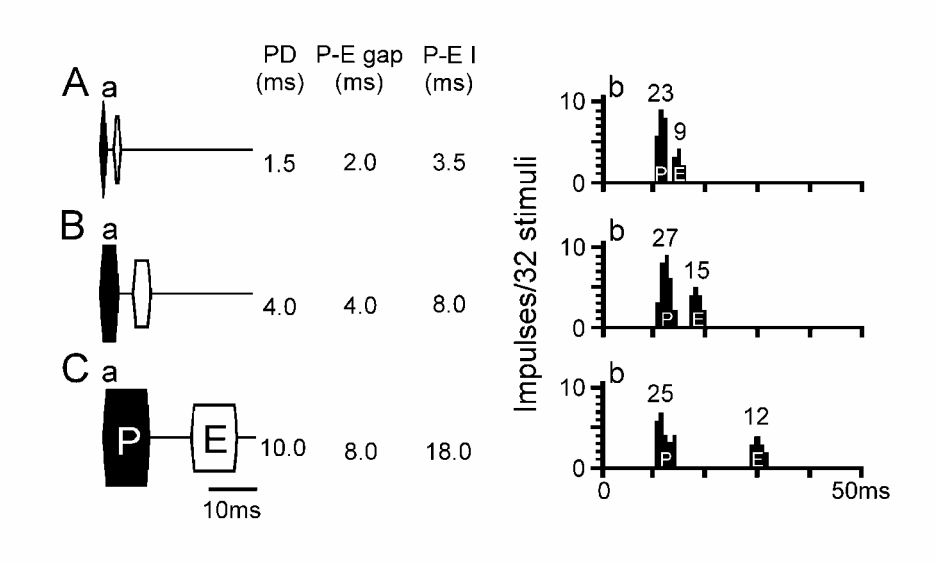
Acoustic stimulation

Acoustic stimuli (4 ms with 0.5 ms rise–decay times, delivered at 2 pulses/s) were generated with an oscillator (KH model 1200) and a homemade electronic switch driven by a stimulator (Grass S88). These stimuli were then amplified after passing through a decade attenuator (HP 350D) before they were fed to a small condenser loudspeaker (AKG model CK 50, 1.5 cm diameter, 1.2 g) that was placed 23 cm away from the bat and 30° contralateral to the recording site. Calibration of the loudspeaker was performed with a ¼-in. microphone (B & K 4135) placed at the position of the bat's head during recording using a measuring amplifier (B & K 2607). The output of the loudspeaker was expressed in dB SPL in reference to 20 µPa root mean square.

Upon isolation of an IC neuron with 4 ms sound pulses, its best frequency (BF) was determined by changing the pulse frequency and intensity. The minimum threshold (MT) at the BF was defined as the sound level that elicited 50% response probability from the neuron. The echo duration selectivity of the IC neuron was studied by plotting its duration tuning curves using the number of impulses in response to eight durations (1, 1.5, 2, 4, 6, 8, 10 and 20 ms) of single pulses and the echo pulses of three P–E pairs. Rise–decay times for these different single pulses and echo durations were typically 0.5 ms but they were 0.25 ms for 1-ms pulse duration. The amplitude of single echo pulses was set at 10 dB above the MT while the

amplitude of pulse and echo was, respectively, set at 30 and 10 dB above the neuron's MT as used in previous studies (Suga et al. 1983; Tanaka et al. 1992; Wong et al. 1992). The left panel of ***Figure 5.1*** shows the sketches of three P–E pairs with duration, gap (time period between the offset of pulse and onset of echo) and interval (time period between onset of pulse and echo) used in this study. These three P–E pairs are comparable to the P–E pairs occurring during search, approach and terminal phases of hunting by *E. fuscus* (Griffin 1958; Surlykke and Moss 2000). When these three P–E pairs were used to study the echo duration selectivity, the pulse was always fixed at a constant value (i.e., 1.5, 4.0 or 10 ms) while the echo duration was varied at eight different durations. Because these bat species uses frequency modulated (FM) pulses during echolocation, we also studied echo duration selectivity of IC neurons using downward sweeping FM P–E pulses generated by means of ramp signals. Each FM pulse swept one octave downward across the BF of the IC neuron.

Figure 5.1 A *a*, B *a*, C *a* Sketches showing the envelops of three pulse–echo (P–E) pairs with P duration (*PD*), P–E gap and P–E interval (*P–E I*) comparable to that occurring during search (C *a*), approach (B *a*) and terminal phases (A *a*) of hunting by the big brown bat, *E. fuscus*. The pulse and echo were set at 30 and 10 dB above the minimum threshold (MT) of each investigated inferior collicular (IC) neuron. A *b*, B *b*, C *b* Peri-stimulus-time (PST) histograms (bin width: 500 μs and sampling period: 50 ms) showing the discharge patterns of an IC neuron obtained with 32 presentations of three P–E pairs. The neuron's number of impulses in response to each pulse (P) and echo (E) are shown *atop*


PD (ms)
P-E gap (ms)
P-E I (ms)
A a
1.5
2.0
3.5
B a
4.0
4.0
8.0
C a
P
E
10.0
8.0
18.0
10ms
Impulses/32 stimuli
b
23
9
27
15
25
12
10
0
50ms

Iontophoresis and recording

Iontophoretic application of bicuculline and GABA to recorded IC neurons has been described in previous studies (Lu et al. 1997, 1998). Briefly, a three-barrel or five-barrel electrode (tip: 10–15 μm) was piggybacked to a 3 M KCl single-barrel electrode (tip: less than 1 μm; impedance: 5–10 MΩ) whose tip was extended about 10 μm from the tip of the three-barrel electrode. The 3 M KCl single-barrel recording electrode was used to record neural responses. One of the barrels of the three-barrel electrode was filled with bicuculline methiodide (10 mM in 0.16 m NaCl, pH 3.0; Sigma) or gamma-aminobutyric acid (GABA, 500 mM in distilled water, pH 3.5; Sigma). However, when a five-barrel electrode was used, two barrels were filled with both drugs, respectively, such that both drugs could be applied to the recorded neuron. The bicuculline and GABA were prepared just prior to each experiment and the electrode filled immediately before use. The drug channel was connected via silver–silver chloride wire to a microiontophoresis constant current generator (Medical Systems Neurophore BH-2) that was used to generate and monitor iontophoretic currents. During drug application, a 1-s pulse of positive 40 nA at 0.5 pps was applied for 1 min before data acquisition. The application current was changed to 10 nA during data acquisition. The other two barrels were filled with 1 M NaCl (pH 7.4), one of which was used as the ground and another as the balanced barrel. The balance electrode was connected to a balance module. The

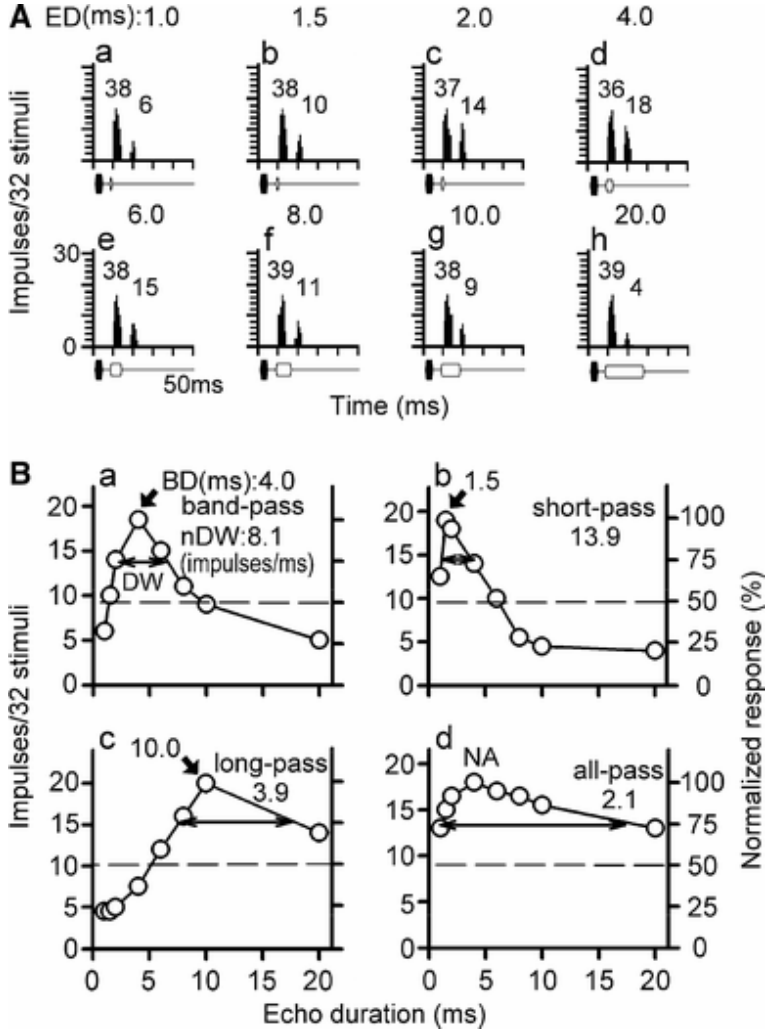
retaining current was negative 8–10 nA.

Recorded action potentials were amplified, band-pass filtered (Krohn-Hite 3500), and then fed through a window discriminator (WPI 121) before being sent to an oscilloscope (Tektronix 5111) and an audio monitor (Grass AM6). They were then sent to a computer (Gateway 2000, 486) for acquisition of peri-stimulus-time (PST) histograms (bin width: 500 μs and sampling period: 300 ms) to 32 presentations of stimuli. The right panel of ***Figure 5.1*** shows the PST histograms and the number of impulses of a representative IC neuron obtained with the three P–E pairs.

Data analysis

When IC neurons received drug application, their echo duration tuning curves were plotted before and during drug application. The tuning properties of a duration tuning curve were expressed with a best duration (BD) and a normalized duration width (nDW) (see ***Figure 5.2***). These two duration tuning properties of IC neurons obtained under different stimulation conditions were then quantitatively studied and statistically compared using repeated measures one-way or two-way ANOVA followed with a Student–Newman–Keuls multiple comparisons post-test at $P < 0.05$.

Figure 5.2 A *a–h* PST histograms showing the discharge pattern of an IC neuron determined with P–E pairs. The envelope of each P–E pair is shown below the PST histogram. The PD was always 4 ms while the echo duration (*ED*) varied from 1 to 20 ms. B *a–d* Four types of *ED* tuning curves plotted with the number of impulses discharged to the echo of each P–E pair against *ED*. These *ED* tuning curves are described as band pass (B *a*), short pass (B *b*), long pass (B *c*) and all pass (B *d*). Each *horizontal dashed line* indicates the 50% maximal response. The sharpness of each *ED* curve is expressed with a best duration (*BD* indicated with an *arrowhead*) and a normalized duration width (*nDW* indicated with a *double arrowhead*) of an *ED* tuning curve at 75% of maximum. *NA* a *BD* is not available. The BF (kHz), latency (ms), MT (dB SPL) and recording depth (μm) of these neurons were 36.9, 11.5, 37.0, 438 (B *a*); 34.6, 12.0, 36.0, 334 (B *b*); 38.6, 12.5, 41.0, 753 (B *c*); and 42.5, 13.5.0, 44.0, 1,286 (B *d*) (see the text for details)


A
ED(ms):1.0
1.5
2.0
4.0
a
b
c
d
38 6
38 10
37 14
36 18
6.0
8.0
10.0
20.0
e
f
g
h
38 15
39 11
38 9
39 4
30
0
50ms
Impulses/32 stimuli
Time (ms)
B
a
BD(ms):4.0
band-pass
nDW:8.1
(impulses/ms)
DW
b
1.5
short-pass
13.9
c
10.0
long-pass
3.9
d
NA
all-pass
2.1
Impulses/32 stimuli
Normalized response (%)
Echo duration (ms)

Results

In this study, 98 IC neurons were isolated at depths between 119 and 1,898 μm. Their BFs and MTs ranged 20.3–73.9 kHz (36.5 ± 7.4 kHz) and 18–55 dB SPL (42.5 ± 7.1 dB SPL). The first-spike latencies were between 9 and 22 ms (12.6 ± 1.7 ms). Echo duration selectivity was studied for all 98 neurons. However, echo duration selectivity of only 54 neurons was studied during drug application because of loss of neurons throughout the course of study. Among them, 22 neurons received bicuculline application and 14 neurons received GABA application. The remaining 18 received bicuculline application first. After recovery from the drug effect, they then received GABA application. Therefore, echo duration selectivity was studied in 40 neurons during bicuculline application and in 32 neurons during GABA application.

In the following, we first describe different types of echo duration tuning curves and compare the echo duration selectivity of IC neurons determined with BF and FM pulses. We then compare echo duration selectivity of IC neurons determined with single pulses and echo pulses of P–E pairs. The description is followed with a presentation of the effect of bicuculline and GABA application on echo duration selectivity. Finally, we describe the relationship between echo duration selectivity, BF and recording depth. For convenience of description and comparison, we use the term "echo duration selectivity" to describe the duration

selectivity of IC neurons determined with both single pulses and echo pulses of P–E pairs.

Echo duration tuning curves of IC neurons

The discharge patterns of a representative IC neuron obtained with P–E pairs of 4 ms pulse at varied echo durations are shown in ***Figure 5.2***A. The neuron's number of impulses in response to the 4 ms pulse did not differ by three pulses while its number of impulses in response to different echo durations varied as many as 14 impulses. This neuron's echo duration tuning curve is shown in ***Figure 5.2***Ba.

In total, 928 echo duration tuning curves were plotted with the number of impulses discharged to single echo pulses and to the echo pulses of P–E pairs under different stimulation conditions. These duration tuning curves can be described as band-, short-, long- and all-pass using the same criterion adopted in previous studies (Jen and Feng 1999; Jen and Zhou 1999; Wu and Jen 2006). The band-pass duration tuning curves showed a maximal number of impulses to a specific duration and the maximal number of impulses decreased at least 50% at both limbs (***Figure 5.2***, Ba, $n = 215$, 23%). The short-pass duration tuning curves showed a maximal number of impulses to a short duration and the maximal number of impulses decreased more than 25% at a short duration and more than 50% at a long duration

(***Figure 5.2***, Bb, *n* = 195, 21%). Conversely, the long-pass duration tuning curves showed a maximal number of impulses to a long duration and the maximal number of impulses decreased more than 25% at a long duration and more than 50% at a short duration (***Figure 5.2***, Bc, *n* = 104, 11%). The number of impulses of all-pass echo duration tuning curves often differed by more than 25% but never by more than 50% at all durations tested (***Figure 5.2***, Bd, *n* = 414, 45%).

In this study, the pulse duration that elicited the maximal number of impulses in the band-, short- and long-pass duration tuning curves is defined as the BD (indicated by an arrowhead in ***Figure 5.2***, Ba–c). For convenience of description, IC neurons with any of these three types of duration tuning curves are called duration selective neurons. Conversely, IC neurons with all-pass duration tuning curves are called duration non-selective neurons.

Because the maximal number of impulses in response to the preferred pulse duration varied greatly among individual IC neurons and during drug application, we used a nDW to express the sharpness of a duration tuning curve. An nDW was obtained by dividing the maximum by the width of a duration tuning curve at 75% of the maximum (***Figure 5.2***, Ba–d, DW indicated by a double arrowhead). By obtaining the nDW, we exclude the possibility that a change in the duration width of echo duration tuning curves plotted under different stimulation conditions might only reflect a change in response size to presented echo pulses rather than a true

change in echo duration selectivity. Thus, a neuron with a large nDW has a narrow duration tuning curve and sharp duration selectivity. Every band-, short- and long-pass echo duration tuning curve has an nDW. Although an all-pass echo duration curve is duration non-selective according to our criterion, an all-pass echo duration curve may have an nDW when the maximum decreases by more than 25% at the two limbs (e.g., ***Figure 5.2***, Bd). As described below, this nDW becomes useful to compare the echo duration selectivity of IC neurons that changed from duration non-selective into duration selective during GABA application.

Echo duration selectivity of IC neurons determined with BF and FM echo pulses

To determine if echo duration selectivity of IC neurons might differ when determined with pure tone pulses and FM pulses, we compared echo duration selectivity of 38 IC neurons determined with P–E pairs of BF pulses and FM pulses. As shown in ***Figure 5.3***, similar discharge patterns and number of impulses were obtained from a representative IC neuron with P–E pairs (pulse: 1.5 ms, P–E gap: 2 ms) of BF and FM pulses. As such, the neuron had similar echo duration tuning curve when plotted with the number of impulses in response to BF and FM echo pulses in varied duration (***Figure 5.3***, A2a vs. B2a). In the same token, the neuron's duration tuning curves obtained with the other two P–E pairs (pulse: 4 or 10 ms, P–E

gap: 4 or 8 ms) were also similar (***Figure 5.3***, A2b, c vs. B2b, c). Overall, the neuron's duration selectivity obtained with both BF and FM echo pulses decreased with lengthening of PD and P–E gap as evident by broadened duration tuning curves, lengthened BD and decreased nDW (***Figure 5.3***, A2, B2).

The average BD of these 38 IC neurons obtained with both BF and FM pulses significantly increased and the average nDW decreased with lengthening of pulse duration (PD) and P–E gap (***Figure 5.3***, Ca, b, repeated measures two-way ANOVA; $P < 0.01$ for unfilled and filled bars). A Student–Newman–Keuls multiple comparisons post-test showed significant differences between each set of the BD and the nDW (**$P < 0.01$ and *$P < 0.05$). However, the BD and nDW obtained from BF and FM pulses did not differ significantly (***Figure 5.3***, Ca, b, repeated measures two-way ANOVA; $P > 0.05$ between filled and unfilled bars).

As shown in ***Table 1***, the percent distribution of all four types of duration tuning curves of these 38 IC neurons obtained from BF and FM pulses is very similar. The number of band- and short-pass echo duration tuning curves decreases while that of long- and all-pass echo duration tuning curves increases with lengthening of PD and P–E gap.

Because echo duration selectivity of IC neurons determined with BF and FM echo pulses did not differ significantly and generation of FM pulses at varied duration was time consuming such that recorded IC neurons often lost before

completion of study, we only used BF echo pulses to study duration selectivity of the remaining 60 neurons. The following presentation therefore is based on the data collected from all 98 IC neurons studied with BF pulses.

Figure 5.3 A1, B1 The PST histograms of an IC neuron in response to three P–E pairs of best frequency (*BF*) tones and frequency modulated (*FM*) pulses. The *PD* and P–E gap were both 4 ms while the echo duration (*ED*) varied between 1 and 20 ms. The *BF* (kHz), latency (ms), MT (dB SPL) and recording depth (μm) of the neuron were 32.5, 10.5, 35.0 and 424. A2, B2 The neuron's echo duration tuning curves plotted with the number of impulses in response to varied echo durations of these three P–E pairs of *BF* and *FM* pulses. C *Bar histograms* showing the average *BD* and *nDW* of echo duration tuning curves determined with three P–E pairs of BF (*unfilled bars*) and FM pulses (*filled bars*) (see the text for details)

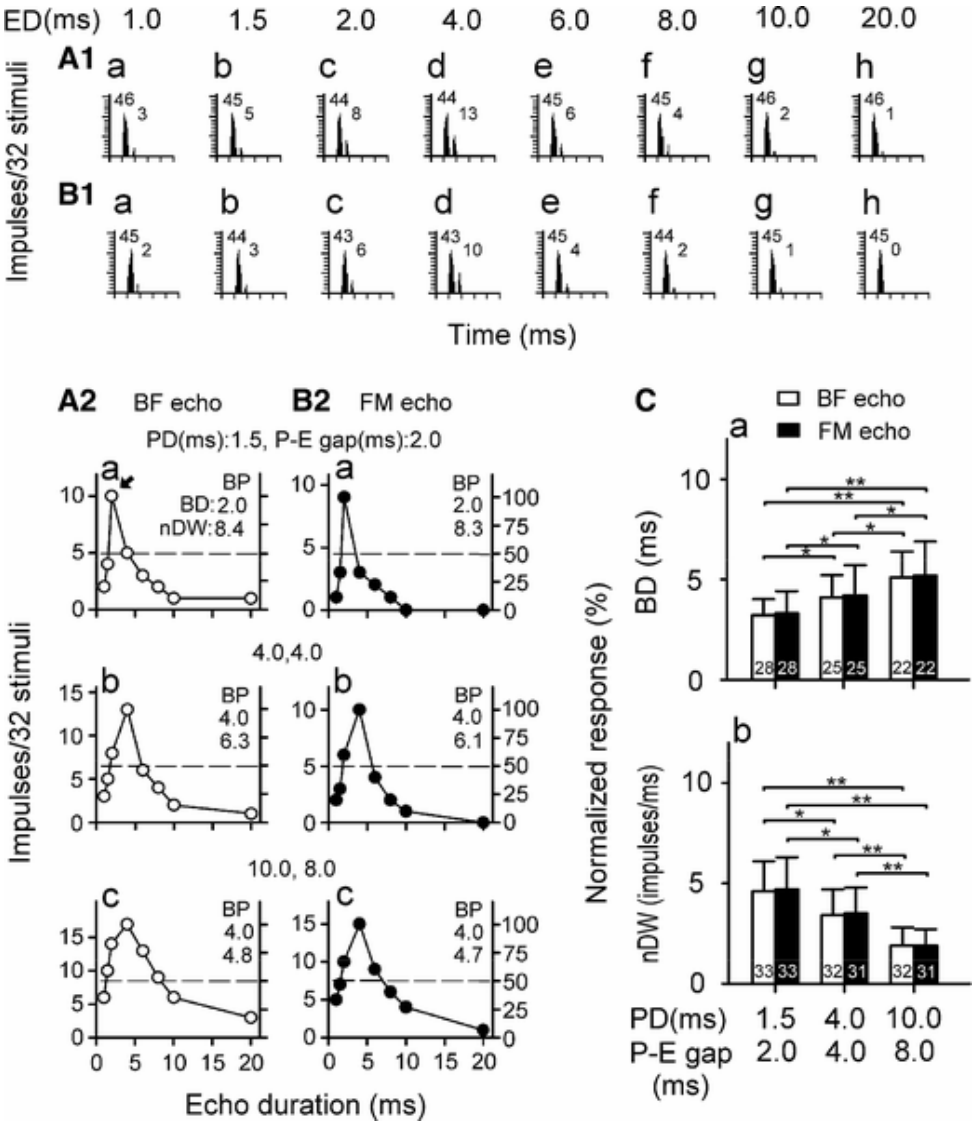

ED(ms) 1.0 1.5 2.0 4.0 6.0 8.0 10.0 20.0
A1
B1
Impulses/32 stimuli
Time (ms)
A2 BF echo
B2 FM echo
PD(ms):1.5, P-E gap(ms):2.0
BD:2.0
nDW:8.4
4.0,4.0
10.0, 8.0
Impulses/32 stimuli
Echo duration (ms)
C
BF echo
FM echo
BD (ms)
nDW (impulses/ms)
Normalized response (%)
PD(ms) 1.5 4.0 10.0
P-E gap 2.0 4.0 8.0
(ms)

Table 5.1 Echo duration tuning curves of 38 IC neurons plotted with the number of impulses discharged to echoes of BF and FM pulse–echo (P–E) pairs

PD (ms)	P-E gap (ms)	stimuli	type of duration tuning curves			
			BP	SP	LP	AP
1.5	2	BF	14 (37%)	12 (32%)	2 (5%)	10 (26%)
		FM	15 (39%)	11 (29%)	2 (5%)	10 (26%)
4	4	BF	12 (32%)	11 (29%)	2 (5%)	13 (34%)
		FM	13 (34%)	10 (26%)	2 (5%)	13 (34%)
10	8	BF	9 (24%)	10 (26%)	3 (10%)	16 (42%)
		FM	10 (26%)	10 (26%)	3 (10%)	15 (39%)

BP: Band-pass; SP: Short-pass; LP: Long-pass; AP: All-pass.

Echo duration selectivity of IC neurons determined with echo pulses of P–E pairs and single echo pulses

IC neurons had sharper echo duration selectivity when determined with echo pulses of P–E pairs than with single echo pulses. As shown in ***Figure 5.4***, a representative IC neuron always discharged more impulses to 4 ms pulse than to 1.5 and 10 ms pulses regardless of the variation in echo duration at each P–E pair. However, the neuron always discharged fewer impulses to echo pulses of P–E pairs than to single echo pulses (***Figure 5.4***, A–C vs. D). When stimulated with three P–E pairs, the neuron's number of impulses discharged to each echo duration became larger with lengthening of P–E gap (***Figure 5.4***, A–C). The neuron discharged maximally to 4 ms BD echo pulses and the maximum progressively decreased as the echo duration became shorter or longer than the BD. The decrease from the maximum was larger for shorter than for longer pulse duration and P–E gap. For example, when the 4 ms echo pulse increased to 6 ms, the number of impulses decreased from 8 to 3 (−62.5%) when tested with 1.5 ms pulse at 2 ms P–E gap; decreased from 12 to 6 (−50%) when tested with 4 ms pulse at a 4 ms P–E gap and decreased from 16 to 12 (−25%) when tested with 10 ms pulse at 8 ms P–E gap (***Figure 5.4***, A–C, at echo duration of 4 vs. 6 ms). As such, the neuron's echo duration selectivity progressively increased with shortening of pulse duration and P–E gap as evident by increasing nDW (***Figure 5.4***, E–G). However, the neuron had the least duration selectivity when obtained with single echo pulses (***Figure 5.4***, H). The

neuron had a short-pass duration tuning curve when obtained with single echo pulses but had a band-pass duration tuning curve when obtained with all three P–E pairs (***Figure 5.4***, E–H). The neuron had a BD of 4 ms when obtained under all four stimulation conditions.

Figure 5.5 shows the discharge patterns and echo duration tuning curves of another IC neuron with varied BD under four different stimulation conditions. This neuron also discharged more impulses to single echo pulses than to echo pulses of P–E pairs (***Figure 5.5***, A–C vs. D). When stimulated with three P–E pairs, the neuron always discharged more impulses to 10 ms pulses than to 4 and 1.5 ms pulses. The neuron's number of impulses discharged to each echo pulses became smaller with shortening of pulse duration and P–E gap (***Figure 5.5***, A–C). The decrease from the maximum obtained at BD was greater for shorter than for longer pulse duration and P–E gap (***Figure 5.5***, A vs. B vs. C). As such, the neuron's echo duration selectivity progressively increased with shortening of pulse duration and P–E gap as evident by increasing nDW (***Figure 5.5***, E–G). The neuron had an all-pass echo duration tuning curve and was duration non-selective when obtained with single echo pulses (***Figure 5.5***, H). When determined with three P–E pairs, the neuron's short-pass duration tuning curve obtained at 8 ms P–E gap changed into band-pass at shorter P–E gap and the BD shortened from 8 to 4 ms (***Figure 5.5***, E–G).

Among the 98 IC neurons studied, 45 neurons had BDs between 1.5 and

10 ms covering the duration of pluses emitted by *E. fuscus* during three phases of hunting. The BD of 27 (60%) neurons did not change (e.g., ***Figure 5.4***) while the BD of other 18 (40%) neurons shortened with decreasing pulse duration and P–E gaps (e.g., ***Figure 5.5***).

The distribution of four types of echo duration tuning curves obtained with echo pulses of three P–E pairs and single echo pulses is shown in ***Table 2***. It is clear that more duration selective neurons were obtained with the echo pulses of P–E pairs than with single echo pulses. It is also clear that many all-pass duration non-selective neurons obtained with single echo pulses changed into band-pass duration selective neurons when obtained with echo pulses of P–E pairs. As shown in ***Figure 5.6***, the number of duration selective neurons progressively decreases while the number of duration non-selective neurons increases with lengthening of pulse duration and P–E gap of the P–E pairs. The variation reaches the maximum when obtained with single echo pulses (***Figure 5.6***, a, filled vs. unfilled circles). As such, the difference between the number of duration selective and non-selective neurons progressively decreases with these different stimulation conditions (***Figure 5.6***, a, dashed line).

Variation in the sharpness of echo duration tuning curves under these four different stimulation conditions can be seen in the variation of the BD and nDW of these IC neurons. The average BD significantly increases and nDW decreases with

lengthening of pulse duration and P–E gap of the P–E pairs and the variation reaches the maximum when stimulated with single echo pulses (***Figure 5.6***, b, c, repeated measures one-way ANOVA, $P < 0.01$). Newman–Keuls multiple comparisons post-test showed significant differences between each set of the BD and the nDW (***$P < 0.001$, **$P < 0.01$ and *$P < 0.05$).

Figure 5.4 A–D PST histograms of an IC neuron obtained with three P–E pairs and single echo pulses. The *PD*, *ED* and P–E gap are, respectively, shown. The BF (kHz), latency (ms), MT (dB SPL) and recording depth (μm) of the neuron were 33.8, 10.5, 37.0 and 446. E–H The neuron's echo duration tuning curves determined with the echo pulses of three P–E pairs and with single echo pulses. The duration tuning properties, the *BD* and the *nDW* are shown within each plot. Note that the IC neuron always had a *BD* of 4 ms when determined at all four stimulation conditions. The neuron had the smallest *nDW* when determined with single echo pulses. The *nDW* progressively increased with shortening of *PD* and P–E gap when tested with three P–E pairs

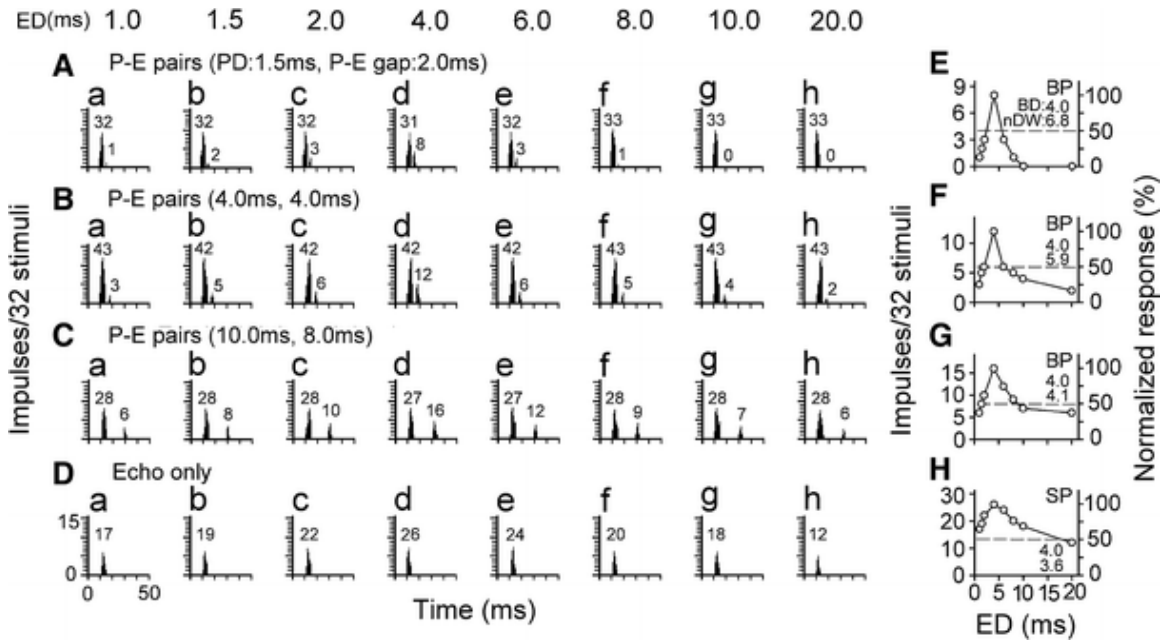

ED(ms) 1.0 1.5 2.0 4.0 6.0 8.0 10.0 20.0
A P-E pairs (PD:1.5ms, P-E gap:2.0ms)
B P-E pairs (4.0ms, 4.0ms)
C P-E pairs (10.0ms, 8.0ms)
D Echo only
Impulses/32 stimuli
Time (ms)
E
F
G
H
BP
BD:4.0
nDW:6.8
4.0
5.9
4.0
4.1
SP
4.0
3.6
Impulses/32 stimuli
Normalized response (%)
ED (ms)

Figure 5.5 The echo duration selectivity of another IC neuron determined with three P–E pairs and single echo pulses. The BF (kHz), latency (ms), MT (dB SPL) and recording depth (μm) of the neuron were 37.6, 12.5, 41.0 and 896. Note that the neuron also had the smallest *nDW* when determined with single echo pulses. When determined with three P–E pairs, the *nDW* progressively increased and the *BD* decreased from 8 to 4 ms with shortening of *PD* and P–E gap (see ***Figure 5.4*** for legends).

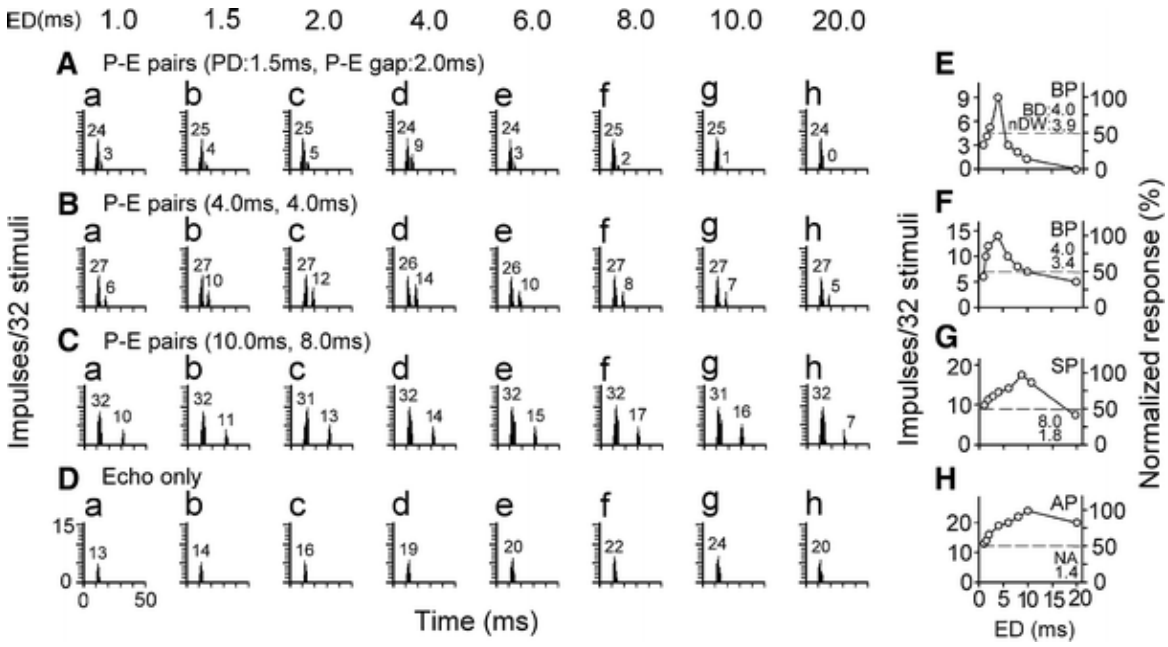

ED(ms) 1.0 1.5 2.0 4.0 6.0 8.0 10.0 20.0
A P-E pairs (PD:1.5ms, P-E gap:2.0ms)
B P-E pairs (4.0ms, 4.0ms)
C P-E pairs (10.0ms, 8.0ms)
D Echo only
Impulses/32 stimuli
Time (ms)
E
F
G
H
BP
BD:4.0
nDW:3.9
SP
AP
NA
1.4
ED (ms)
Normalized response (%)

Figure 5.6 A: Variation in the number of duration selective (band pass, short pass and long pass, *unfilled circles*) and non-selective (all pass, *filled circles*) IC neurons determined with three P–E pairs and single echo pulses. The *dashed line* indicates the difference in the number between the two groups of IC neurons. B, C: *Bar histograms* showing the average *BD* and *nDW* of IC neurons determined under different stimulation conditions. The *BD* and *nDW* obtained under all four stimulation conditions differ significantly (repeated measures one-way ANOVA, $P < 0.01$). Newman–Keuls multiple comparisons post-test showed significant differences between each set of the *BD* and the *nDW* (**$P < 0.01$ and *$P < 0.05$)

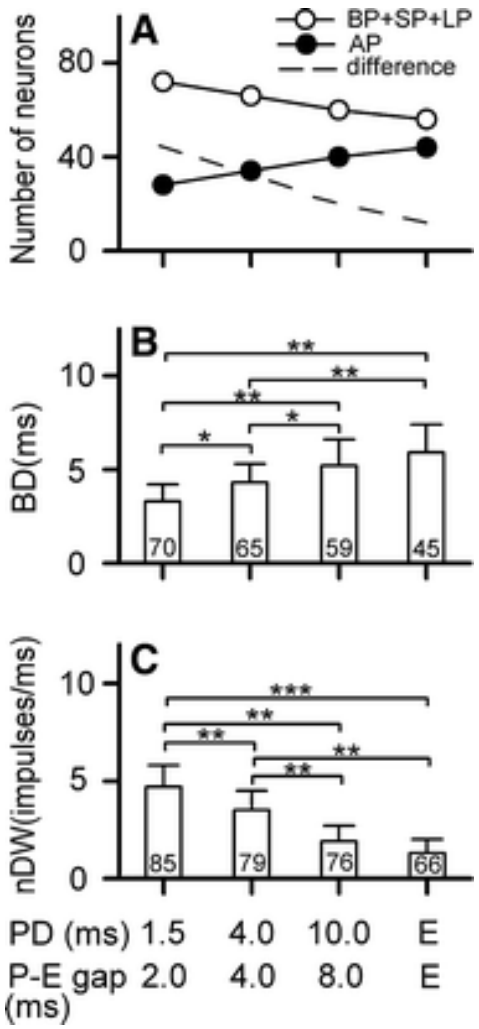

A
BP+SP+LP
AP
difference
Number of neurons
80
40
0
B
BD(ms)
10
5
0
**
**
**
*
*
70
65
59
45
C
nDW(impulses/ms)
10
5
0
***
**
**
**
**
85
79
76
66
PD (ms) 1.5 4.0 10.0 E
P-E gap (ms) 2.0 4.0 8.0 E

Table 5.2 Echo duration tuning curves of IC neurons plotted with the number of impulses discharged to the echoes of P–E pairs and to single pulses

PD (ms)	1.5	4.0	10.0	Single
P–E gap (ms)	2.0	4.0	8.0	pulses
SP	28 (26%)	27 (27%)	27 (27%)	26 (27%)
BP	34 (35%)	28 (29%)	22 (22%)	17 (17%)
LP	8 (8%)	10 (10%)	10 (10%)	12 (12%)
SP + BP + LP (duration selective neurons)	70 (72%)	65 (66%)	59 (60%)	55 (56%)
AP (duration non-selective neurons)	28 (28%)	33 (34%)	39 (40%)	43 (44%)

See ***Table 5.1*** for legends

Echo duration selectivity of IC neurons in relation to pulse duration, P–E interval and P–E gap

Because we examined the echo duration selectivity with the P–E pairs that varied in pulse duration, P–E interval and P–E gap, we were able to examine the effect of these varied parameters on echo duration selectivity of 48 IC neurons. ***Figure 5.7*** shows the nine echo duration tuning curves of a representative IC neuron obtained with the P–E pairs under different combinations of pulse duration and P–E interval. The neuron's discharge patterns to the P–E pairs at two representative P–E intervals with and without P–E overlap are shown in ***Figure 5.7*** B. When stimulated with 10 ms pulse duration at 8 and 18 ms P–E intervals, the neuron's responses to pulse and varied echo durations were always recognizable although P–E overlap occurred when stimulated at 18 ms P–E interval (***Figure 5.7***, Ba vs. Bb). However, response to each echo pulse was greatly reduced when P and E overlapped such that the neuron had poor echo duration selectivity (i.e., small nDW)(***Figure 5.7***, Ah vs. Ai, nDW: 0.9 vs. 3.0). Similarly, the neuron's nDW was 9.1 when tested with 1.5 ms pulse at 3.5 ms P–E interval (i.e., no P–E overlap) but its nDW was only 0.8 when tested with 10 ms pulse at 3.5 ms P–E interval (i.e., P–E overlap) (e.g., ***Figure 5.7***, Aa vs. g).

When tested with P–E pairs, variation in echo duration selectivity of the IC neuron with the pulse duration and P–E interval is unsystematic. For example, when

determined at 3.5 or 8 ms P–E interval, echo duration selectivity (i.e., nDW) varied unsystematically with lengthening of pulse duration (***Figure 5.7***, Aa vs. d vs. g, b vs. e vs. h, Ca solid and unfilled circles). When determined at 18 ms P–E interval, duration selectivity hardly changed with pulse duration (***Figure 5.7***, Ac vs. f vs. I, Ca solid triangles). Similarly, when determined with 1.5-, 4- and 10-ms pulse duration at varied P–E interval, the nDW changed unsystematically with increasing P–E interval (***Figure 5.7***, Aa vs. b vs. c, d vs. e vs. f, g vs. h vs. i, Cb).

We also plotted the echo duration tuning curve of the same IC neuron under different combinations of pulse duration and P–E gap. Different from the above observation; the echo duration selectivity of the same IC neuron varied systematically with pulse duration and P–E gap. When determined at 2 or 4 ms P–E gap, the nDW progressively decreased with lengthening of pulse duration (***Figure 5.8***, Aa vs. d vs. g; b vs. e vs. h, Ba solid and unfilled circles). However, when determined at 8 ms P–E gap, duration selectivity hardly changed with pulse duration (***Figure 5.8***, Ac vs. f vs. I, Ba solid triangles). Alternatively, when determined with 1.5-, 4- and 10-ms pulse duration, the nDW progressively increased with shortening of P–E gap (***Figure 5.8*** Aa vs. b vs. c, d vs. e vs. f, g vs. h vs. i). However, the change in the nDW was greater for shorter PD than long PD (***Figure 5.8***, Bb).

Figure 5.9 summarizes the variation in the average BD and nDW of 42 IC

neurons with P–E gap and interval. It is clear that when stimulated with 1.5 and 4 ms pulses at 2 ms P–E gap, these neurons had the smallest average BD which significantly increased with shortening and lengthening of P–E gap and P–E interval (***Figure 5.9***, Aa, Ba, one-way ANOVA, $P < 0.05$). However, the average BD hardly changed when pulse and echo overlapped (***Figure 5.9***, Aa, Ba, shaded). The average BD hardly varied with the P–E gap and interval when tested with 10 ms pulses (***Figure 5.9***, Ca, one-way ANOVA, $P > 0.05$).

Conversely, when stimulated with 1.5 and 4 ms pulses at 2 ms P–E gap, these neurons had the largest average nDW which significantly decreased with shortening and lengthening of P–E gap and P–E interval (***Figure 5.9***, Ab, Bb, Cb, one-way ANOVA, $P < 0.01–0.05$). Variation of the average nDW with P–E gap and interval was minimal when pulse and echo overlapped (***Figure 5.9***, Ab, Bb, Cb, shaded).

Figure 5.7 A *a*–*i* Nine echo duration tuning curves of an IC neuron obtained with P–E pairs at different combinations of *PD* and *P–E I*. B The discharge patterns of the IC neurons obtained with P–E pairs with (B *a*) and without (B *b*) the overlap between P and E. The plotted echo duration tuning curves are, respectively, shown in (A *h*, A *i*). Note that the neuron had poor *ED* selectivity when P and E overlapped [as shown by smaller *nDW*, 0.9 in (A *h*) than 3.0 in (A *i*)]. C Variation in *nDW* with *PD* (B *a*) and *P–E I* (B *b*). Note that the *nDW* varied unsystematically with increasing *PD* and *P–E I* (see the text for details)

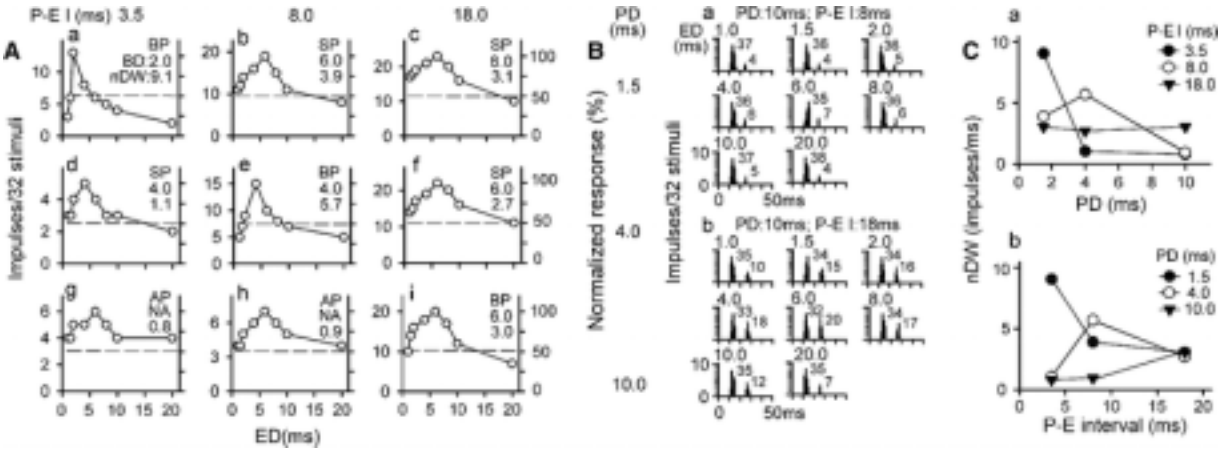

A
P-E I (ms) 3.5
8.0
18.0
Impulses/32 stimuli
ED(ms)
B
PD (ms)
Normalized response (%)
PD:10ms; P-E I:8ms
PD:10ms; P-E I:18ms
ED (ms)
50ms
C
P-E I (ms)
PD (ms)
nDVV (impulses/ms)
P-E interval (ms)

Figure 5.8 A *a–i* Nine echo duration tuning curves of an IC neuron obtained with P–E pairs at different combinations of *PD* and P–E gap. The duration tuning properties, the *BD* and the *nDW* are shown within each plot. B Variation in *nDW* with *PD* (B *a*) and P–E gap (B *b*). Note that the *nDW* mostly decreases with increasing *PD* and P–E gap (see the text for details)

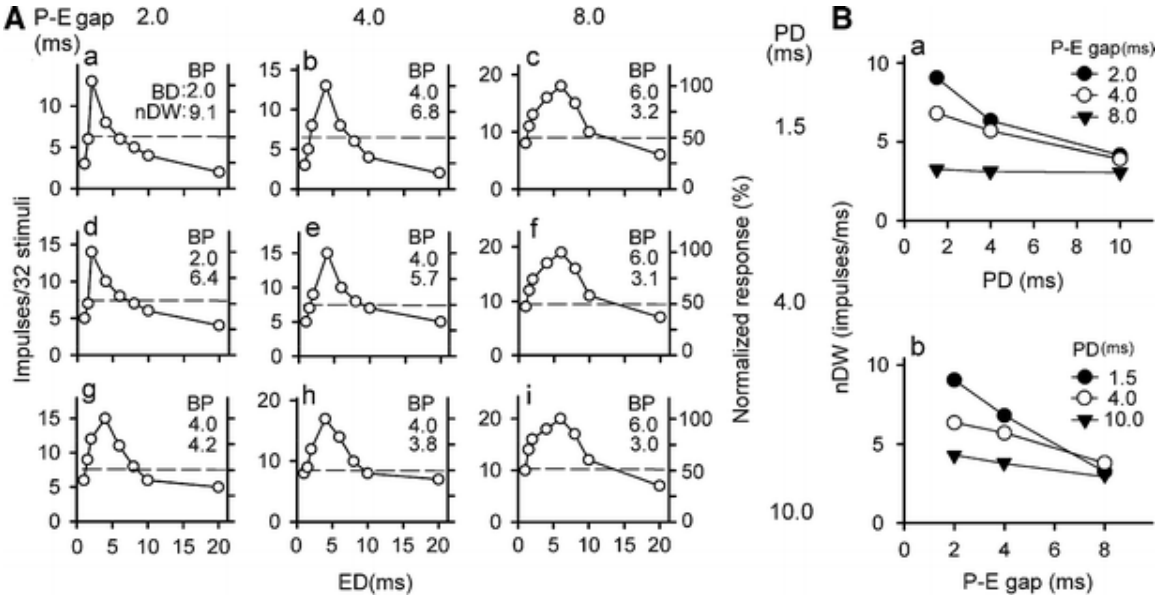
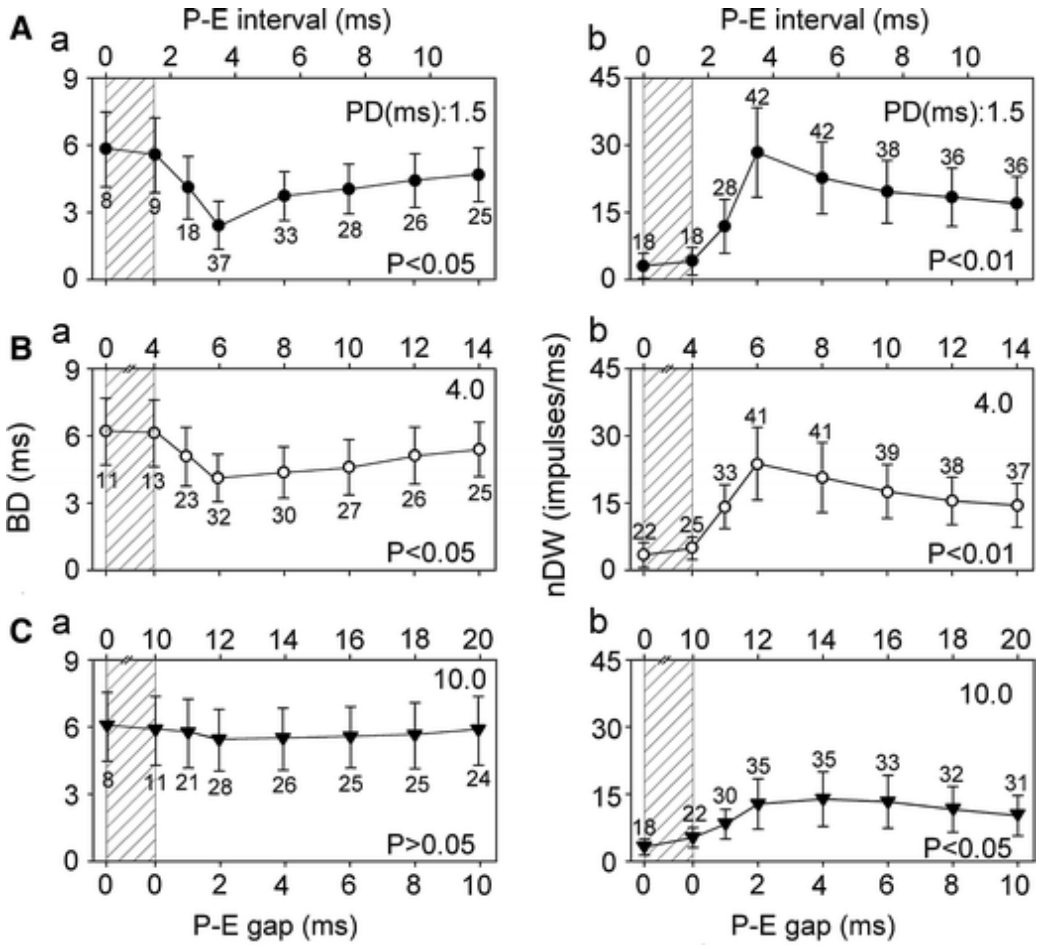

A
P-E gap (ms)
2.0
4.0
8.0
a
BP
BD:2.0
nDW:9.1
b
BP
4.0
6.8
c
BP
6.0
3.2
d
BP
2.0
6.4
e
BP
4.0
5.7
f
BP
6.0
3.1
g
BP
4.0
4.2
h
BP
4.0
3.8
i
BP
6.0
3.0
Impulses/32 stimuli
ED(ms)
Normalized response (%)
PD (ms)
1.5
4.0
10.0
B
a
P-E gap(ms)
2.0
4.0
8.0
PD (ms)
b
PD(ms)
1.5
4.0
10.0
nDW (impulses/ms)
P-E gap (ms)

Figure 5.9 Variation in the *BD* (A *a*, B *a*, C *a*) and *nDW* (A *b*, B *b*, C *b*) of echo duration tuning curves of IC neurons plotted with different combinations of the *PD* (A 1.5 ms, B 4.0 ms and C 10.0 ms) and P–E gap (0, 1, 2, 4, 6, 8 and 10 ms). The *top abscissa* represents the P–E interval corresponding P–E gap shown in the *bottom abscissa*. *Shaded areas* indicate overlap between P and E (see the text for details)


A
a
P-E interval (ms)
PD(ms):1.5
P<0.05
b
P-E interval (ms)
PD(ms):1.5
P<0.01
B
BD (ms)
4.0
P<0.05
nDW (impulses/ms)
4.0
P<0.01
C
10.0
P>0.05
10.0
P<0.05
P-E gap (ms)
P-E gap (ms)

The effect of bicuculline application on echo duration selectivity determined with P–E pairs

In agreement with previous studies (Casseday et al. 1994, 2000; Jen and Feng 1999), bicuculline application broadened the echo duration tuning curves of IC neurons. As shown in ***Figure 5.10***, a representative IC neuron had a band-pass echo duration tuning curve when plotted with the echo pulses of three P–E pairs before bicuculline application (***Figure 5.10*** , Aa–c). Bicuculline application produced a greater increase in the number of impulses for shorter and longer non-BD echo pulses than for the BD echo pulse (***Figure 5.10***, Aa vs. Ba, Ab vs. Bb, Ac vs. Cc, left ordinate). As a result, all band-pass echo duration tuning curves were broadened and changed into all-pass echo duration tuning curves with decreasing nDW (***Figure 5.10***, A vs. B).

Among 40 neurons studied, bicuculline application significantly increased the BD and decreased the nDW of echo duration tuning curves to varying degree when determined with the echo pulses of all three P–E pairs (***Figure 5.10*** Ca, Da, filled vs. unfilled bars). As such, significant increase in BD and decrease in nDW with lengthening of pulse duration and P–E gap was only observed before but not during bicuculline application (repeated measures two-way ANOVA; unfilled bars, $P < 0.05$ for ***Figure 5.10***, Ca, $P < 0.01$ for ***Figure 5.10***, Da; filled bars, $P > 0.05$ for ***Figure 5.10***, Ca, Da). A Student–Newman–Keuls multiple comparisons post-test showed significant

differences between each set of the BD and the nDW (**$P < 0.01$ and *$P < 0.05$). The average percent change of the BD and nDW during bicuculline application significantly decreased with lengthening of pulse duration and P–E gap (repeated measures one-way ANOVA; $P < 0.05$ for ***Figure 5.10***, Cb, $P < 0.01$ for ***Figure 5.10***, Db). A Student–Newman–Keuls multiple comparisons post-test showed significant differences between each set of the BD and the nDW (**$P < 0.01$ and *$P < 0.05$).

The echo duration tuning curves of 48–58% of these 40 IC neurons changed into all-pass during bicuculline application. As a result, progressive decrease in the number of band- and short-pass echo duration tuning curves and increase in the number of all-pass echo duration tuning curves with lengthening of pulse duration and P–E gap was only observed before but not during bicuculline application (***Table 5.3***, predrug vs. bicuculline).

Figure 5.10 A, B Echo duration tuning curves of an IC neuron determined with three P–E pairs before (predrug, A) and during (B) bicuculline application. The *PD*, P–E gap, *BD* and *nDW* are shown within each plot. C *a*, D *a Bar histograms* showing the average *BD* and *nDW* determined with three P–E pairs before (predrug, *unfilled bars*) and during (*filled bars*) bicuculline application. C *b*, D *b* Percent change in the *BD* and the *nDW* during bicuculline application (see the text for details)

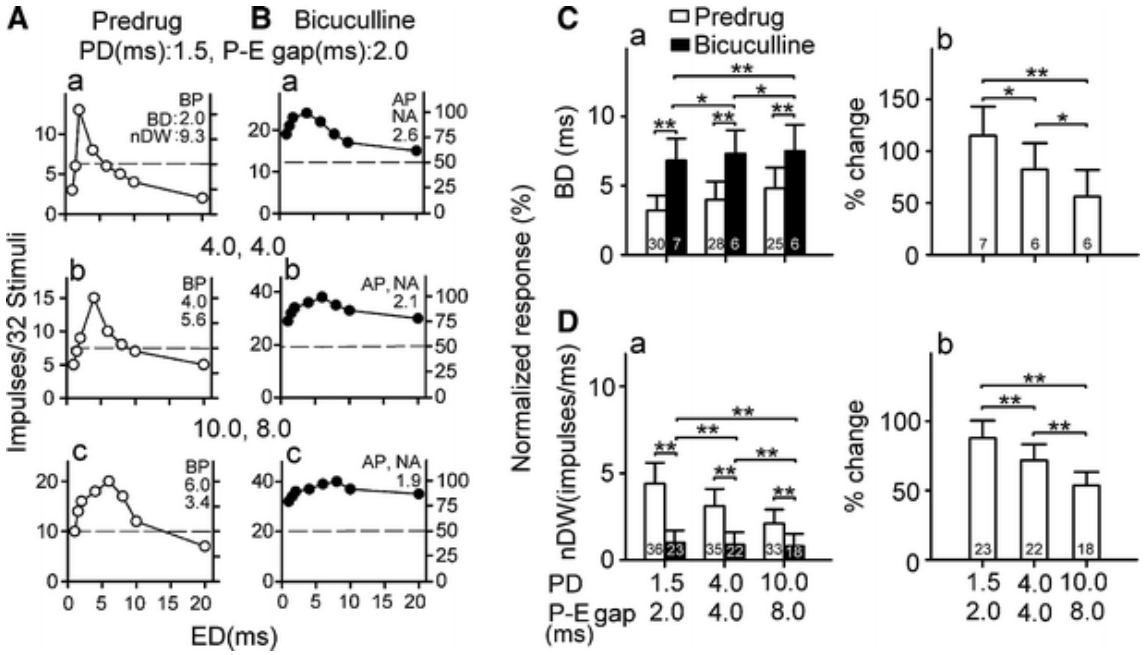

A
Predrug
B
Bicuculline
PD(ms):1.5, P-E gap(ms):2.0
a
BP
BD:2.0
nDW:9.3
AP
NA
2.6
4.0, 4.0
b
BP
4.0
5.6
AP, NA
2.1
10.0, 8.0
c
BP
6.0
3.4
AP, NA
1.9
Impulses/32 Stimuli
ED(ms)
C
Predrug
Bicuculline
BD (ms)
% change
Normalized response (%)
D
nDW(impulses/ms)
PD
P-E gap (ms)
1.5 4.0 10.0
2.0 4.0 8.0

Table 5.3 The effect of bicuculline application on echo duration tuning curves of 40 IC neurons determined with three P–E pairs

PD	P-E gap	Tuning properties	Predrug	Bicuculline			
				BP	SP	LP	AP
		BP	17 (43 %)	3		1	13
1.5 ms	2.0 ms	SP	11 (28 %)		1		10
		LP	2 (5 %)			2	
		AP	10 (25 %)				10
		Total	40	3 (8 %)	1 (3 %)	3 (8 %)	33 (83 %)
		BP	15 (38 %)	3			12
4.0 ms	4.0 ms	SP	11 (28 %)		1		10
		LP	2 (5 %)			2	
		AP	12 (30 %)				12
		Total	40	3 (8 %)	1 (3 %)	2 (5 %)	34 (85 %)
		BP	13 (33 %)	3			10
10.0 ms	8.0 ms	SP	10 (25 %)		1		9
		LP	2 (5 %)			2	
		AP	15 (37 %)				15
		Total	40	3 (8 %)	1 (3 %)	2 (5 %)	34 (85 %)

See ***Table 5.1*** for legends. Underlined values indicate no change in duration tuning properties

The effect of GABA application on echo duration selectivity determined with P–E pairs

Contrary to the effect of bicuculline application, GABA application sharpened the echo duration tuning curves of all IC neurons. ***Figure 5.11*** shows the echo duration tuning curves of an IC neuron that had band-, short- and all-pass echo duration tuning curves when plotted with the echo pulses of three P–E pairs before GABA application (***Figure 5.11***, Aa–c). GABA application produced a greater decrease in the number of impulses for non-BD echo pulses than for BD echo pulse (***Figure 5.11***, Aa vs. Ba, Ab vs. Bb, Ac vs. Cc, left ordinate). As a result, the neuron's band-pass echo duration tuning curve became even sharper while the short- and all-pass echo duration tuning curves changed into band-pass echo duration tuning curves (***Figure 5.11***, Aa–c vs. Ba–c). The increase in the neuron's nDW during GABA application was greater when tested with longer pulse duration and P–E gap (***Figure 5.11***, Ba, 6.25%; Bb, 23.1%; Bc, 61%).

GABA application significantly decreased the BD and increased the nDW of echo duration tuning curves of 32 IC neurons to varying degree when determined with all three P–E pairs (***Figure 5.11***, Ca, Da, filled vs. unfilled bars). As such, significant increase in BD and decrease in nDW with lengthening of pulse duration and P–E gap was only observed before but not during GABA application (repeated measures two-way ANOVA; unfilled bars, $P < 0.05$ for ***Figure 5.11***, Ca, $P < 0.01$ for

Figure 5.11, Da; filled bars, $P > 0.05$ for ***Figure 5.11***, Ca, Da). A Student–Newman–Keuls multiple comparisons post-test showed significant differences between each set of BDs (**$P < 0.01$ and *$P < 0.05$). The average percent change of the BD and nDW during GABA application significantly increased with lengthening of pulse duration and P–E gap (repeated measures one-way ANOVA; $P < 0.05$ for ***Figure 5.11***, Cb, $P < 0.01$ for ***Figure 5.12*** d). A Student–Newman–Keuls multiple comparisons post-test showed significant differences between each set of the BD and the nDW (**$P < 0.01$).

Among 32 IC neurons studied, GABA application changed most all-pass duration tuning neurons into short and band-pass duration tuning neurons. As a result, noticeable decrease in the number of band- and short-pass echo duration tuning curves and increase in the number of all-pass echo duration tuning curves with lengthening of pulse duration and P–E gap was only observed before but not during GABA application (***Table 4***, predrug vs. GABA).

Figure 5.11 A, B: Echo duration tuning curves of an IC neuron determined with echo pulses of three P-E pairs before (predrug, Aa-c) and during GABA application (Ba-c). The types, BD and nDW of echo duration tuning curves are shown in each plot. Ca, Da: Bar histograms showing the average BD and nDW determined with echo pulses of three P-E pairs before and during GABA application. Note that the BD decreases (Ca) and the nDW increases (Da) during GABA application (Repeated measures two-way ANOVA; unfilled vs filled bars, $P<0.01$ for C-Da). Moreover, the BD increases and the nDW decreases with lengthening of PD and P-E gap only before but not during GABA application (Repeated measures two-way ANOVA; unfilled bars, $P<0.05$ for Ca, $P<0.01$ for Da; filled bars, $P>0.05$ for C-Da). Cb, Db: Both percent changes in the BD and nDW during GABA application increase with lengthening of PD and P-E gap (Repeated measures one-way ANOVA, $P<0.05$ for Cb, $P<0.01$ for Db). A Student–Newman–Keuls multiple comparisons post-test showed significant differences between each set of the data (** $P<0.01$).

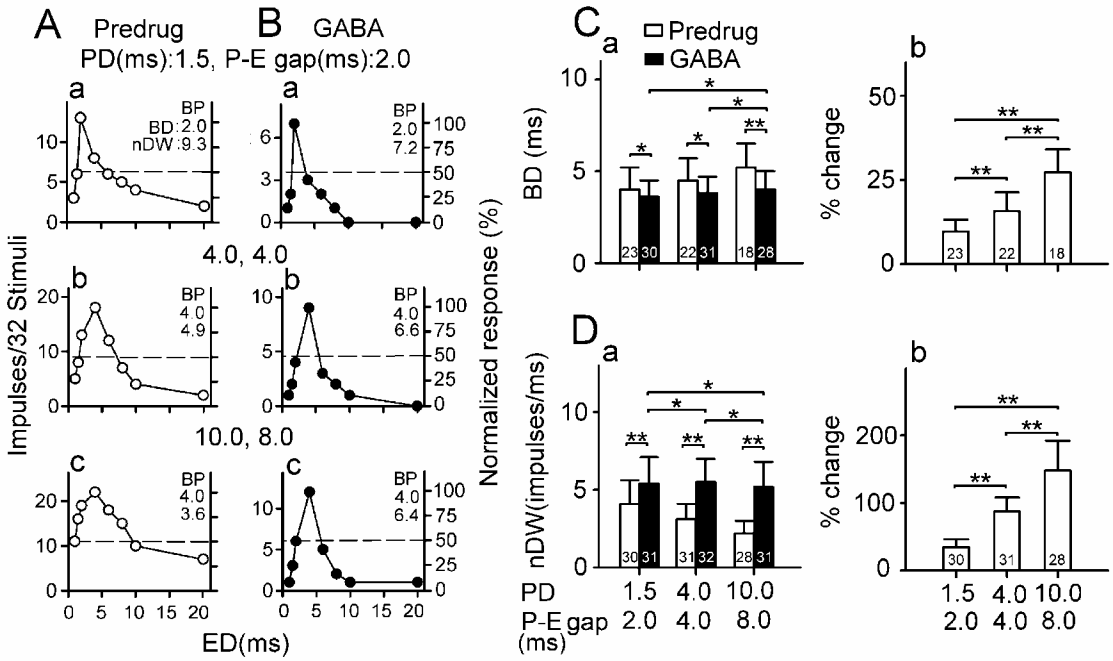

A Predrug
B GABA
PD(ms):1.5, P-E gap(ms):2.0
BP
BD:2.0
nDW:9.3
BP
2.0
7.2
4.0, 4.0
BP
4.0
4.9
BP
4.0
6.6
10.0, 8.0
BP
4.0
3.6
BP
4.0
6.4
Impulses/32 Stimuli
Normalized response (%)
ED(ms)
C
Predrug
GABA
BD (ms)
% change
D
nDW(impulses/ms)
% change
PD 1.5 4.0 10.0
P-E gap (ms) 2.0 4.0 8.0

Table 5.4 The effect of GABA application on echo duration tuning curves of 32 IC neurons determined with three P–E pairs

PD	P-E gap	Tuning properties	Predrug	GABA			
				BP	SP	LP	AP
		BP	11 (34%)	11			
1.5 ms	2.0 ms	SP	10 (31%)	4	6		
		LP	2 (6%)			2	
		AP	9 (28%)	1	6		2
		Total	32	16 (42%)	12 (38%)	2 (6%)	2 (6%)
		BP	10 (31%)	10			
4.0 ms	4.0 ms	SP	10 (31%)	4	6		
		LP	2 (6%)			2	
		AP	10 (31%)	2	6		2
		Total	32	16 (42%)	12 (38%)	2 (6%)	2 (6%)
		BP	7 (22%)	7			
10.0 ms	8.0 ms	SP	9 (28%)	5	4		
		LP	2 (6%)			2	
		AP	14 (44%)	2	7	1	4
		Total	32	14 (44%)	11 (34%)	3 (9%)	4 (13%)

See ***Tables 5.1*** and ***5.3*** for legends.

Echo duration selectivity, BF and recording depth

Because IC neurons differed in BF and recording depth, we studied the echo duration selectivity in relation to BF and recording depth. Regardless of stimulus conditions, high BF neurons tend to have long BD and small nDW than low BF neurons had (***Figures 5.12-5.13***). Linear regression analyses of scatter plots of BD and nDW in relation to BF revealed that the BD significantly increased and the nDW decreased with BF ($P < 0.01$).

Consonant with previous studies (Jen and Schlegel 1982; Pinheiro et al. 1991; Poon et al. 1990, Wu and Jen 1991), the BF of sequentially isolated neurons increased with recording depth (***Figure 5.14***, Aa, Ba). Furthermore, linear regression analyses of the scatter plots of BD and nDW in relation to recording depth revealed that the BD significantly increased and the nDW decreased with recording depth ($P < 0.01–0.05$; for simplicity, we only show the scatter plots obtained with single echo pulses and echo pulses of one P–E pair). Similar observations were also obtained when echo duration selectivity of IC neurons was studied with other two P–E pairs.

Figure 5.12 Scatter plots showing the distribution of *BD* of IC neurons in relation to the *BF* under four different stimulation conditions. The linear regression line is shown with a *solid line*. *N* is the number of neurons, *r* the correlation coefficient and *P* the significance level.

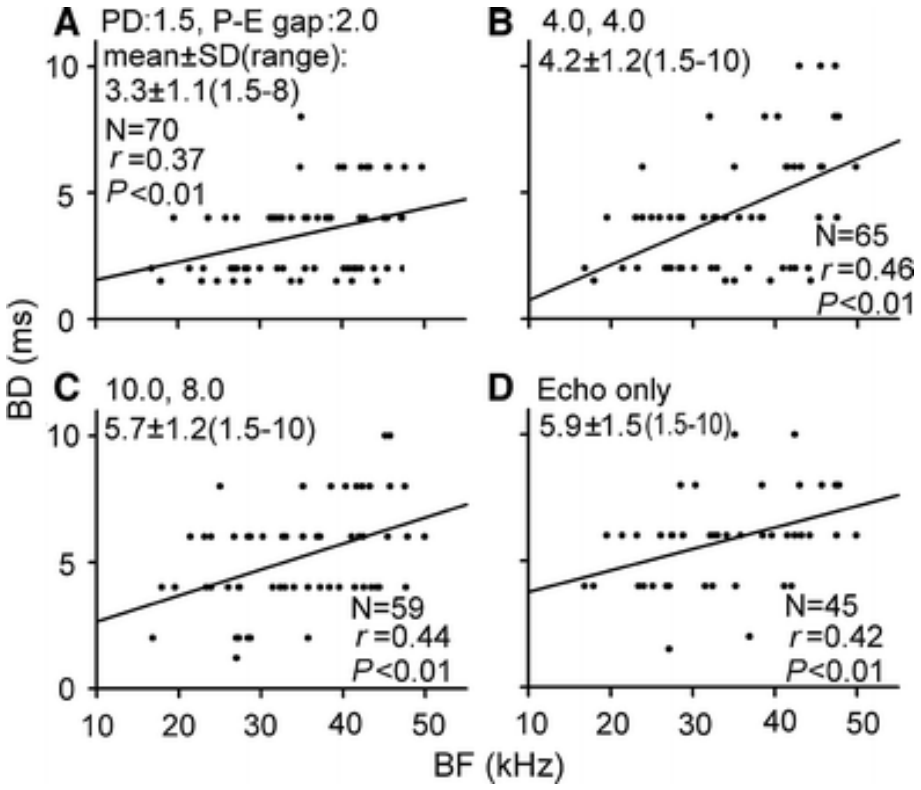

A
PD:1.5, P-E gap:2.0
mean±SD(range):
3.3±1.1(1.5-8)
N=70
r=0.37
P<0.01
B
4.0, 4.0
4.2±1.2(1.5-10)
N=65
r=0.46
P<0.01
C
10.0, 8.0
5.7±1.2(1.5-10)
N=59
r=0.44
P<0.01
D
Echo only
5.9±1.5(1.5-10)
N=45
r=0.42
P<0.01
BD (ms)
BF (kHz)
0
5
10
10
20
30
40
50

Figure 5.13 Scatter plots showing the distribution of *nDW* of IC neurons in relation to *BF* under four different stimulation conditions (see ***Figure 5.12*** for legends)

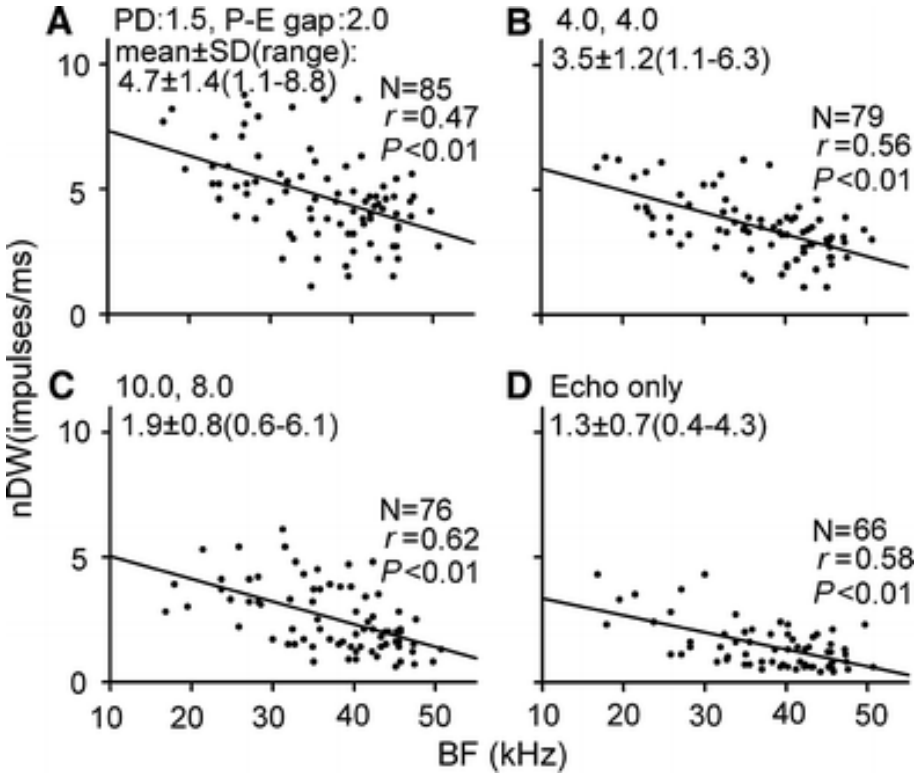
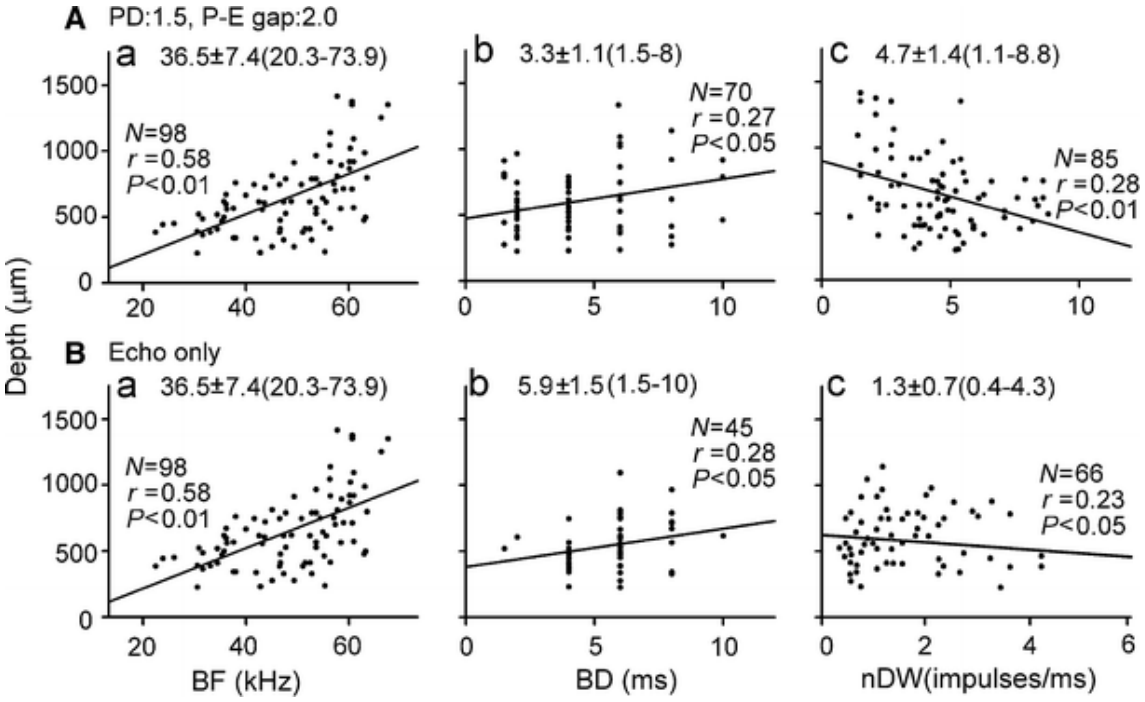

A
PD:1.5, P-E gap:2.0
mean±SD(range):
4.7±1.4(1.1-8.8)
N=85
r=0.47
P<0.01
B
4.0, 4.0
3.5±1.2(1.1-6.3)
N=79
r=0.56
P<0.01
C
10.0, 8.0
1.9±0.8(0.6-6.1)
N=76
r=0.62
P<0.01
D
Echo only
1.3±0.7(0.4-4.3)
N=66
r=0.58
P<0.01
nDW(impulses/ms)
BF (kHz)
0
5
10
10
20
30
40
50

Figure 5.14 Scatter plots showing the distribution of *BF*, *BD* and *nDW* of IC neurons in relation to recording depth under different stimulation conditions. Note that the scale is different in (A *c*, B *c*)


A PD:1.5, P-E gap:2.0
a 36.5±7.4(20.3-73.9)
N=98
r =0.58
P<0.01
b 3.3±1.1(1.5-8)
N=70
r =0.27
P<0.05
c 4.7±1.4(1.1-8.8)
N=85
r =0.28
P<0.01
B Echo only
a 36.5±7.4(20.3-73.9)
N=98
r =0.58
P<0.01
b 5.9±1.5(1.5-10)
N=45
r =0.28
P<0.05
c 1.3±0.7(0.4-4.3)
N=66
r =0.23
P<0.05
Depth (μm)
BF (kHz)
BD (ms)
nDW(impulses/ms)

Discussion

Echo duration selectivity of IC neurons determined with single echo pulses and echo pulses of P–E pairs

In the present study, we showed that the duration selective IC neurons had band-, short-, or long-pass duration tuning curves (***Figures 5.2-5.6***). These neurons responded maximally to a specific duration and showed high sensitivity to change in the pulse duration. We observed that the echo duration selectivity of IC neurons was sharper when determined with echo pulses of P–E pairs than with single echo pulses (***Figures 5.4-5.6***). These observations suggest that a bat's echo duration selectivity in the real world is sharper than what is shown by earlier studies using single pulses (Casseday et al. 1994, 2000; Ehrlich et al. 1997; Fuzessery and Hall 1999; Galazyuk and Feng, 1997; Jen and Feng 1999; Jen and Schlegel 1982; Pinheiro et al. 1991; Zhou and Jen 2001).

A previous study examined the interaction of excitation and inhibition in IC neurons using a probe (excitatory pulse) and a masker (inhibitory pulse) (Lu and Jen 2002). This study showed that masking of probe-elicited responses of IC neurons occurs when a masker is presented within a certain inter-pulse intervals (the temporal window) in relation to the probe. Within the temporal window, the strength of this forward masking increases with shortening of inter-pulse interval. Similarly, many studies showed that a neuron's response to a single pulse could be

suppressed when the single pulse is paired with another pulse within a temporal window (Brosch and Schreiner 1997; Calford and Semple 1995; Faure et al. 2003; Hocherman and Gilat 1981; Litovs.ky and Yin 1998). As such, neurons show larger responses to single pulses presented in temporal isolation than to the same pulse presented in temporally patterned pulse trains (Moriyama et al. 1994). It is therefore possible that this forward neural masking may also account for the sharper echo duration selectivity of IC neurons obtained with echo pulses of P–E pairs than with single echo pulses.

In the present study, the P served as the masker and the E served as the probe. When determined with the P–E pairs, the forward masking and the recovery property, which determines a neuron's ability in response to a succeeding pulse, may be the two predominant factors to shape a neuron's echo duration selectivity. The shorter the P–E gap is, the stronger the forward masking becomes to shape the echo duration selectivity. However, our earlier studies in E. fuscus show that the recovery property of IC neurons becomes poor with shortening of P–E gap (Lu et al. 1997; Wu and Jen 1998; Zhou and Jen 2003). For this reason, the echo duration selectivity of IC neurons is shaped by these two opposing forces in relation to the P–E gap. Conceivably, there is an optimal P–E gap when forward masking and a neuron's recovery ability complement to each other to shape the neuron's sharpest echo duration selectivity.

For example, when the P–E gap is within the temporal window of forward masking, a neuron's echo duration selectivity becomes sharper with shortening of P–E gap because of increasing strength of forward masking (***Figures 5.6***c, ***5.8***Bb, ***5.9***). However, when the P–E gap becomes smaller than the optimal P–E gap, the increasing strength of forward masking would be counterbalanced by the increasingly poor recovery ability of the IC neuron. As a result, the neuron's echo duration selectivity becomes poor, in particular when P and E overlap (***Figures 5.7***, ***5.9***). Conceivably, echo duration selectivity determined with overlapping P–E pairs is comparable to that determined with single echo pulses. As shown earlier, echo duration selectivity of IC neurons is sharper when determined with non-overlapping P–E pairs than with single echo pulses (***Figures 5.4***-***5.6***).

Alternatively, previous studies have shown that masking effect is maximal when the masker and probe overlap (i.e., simultaneous masking) (Faure et al. 2003; Lu and Jen 2002). It is possible that the simultaneously masking during P–E overlap may produce similar degree of decrease in the number of impulses to BD and non-BD echo durations. As such, the simultaneous masking only lowers but does not change the sharpness of the echo duration tuning curve.

We observed that when the P–E gap is larger than the temporal window, forward masking becomes ineffective. For example, echo duration selectivity of IC neurons changed in a small degree with pulse duration when determined at 8 ms

P–E gap or at 10-ms pulse duration (***Figure 5.8***, Ba, solid triangles; ***Figure 5.9*** C). These observations suggest that the temporal window for forward masking is smaller than 10 ms which is the inter-pulse interval for 1.5 ms pulses at 8 ms P–E gap. Since the inter-pulse intervals for 4 and 10 ms pulses at 8 ms P–E gap are 12 and 18 ms, forward masking on echo duration selectivity would be also ineffective (***Figure 5.8***, Ba, filled triangles). These data are comparable to two previous studies which show that the temporal window for forward masking of response properties (i.e., number of impulses, latency, frequency tuning curves and directional selectivity) of IC neurons of the same bat species is 6.3–7.0 ms (Lu and Jen 2002; Zhou and Jen 2000).

The role of GABAergic inhibition in shaping echo duration selectivity of IC neurons

The role of GABAergic inhibition in shaping duration selectivity of IC neurons has been reported in previous studies (Casseday et al. 1994, 2000; Ehrlich et al. 1997; Fuzessery and Hall 1999, Jen and Feng 1999, Wu and Jen 2006). In the present study, we plotted the echo duration tuning curves of IC neurons with the P–E pairs before and during application of GABA and its antagonist, bicuculline (Bormann 1988; Cooper et al. 1982). The opposite effect on the echo duration tuning curves of IC neurons during both drug applications allowed us to double confirm

the role of GABAergic inhibition in shaping the echo duration selectivity of IC neurons (***Figures 5.10-5.11***). Our data indicate that GABAergic inhibition sharpens echo duration selectivity of IC neurons by producing a greater decrease in the number of impulses for non-BD echo pulses than for BD echo pulse when stimulated with all three P–E pairs. As such, many duration non-selective IC neurons became duration selective during GABA application (***Figure 5.11***).

Our data also indicate that GABAergic inhibition contributes to improving echo duration selectivity of IC neurons with shortening of pulse duration and P–E gap (***Figures 5.4-5.6***). This is supported as follows. (1) The degree of broadening of echo duration tuning curves progressively increased with shortening of pulse duration and P–E gap during bicuculline application (***Figure 5.10***, Cb, Db). (2) The degree of sharpening of echo duration tuning curves progressively decreased with shortening of pulse duration and P–E gap during GABA application (***Figure 5.11***, Cb, Db). (3) The number of band- and short-pass echo duration tuning curves progressively increases and the number of all-pass echo duration tuning curves decreases with shortening of PD and P–E gap was only observed before but not during bicuculline and GABA application (***Tables 3, 4***).

Because shortening of pulse duration and P–E gap is in essence equivalent to increase pulse repetition rate, all these data are in parallel with our most recent study which shows that the improvement of duration selectivity is due to

progressively increasing GABAergic inhibition with pulse repetition rate (Wu and Jen 2006). In agreement with our present data, this study showed that bicuculline application produces more pronounced broadening of duration tuning curves at high than at low pulse repetition rate while GABA application produces more pronounced narrowing of duration tuning curves at low than at high pulse repetition rate.

A previous study reported that forward masking is based on the combination of IPSP and after-hyperpolarization to create a shorter recovery cycle for shorter than for long duration stimuli such that forward masking is greater for shorter than for long duration stimuli (Eggermont 2000). Based on all these studies and our present data, we suggest that increasing strength of GABAergic inhibition may be the underlying mechanism for increasing forward masking with shortening of pulse duration and P–E gap to sharpen the echo duration selectivity. On the other hand, we have shown that the GBAergic inhibition also shapes the recovery property of IC neurons of this bat species (Lu et al. 1997; Zhou and Jen 2003). For this reason, increasing strength of GABAergic inhibition with shortening of P–E gap would inevitably deteriorate the recovery property of IC neurons. As such, the sharpest echo duration selectivity is only obtained at the optimal P–E gap as described above.

We observed that the BD became shorter in 40% of neurons (e.g., ***Figure 5.5***)

but remained unchanged in 60% neurons (e.g., ***Figure 5.4***) with shortening of the pulse and P–E gap. This observation suggests that neural mechanism underlying the formation of BD of individual IC neurons is dependent on pulse duration and P–E gap. Future works are needed to determine the difference in the neural mechanisms underlying the formation of BD of these two types of IC neurons.

Echo duration selectivity, stimulus frequency and recording depth

We showed that IC neurons with short BD and large nDW typically had low BF while IC neurons with long BD and small nDW had high BF (***Figures 5.12-5.13***). Furthermore, the BD of tonotopically organized IC neurons significantly increased and the nDW decreased with recording depth (***Figure 5.14***). These findings suggest that neurons at upper IC have shorter BD and larger nDW than neurons at deep IC have. A previous study shows that neurons with $GABA_A$ receptors are mostly distributed in the dorso-medial region of the IC but are sparsely distributed in the ventro-lateral region (Fubara et al. 1996). For this reason, high BF neurons at deeper IC would conceivably receive fewer GABAergic inhibitory inputs than low BF neurons at upper IC. As such, drug application would produce greater change in nDW of duration tuning curves of low BF neurons at upper IC than high BF neurons at deeper IC. In sum, our data show that echo duration selectivity of IC neurons shaped by GABAergic inhibition appears to be systematically organized along the

dorso-ventral axis of the IC.

Previous studies show that duration selectivity of IC neurons can also be shaped by glycinergic inhibition and neurons with glycine receptors are mostly distributed at the ventro-lateral region of the IC but are sparsely distributed at dorso-medial region of the IC (Ehrlich et al. 1997; Fubara et al. 1996). How echo duration selectivity of IC neurons shaped by glycinergic inhibition is organized in the IC remains to be studied.

Behavioral relevance

Consonant with our previous study (Jen and Zhou 1999), we found that discharge patterns and echo duration curves obtained with both BF and FM pulses did not differ significantly (***Figure 5.3*** and ***Table 1***). Since FM pulses of different duration differ in frequency sweep direction and rate, duration selectivity of IC neurons determined with FM pulses represents the result of interactions between the sweep rate and variation in frequency within the pulse duration. Our data suggest that variation in pulse duration rather than frequency sweep rate is the predominant factor in determining the duration selectivity of IC neurons.

During hunting, *E. fuscus* progressively increase the repetition rate, shorten the duration, decrease the amplitude and lower the frequency of emitted pulses as

they search, approach and finally intercept the insects or avoid obstacles (Griffin 1958; Jen and Kamada 1982; Surlykke and Moss 2000). In this study, we determined echo duration selectivity with P–E pairs mimicking those occurring at search, approach and terminal phase of echolocation (***Figure 5.1***). We showed that the echo duration selectivity of bat IC neurons became sharper with shortening of pulse duration and P–E gap but became poor when pulse and echo overlap (***Figures 5.4-5.9***). These data suggest that shortening pulse duration by *E. fuscus* during hunting to avoid overlap between pulse and echo is crucial for accurate echo recognition. These data also suggest that as bats progressively increase pulse emission rate and shorten emitted pulses during of hunting, echo duration selectivity would improve and therefore facilitate echo recognition.

The BD of IC neurons we studied ranged between 1.5 and 10 ms covering the duration of pluses emitted by *E. fuscus* during three phases of hunting. We showed that the BD in 40% of neurons became shorter (e.g., ***Figure 5.5***) while the BD in 60% of neurons remained unchanged (e.g., ***Figure 5.4***) with shortening of the pulse and P–E gap. Presumably, IC neurons with unchanged BD can be utilized by bats to encode echoes at each phase of hunting. Conversely, IC neurons with shortened BD and improved duration selectivity during hunting can be utilized by bats to encode the progressively shortening echo throughout the entire course of hunting.

We showed that IC neurons are systematically organized along the

dorsoventral axis of the IC based on the BF, BD and nDW (***Figures 5.12-5.14***). Conceivably, low BF neurons with shorter BD and sharper duration selectivity would appear suitable for echo recognition during the terminal phase of hunting when the highly repetitive echoes are low in frequency and short in duration. Conversely, high BF neurons with long BD would be suitable for echo recognition during search phase of hunting when the returning echoes are high in frequency and long in duration.

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CHAPTER 6

SUMMARY

The dissertation contains four studies. The first study examined the duration selectivity organization in the inferior colliculus of the big brown bat, *Eptesicus fuscus*. The last three studies examined the role of GABAergic inhibition in shaping duration selectivity of inferior collicular (IC) neurons in this bat species using temporally isolated single pulses, temporally patterned pulses and pulse-echo pairs.

The first study specifically examined the organization of duration selectivity of IC neurons of the big brown bat in relation to graded spatial distribution of $GABA_A$ receptors, which are mostly distributed in the dorsomedial region of the IC but are sparsely distributed in the ventrolateral region. This study shows that bicuculline application produces more pronounced broadening of the duration tuning curves of neurons at upper IC than at deeper IC but the opposite is observed during GABA application. The best duration of IC neurons progressively lengthens and duration selectivity decreases with recording depth both before and during drug application. As such, low best frequency neurons at upper IC have shorter best duration and sharper duration selectivity than high best frequency neurons in the deeper IC have. All data in this study suggest that duration selectivity of IC neurons systematically varies with $GABA_A$ receptor distribution gradient within the IC.

The second study examined the role of GABAergic inhibition in shaping duration selectivity of bat IC neurons to sound pulses in rapid sequences. This study shows that the response size of IC neurons progressively decreases and duration selectivity increases when determined with sequentially presented sound pulses. This variation in the response size and duration selectivity of IC neurons with sequentially presented sound pulses is abolished or reduced during bicuculline and GABA application. Bicuculline application increases the response size and broadenes the duration tuning curve of IC neurons while GABA application produced opposite results. All these data indicate that increasing strength of GABAergic inhibition contributes to progressive sharpening of duration selectivity of IC neurons with sequentially presented pulses.

The third study examined the role of GABAergic inhibition in shaping duration selectivity of bat IC neurons determined with temporally patterned sound trains. This study shows that the number of all-pass duration tuning curves progressively decreases and the number of band-pass duration tuning curves increases with pulse repetition rate (PRR) of pulse trains. Duration selectivity of IC neurons also progressively improves and the best duration shortens with increasing PRR of pulse trains. Bicuculline application produces more pronounced broadening of duration tuning curves at high than at low PRR. Conversely, GABA application produces more pronounced narrowing of duration tuning curves at low than at high PRR. In either case, improving duration selectivity of IC neurons with increasing

PRR of pulse trains is abolished during drug application. These data indicate that increasing strength of GABAergic inhibition contributes to progressive sharpening of duration selectivity of IC neurons with increasing PRR of pulse trains. Furthermore, the duration tuning curves of IC neurons progressively broadenes with recording depth at all PRRs tested. Broadening of duration tuning curves during bicuculline application is more pronounced for neurons at upper than at deep IC.

The last study examined the GABA-mediated echo duration selectivity of bat IC neurons. Echo duration selectivity was studied by plotting echo duration tuning curves with the number of impulses discharged to echo pulses of pulse-echo (P-E) pairs or isolation echo pulses against echo durations. The pulse duration (PD) and P-E gap within P-E pairs were comparable to those occurring at search, approach and terminal phase of echolocation in the big brown bat. This study shows that the echo duration selectivity determined with the echo pulses of P-E pairs systematically sharpens with shortening of PD, P-E gap or both. Moreover, echo duration selectivity of bat IC neurons determined with the echo pulse of P-E pairs is always greater than that determined with isolation echo pulses. Bicuculline application produces more pronounced broadening of echo duration tuning curves at short PD and gap while GABA application produces opposite results. This study also examined the correlation of the best duration (BD) and the sharpness of echo duration tuning curves with the best frequency (BF) and the recording depth. When

determined with the echo pulses of different P-E pairs or isolation echo pulses, auditory neurons at upper IC always have low BF, short BD and sharp echo duration selectivity while auditory neurons in deeper IC show the opposite results. These observations suggest that GABAergic inhibition and forward masking contribute to improving echo duration selectivity with shortening of PD and P-E gap. The improvement of echo duration selectivity may potentially help bats facilitate echo duration recognition throughout different phases of hunting.

ABBREVIATIONS

AP	all-pass
BD	best duration
BF	best frequency
BP	band-pass
CF	constant frequency
db SPL	decibel sound pressure levele
DW	duration width
EA	echo amplitude
ED	echo duration
EF	echo frequency
FM	frequency modulated
GABA	gamma-aminobutyric acid
IC	inferior colliculus
LP	long-pass
MT	minimum threshold
nDW	normalized duration width
PD	pulse duration
P-E	pulse-echo

P-E gap	pulse-echo gap
pps	pulse per second
PRR	pulse repetition rate
PST	peri-stimulus-time
SP	short-pass

VITA

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