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Multi-sensory integration in brainstem and auditory cortex

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Abstract

Tinnitus is the perception of sound in the absence of a physical sound stimulus. It is thought to arise from aberrant neural activity within central auditory pathways that may be influenced by multiple brain centers, including the somatosensory system. Auditory-somatosensory (bimodal) integration occurs in the dorsal cochlear nucleus (DCN), where electrical activation of somatosensory regions alters pyramidal cell spike timing and rates of sound stimuli. Moreover, in conditions of tinnitus, bimodal integration in DCN is enhanced, producing greater spontaneous and sound-driven neural activity, which are neural correlates of tinnitus. In primary auditory cortex (A1), similar auditory-somatosensory integration has been described in the normal system (Lakatos et al. 2007), where sub-threshold multisensory modulation may be a direct reflection of subcortical multisensory responses (Tyll et al. 2011). The present work utilized simultaneous recordings from both DCN and A1 to directly compare bimodal integration across these separate brain stations of the intact auditory pathway. Four-shank, 32-channel electrodes were placed in DCN and A1 to simultaneously record tone-evoked unit activity in the presence and absence of spinal trigeminal nucleus (Sp5) electrical activation. Bimodal stimulation led to long-lasting facilitation or suppression of single and multi-unit responses to subsequent sound in both DCN and A1. Immediate (bimodal response) and long-lasting (bimodal plasticity) effects of Sp5-tone stimulation were facilitation or suppression of tone-evoked firing rates in DCN and A1 at all Sp5-tone pairing intervals (10, 20, & 40ms), and greater suppression at 20ms pairing-intervals for single unit responses.. Understanding the complex relationships between DCN and A1 bimodal processing in the normal animal provides the basis for studying its disruption in hearing loss and tinnitus models.

Keywords

Primary auditory cortex; Bimodal integration; Dorsal cochlear nucleus; Somatosensory; Tinnitus

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1. Introduction

Modulation of central auditory pathway neurons by multiple non-auditory systems has emerged as a fascinating concept in brain processing (Ghanzanfar and Schroeder, 2006). Observations that the somatosensory system can modulate activity in auditory brain stations like the dorsal cochlear nucleus (DCN), inferior colliculus (Jain and Shore, 2006), and primary auditory cortex (A1) become clinically relevant since many individuals with tinnitus can alter the pitch and loudness of tinnitus percepts through somatic maneuvers including jaw or neck muscle clenching (Pinchoff et al. 1998; Levine, 1999). Animal studies have demonstrated that DCN pyramidal cells receive somatosensory input from dorsal column and trigeminal brainstem nuclei (Kirzinger & Jurgens, 1991; Wright & Ryugo, 1996; Luthe et al. 2000; Zhou & Shore, 2004; Haenggeli et al. 2005; Kanold et al., 2010) that can elicit cellular excitation or inhibition (Saade et al. 1989; Davis et al. 1996; Kanold & Young, 2001; Shore, 2005; Koehler et al. 2011). Interestingly, bimodal stimulation with sound and somatosensory activation can facilitate or suppress the firing rates of DCN pyramidal cell neurons to the sound stimulus (Shore, 2005; Shore et al. 2008; Dehmel et al., 2012), demonstrating that these neurons are capable of multisensory integration (Stein & Meredith, 1990, 1993; Populin & Yin, 2002; Meredith et al. 2006). Moreover, after noise damage and tinnitus induction, bimodal integration in the DCN is significantly enhanced, leading to large increases in the firing rates of output neurons to higher auditory centers (Dehmel et al., 2012). Since hyperactivity and neural synchrony are physiologic correlates of tinnitus, bimodal facilitation by the somatosensory system after noise damage may be a major factor in tinnitus generation.

Several electrophysiology studies using local field potentials or current source densities have revealed widespread influences of somatosensory stimuli on sound-driven properties in association auditory cortex and A1 (Ghanzanfar et al. 2008; 2005; Lakatos et al. 2007; Schroeder and Foxe, 2002; Schroeder et al. 2001, 2003). In A1 outer supragranular layers, somatosensory stimulation modulates early event-related field potentials (Lakatos et al. 2007). Like the DCN, A1 neurons are capable of multisensory integration since somatosensory-auditory stimulation results in a supra-additive interaction (Lakatos et al. 2007; Kayser et al. 2005). These studies, performed in non-human primates, utilized tactile stimulation of the hand and foot and found somatosensory integration to be stronger for temporally coincident stimuli (Kayser et al. 2005). Previous studies promoted a hierarchical viewpoint, suggesting that sensory information converges only in higher association areas of the auditory cortex (Felleman and Van Essen, 1991), while more recent data suggests that multisensory integration takes place at lower cortical levels (Foxe and Schroeder, 2005; Ghanzanfar and Schroeder, 2006; Schroeder et al. 2004), invoking the idea that multisensory interactions within the auditory system occur at the earliest stage of auditory cortical processing.

While it is evident that somatosensory modulation exists in neurons at several brain stations within the central auditory pathway, the understanding of how multisensory integration interacts at different auditory brain stations remains largely unexplored. It is very likely that bimodal effects on spike timing in DCN could alter the way A1 neurons process sound in both the intact pathway and in aberrant circuits leading to tinnitus generation. The hypothesis of the present study is that somatosensory-auditory responses in A1 represent a combination of cortical processing of auditory and somatosensory inputs combined with already-processed multisensory information from subcortical auditory sources. To test this hypothesis, we used multichannel electrodes to simultaneously record extra-cellular neural responses to the same bimodal stimulation in DCN and A1. Measuring concurrent neural responses in DCN and A1 before, during, and after bimodal stimulation provides the opportunity to evaluate multisensory integration as a reflection of subcortical responses

(already-processed) versus independent processing at the cortical level (cortical-processed). In addition, we evaluated whether multisensory interactions are strongest when both modalities are presented simultaneously or at various pairing intervals. The novel approach of the present study utilizing simultaneous recordings from multiple brain stations in the intact guinea pig can provide key information about auditory circuitry to better understand aberrant changes that may occur in tinnitus percept generation.

2. Results

Recordings were obtained from isolated single units (DCN $n=86$; A1 $n=43$) and multi-units (DCN $n=259$; A1 $n=198$) from both brain regions. In DCN, responses were recorded from the pyramidal and deep cell layers (layer II, 200–500 μ m from DCN surface) and in A1 across all cortical layers (approximately 2.5mm below the outer surface) in response to best frequency (BF) tones preceded by Sp5 stimulation and to BF tones alone. The anatomic locations of the stimulating electrodes are shown in Fig. 1.

To assess the effects of bimodal stimulation on DCN and A1 neuron firing rates, responses to Sp5-tone pairings and acoustic stimuli alone following bimodal stimulation were compared with neural responses to acoustic stimuli preceding bimodal stimulation (Fig. 2). Because the long-lasting effects fall within the same time periods as shown for synaptic plasticity (reviewed in Dehmel et al. 2008), we refer to the long-lasting effects as *bimodal plasticity* with the immediate effects termed *bimodal response*.

2.1 Bimodal stimulation can suppress or facilitate immediate responses to sound in DCN and A1

Paired Sp5-auditory (bimodal) stimulation facilitated or suppressed firing rates in both DCN and A1 (Fig. 3) compared to firing rates evoked by sound alone. In DCN (Fig. 3A), bimodal stimulation facilitated or suppressed neuronal firing at all tested pairing intervals (10, 20 & 40ms). The first column of figure 3A demonstrates peri-stimulus time histograms (PSTHs) showing the unimodal response to sound (blue; left column) compared with the response to bimodal stimulation (red; middle column). The third column demonstrates the corresponding bimodal effect for each pairing-interval presented as a percent change in firing rate, whereby positive changes represent facilitation and negative changes signify suppression in neural firing. For DCN, all three pairing intervals produced units that were either suppressed or facilitated (Fig. 3A), with 10 and 40ms being the optimal pairing-intervals for suppression and facilitation, respectively (Fig. 3A).

Figure 3B shows the PSTHs and percentages of A1 unit activity in response to bimodal stimulation. The PSTHs in A1 (Fig. 3B) demonstrate a large initial peak, representing the electrical artifact during the Sp5 stimulus, followed by the bimodal response. As in DCN, neuronal facilitation and suppression with bimodal stimulation were also observed in A1 across all pairing intervals (Fig. 3B). Facilitated spiking activity was greatest at 20 and 40ms. The period of facilitation in A1 was time-limited, whereas in DCN it lasted for the duration of the stimulus (Fig. 3A compared to B). In contrast to DCN, units in A1 showing the greatest immediate suppression following bimodal stimulation occurred during shorter pairing intervals of 10ms (Fig 3B).

2.2 Bimodal stimulation produces long-lasting facilitation or suppression to sound in DCN

DCN responses, 5–10 minutes after multiple pairing trials of Sp5-auditory stimulation, revealed long-lasting suppression or facilitation to subsequent sound-only stimuli compared to pre-bimodal sound alone (Fig. 4A–B). Figure 4A shows one representative PSTH of DCN neural responses before (blue) and after (red) bimodal stimulation with Sp5 stimulation preceding sound by 10ms. The corresponding histogram (Fig. 4B) from the 10ms-pairing

interval demonstrates the percentages of DCN units showing long-lasting facilitation or suppression after bimodal stimulation.

2.3 Bimodal stimulation produces stronger long-lasting facilitation than suppression of responses to sound in A1

A1 responses, 5–10 minutes after paired Sp5-auditory stimulation at one pairing interval, revealed long-lasting facilitation and suppression to subsequent sound-only stimuli compared to pre-bimodal sound alone (Fig. 4C-D). Figure 4C shows one representative PSTH from a multi-unit cluster before (blue) and after (red) Sp5-auditory stimulation at a 10ms-pairing interval. The corresponding histogram from the 10ms-pairing interval demonstrates the percentages of A1 units showing long-lasting facilitation or suppression after bimodal stimulation. No appreciable differences in PSTH profiles were evident when comparing single units to multi-units, confirming that the observed neural responses are representative changes of the sampled neuron populations from both brain stations (Fig 5).

Fig. 6 shows the data from Figures 3 and 4 re-plotted as the mean change in firing rate for single- and multi-units showing facilitation (above zero) or suppression (below zero) for DCN and A1 with bimodal stimulation. The immediate effect, *bimodal response*, is shown in Fig. 6A and the long-lasting effect, *bimodal plasticity* is shown in Fig. 6B..

Immediate Effect (Bimodal Response)—DCN bimodal responses were facilitated across all pairing intervals with the most robust responses at 20ms. In contrast, DCN units showing the most robust suppression of the bimodal response occurred at the 10ms-pairing interval for multi-units. Single-unit DCN responses, while mirroring the multi-units for all other pairing-intervals, showed the greatest suppression at 20ms. In A1, the optimal pairing interval for bimodal facilitation also occurred at 20ms for multi-units, whereas the most robust suppression occurred at 20ms for single-units.

Long-lasting Effect (Bimodal Plasticity)—DCN responses exhibited bimodal plasticity at all intervals tested with a maximum facilitation at 10ms and similar suppression across intervals. The single unit responses showed maximal suppression at 20ms. In A1, bimodal plasticity occurred for facilitation at the 20 and 10 ms pairing intervals whereas minor long-term suppression occurred at all intervals in multi-units but was striking for single units at the 20ms pairing interval.

Overall, the responses in both brain regions at all pairing-intervals were similar between single- and multi-units, with the exception of 20ms, which showed the greatest suppression in bimodal response and plasticity.

The relationship between bimodal (dependent variable) and pre-bimodal responses to sound alone (covariate) as a function of brain region and pairing interval (independent variables) for both DCN and A1 was evaluated using a one-way analysis of covariance (ANCOVA). For multi-units that were either facilitated or suppressed following bimodal stimulation in DCN and A1, the effect was significant, $F(1,450)=6859.1$, $p<.001$; $F(1,318)=2874.5$, $p<.001$, respectively, as were values for single-unit recordings $F(1,28) = 81386$, $p<.001$; $F(1,75)=23788$, $p<.001$. The long-lasting effects (bimodal plasticity) of bimodal stimulation in both DCN and A1 were significant for multi-units that were facilitated and suppressed $F(1,406)=10580.4$, $p<.001$; $F(1,352)=55577.9$, $p<.001$, respectively, and for single-unit recordings $F(1,17)=68109$, $p<.001$; $F(1,2)=5660$, $p<.05$.

2.4 Effect of recording depth on bimodal responses and bimodal plasticity in A1

Single and multi-unit responses were compared across the 8 recording electrodes along each of the 4 shanks from shallow to deep. The immediate effects of bimodal stimulation resulted in the previously-described facilitated and suppressed responses, yet did not show any obvious changes across layers (Fig. 7, top panel A). Likewise, the long-lasting effects of bimodal stimulation did not show A1 layer-specific response properties (Fig. 7 lower panel B).

3. Discussion

The present results demonstrate immediate and long-lasting effects of bimodal, Sp5-auditory-stimulation on neural responses recorded simultaneously from both DCN and A1. The divergent single and multiunit responses to bimodal stimulation between these separate brain regions suggests that common Sp5 activation modulates neuronal activity across the central auditory pathway in different ways depending on the target region.

3.1 Bimodal stimulation produces immediate and long-term facilitation or suppression to sound in DCN

The observed bimodal responses of DCN units in the present study are consistent with previous findings in which preceding sound with Sp5 stimulation altered sound-evoked first spike latencies and firing rates for the duration of the sound stimulus (Shore, 2005; Koehler et al. 2011; Kanold et al. 2011). The long-lasting bimodal effect is similar to our recently described results (Dehmel et al. 2012). The variability in immediate and long-lasting effects of Sp5 stimulation on firing rate in the present study implies multiple underlying mechanisms. This is not unexpected since known anatomical projections and physiologic effects (Davis & Young, 1997; Kanold & Young, 2001; Zhou and Shore, 2004; Shore, 2005) implicate somatosensory activation of glycinergic inhibitory circuits, while the presence of GABA receptors on parallel fibers and apical pyramidal cell dendrites (Evans & Zhao, 1993; Juiz et al., 1994; Lujan et al., 2004) suggest that inhibitory synapses contribute to the suppression of immediate and lasting responses. In addition, glutamate receptors on cartwheel cell dendrites (Wright et al., 1996; Molitor & Manis, 1997; Mugnaini et al., 1997; Rubio & Wenthold, 1997; Petralia et al., 2000; Spatz, 2001) may be driven by somatosensory synaptic activity and potentially regulate the strength (Fujino & Oertel, 2003) of synaptic transmission within DCN. In the present study, the most robust effects on DCN neuronal firing properties were observed with shorter (10–20ms) interval pairings between Sp5 and sound. The responses to a short bimodal pairing interval may reflect differences in processing of auditory versus Sp5 inputs in DCN. For example, fusiform cells respond to acoustic input through auditory nerve fibers, while multimodal inputs are integrated by direct, excitatory input from parallel fibers to fusiform cells and indirectly through inhibitory input from parallel fibers through cartwheel cells (Fujino & Oertel, 2003). These complex excitatory and inhibitory relationships may help explain the bimodal distribution of responses (facilitatory versus suppressive) and temporal integration window to bimodal stimulation in the current results.

3.2 Bimodal stimulation produces more long-lasting suppression of responses to sound in A1

In the present study, bimodal stimulation in A1 also led to immediate suppression and facilitation of neuron spiking compared to that elicited by sound alone. These findings are interesting since A1 is a region traditionally viewed as unimodal (Schroeder and Foxe, 2005). However, A1 receives non-auditory input from multiple sources including feed-forward projections from “non-specific” thalamic inputs (Hackett et al. 1998b; Jones, 1998), feedback projections from higher-order multi-sensory cortical regions (Hackett et al. 1998a),

and direct lateral projections from low-level non-auditory cortices (Falchier et al. 2002; Cappe and Barone, 2005). The immediate facilitating effects of bimodal stimulation on A1 neuron spiking are not surprising since somatosensory stimulation produces perceptual amplification of auditory input (Schurman et al. 2004) and increased neural spiking in both caudal belt and A1 neurons in primate (Kayser et al. 2009), suggesting that multisensory influences are not restricted to secondary auditory areas but also occur in regions anatomically and functionally characterized as A1 (Kayser et al. 2008; 2009; Lakatos et al. 2007). The authors interpreted this as feed-forward somatosensory-related stimulation of A1 neurons that are initially activated within the outer, supra-granular layers of the primate (Lakatos et al. 2007). Our data demonstrating similar neural response properties across superficial and deep layers of A1 (Fig. 7) support the notion of feed-forward, already-processed, subcortical bimodal processing versus independent modulation at the cortical level. However, our data cannot completely exclude an alternate somatosensory pathway including lateral and feedback projections, both requiring transmission through the somatosensory cortex (Fig. 8). Future studies using current source density measures will be necessary to more specifically ascertain A1 layer response properties.

The facilitation of A1 neuronal firing with bimodal stimulation reflects the principles of temporal coincidence and inverse effectiveness (Stein and Meredith, 1993; Lakatos et al. 2007; Kayser et al. 2005), whereby multisensory interactions are strongest when both modalities are presented simultaneously versus sequentially. In the present study, Sp5-stimulation preceded sound stimulation and was thereby likely to modulate the auditory response, supporting the concept of neural oscillation and phase resetting. In that model, somatosensory integration enhances auditory processing by resetting the phase of neural oscillations so that concurrent auditory inputs arrive during a high-excitability phase and are thus amplified (Lakatos et al. 2007). Therefore, auditory inputs that are intimately associated with convergent somatosensory stimuli are enhanced above those inputs that have a random or weaker multisensory association. Alternatively, just as auditory (Lakatos et al. 2005) and non-auditory (Lakatos et al. 2007) stimuli may reset the oscillatory phase to enhance responses, resetting to the low-excitability phase, may result in neuron suppression.

In addition to the immediate effects, bimodal stimulation in the present study led to long-lasting suppression and facilitation of subsequent sound-evoked firing rates in A1. However, compared to the immediate effects, the long-lasting facilitation 5–10 minutes after pairing was not as strong in multi-units, but robust suppression at 20ms was observed in single-units. Previous studies have utilized tactile stimulation (Galambos et al., 1981; Lakatos et al., 2005; 2007) while the described effects in the present study were achieved with electrical activation of a somatosensory structure in an anesthetized preparation. This could lead to simultaneous activation of large volumes of neurons stimulated en masse without the influence of peripheral filtering processing. However, in spite of these technical considerations, an optimal pairing interval for bimodal effects at 20ms in both DCN and A1 in the present study is consistent with the largest super-additive effect in somatosensory-auditory interactions of 30–40ms in primate (Lakatos et al. 2007) and humans (Foxe et al. 2000; Murray et al. 2005).

3.3 Regional depth versus immediate and long-lasting effects in A1

The present data demonstrate that processing of auditory information following bimodal (Sp5-auditory) stimulation, is, at least in part, conserved from the DCN to A1. Facilitated neuronal firing above that elicited by sound alone was observed in both brain stations following bimodal stimulation. Since the response properties from both single- and multi-units were maintained across all layers of A1, the processing of bimodal information may be conveyed along the, “already-processed” lemniscal pathway from brainstem to A1, where enhanced firing is also seen, versus a “non-processed” circuit that depends largely on

integration at the cortical level (Fig. 8). Regardless, evidence of long-lasting enhancement and suppression of DCN and A1 responses to sound following bimodal stimulation suggests that non-auditory systems are able to modulate variable responses to stimuli within the auditory circuit with effects that may be persistent.

In animal models of noise-over-exposure that induce cochlear damage, increases in spontaneous rates, sound-driven activity and synchrony between neurons in DCN (Koehler et al., 2011; Shore et al., 2008), and auditory cortex (Eggermont, 2005) have been observed and proposed as neural correlates of tinnitus (Bauer et al., 2008; Brozoski et al., 2002; Kaltenbach et al., 1998). These changes, coupled with concurrent increases in excitatory inputs from the somatosensory system (Zeng et al., 2009) implicate a shift in the balance of bimodal integration, with a greater influence from somatosensory inputs. A shift from bimodal suppression in the intact DCN to bimodal facilitation in a tinnitus model (Dehmel et al., 2012) could, in part, account for the observed increases in spontaneous neural activity and sound driven responses in animals showing behavioral evidence of tinnitus. These changes at the level of the brainstem could have similar influences in A1, giving rise to tonal perception at the affected frequencies (Eggermont, 2008). The novel data in the present study, derived from simultaneous recordings from both DCN and A1, demonstrate that neuronal firing rates across both brain stations are influenced by the temporal interactions of multiple systems, both auditory and non-auditory. These results may have clinical implications for revealing the underlying mechanisms contributing to normal auditory processing and also in conditions of aberrancy including the generation of tinnitus perception.

4. Experimental Procedure

4.1 Surgical preparation

Experiments were performed on five mature, female, pigmented guinea pigs (250–350g; Elm Hill). All procedures were approved by the University of Michigan Committee on the Use and Care of Animals (UCUCA). Animals were anesthetized with ketamine (40 mg/kg) and xylazine (10 mg/kg), and held in a stereotaxic device (Kopf) with hollow ear bars for sound delivery. Rectal temperature was monitored and maintained at 38 ± 0.5 °C with a thermostatically controlled heating pad. Supplemental anesthesia (0.25–0.5mls initial dose) was given approximately hourly, after performing a digital pinch test to elicit paw withdrawal. Unit thresholds to broadband noise were monitored throughout the experiment to assess the physiologic condition of the animals. The bone overlying the cerebellum and posterior occipital cortex was removed, and a small amount of cerebellum was aspirated to reveal the surface of DCN. A contralateral craniotomy was subsequently performed over the right auditory cortex to identify the middle cerebral artery (Fig. 1C,D).

4.2 Acoustic stimulation

Acoustic stimuli were 50-ms tone bursts (1.5 ms rise fall times) presented at different levels to assess neural thresholds, best frequencies (BFs), latencies and rate-level functions. Stimuli were delivered to the left ear with a Beyer dynamic earphone (DT-770) coupled to the hollow ear bar using Tucker-Davis Technologies (TDT) system III hardware for digital-to-analog conversion and analog attenuation. Digital signals were generated and delivered to the TDT hardware by TDT software on a PC. Stimuli were generated using a sampling rate of 50kHz with 16-bit resolution. Tones were calibrated using a 1–4-inch condenser microphone (Bruel & Kjaer; Mic: 4136; Preamp: 2619; Power Supply: 2804) coupled to the ear bar with a 0.2-mL tube. Noise was calibrated with the 1–4-inch microphone and coupler attached to a sound level meter set to measure the bandwidth of interest (200Hz–20kHz for broadband noise). Equalization to correct for the system response was performed in the

frequency domain using digital filters implemented in TDT hardware. The stimulus variable sequences were generated in pseudorandom order from within Matlab. The maximum output of the system was 85dB SPL.

4.3 Trigeminal nucleus (Sp5) stimulation

Sp5 neurons were activated by passing current through a bipolar concentric stimulating electrode (Frederick Haer) directed towards the left Sp5 using stereotaxic coordinates (0.28cm left of midline, 0.2–0.3cm caudal to the transverse sinus, 0.9cm below surface of cerebellum; Fig. 1B,C). Current (5 pulses, 1000 Hz sec) amplitudes ranged from 50 to 80 μ A. The tip of the stimulating electrode was dipped in fluorogold before brain insertion to enable post mortem reconstruction of the electrode placements (Fig. 1A).

4.4 Bimodal stimulation

Paired Sp5-auditory (bimodal) stimulation consisted of a short burst of electrical activity in Sp5 followed 10–40ms later by a short BF tone burst (50ms, 20dB SL). To assess the effects of bimodal stimulation on DCN and A1 neural firing rates, responses to Sp5-tone pairings and acoustic stimuli alone following bimodal stimulation were compared with neural responses to acoustic stimuli preceding bimodal stimulation (50ms, BF tone bursts at 20dB SL; Fig. 2). Unimodal acoustic and bimodal trials were performed as test–retest blocks. Specifically, multiple presentations/test blocks (500 trials at 2/sec) of bimodal stimulation were presented at the same pairing interval while acoustic stimulation was presented (200 trials at 5/sec) alone.

4.5 Data acquisition

Recordings were made in a sound-attenuating single-walled booth. One separate four-shank, 32-channel silicon substrate electrode (100 μ m between sites, 250 μ m between shanks, 177 μ m² site area; Neuro-Nexus, Michigan, USA; Fig. 1C) was placed in the DCN (ipsilateral to the sound source) with a second, separate electrode placed in the contralateral A1 were used to record activity from DCN and A1 single- and multi-units simultaneously. Prior to brain insertion, the tips of the recording electrodes were dipped in fluorogold to enable post mortem reconstruction of electrode placements (Fig. 1A). The electrode was inclined to an angle of 35–45° from vertical and positioned on the DCN surface 0.5–0.75mm medial to the parafloccular recess (Fig. 1B,C). The tip of the electrode array was advanced 600 μ m below the surface of the DCN in a ventral-rostral direction. When necessary, the electrode was repositioned until robust responses to ipsilateral acoustic stimulation were obtained. The 4 shank probes were also placed in the contralateral A1, and penetrated approximately 2mm below the surface of the cortex (Fig. 1A,C) based on surface anatomy from previously published data (Wallace et al. 2000), to achieve optimal placement in a mid-range frequency. As with DCN, the electrode was repositioned only when needed until robust responses were obtained. After achieving final multi-site probe placement (Fig. 1C), an optimal stimulating frequency was chosen based on response maps to elicit responses from the maximum number of neurons from both DCN and A1. The 32-channel electrodes were each connected by a 32-channel pre-amplifier and digitizer to a TDT data-acquisition system. The signals were filtered from 300 to 7500Hz prior to analog-to-digital conversion. Analog-to-digital conversion was performed by simultaneous-sampling 12-bit converters at 25kHz per channel. A spike detection threshold was set independently for each recording channel to four standard deviations (SDs) above the mean background noise voltage. Time stamps and associated waveforms were recorded at each threshold crossing.

3.6 Offline Sorting

Voltages recorded from the multi-channel recording electrodes were digitized by a PZ2 preamp (Fs=12kHz, TDT, Alachua, FL, USA) and band-pass filtered (300 Hz 3 kHz) before online spike detection using a fixed voltage threshold set at 2.5 standard deviations above background noise (RZ2, TDT, Alachua, FL, USA). Spike waveform snippets and timestamps were saved to a PC using Open Explorer (TDT, Alachua, FL, USA). Waveform snippets were sorted using principle components of the waveform shape and Kmeans cluster analysis with fixed variance (95%) and 5 clusters (OpenSorter, TDT, Alachua, FL, USA). Clusters with a J2 value (4) above $1e-5$ were not considered well isolated and were combined. Single units were identified by consistency of waveform shape and amplitude. Spikes up to 15ms after the onset of current stimulation were contaminated by electrical artifact and ringing that occurred following current pulses and excluded from all analyses. While multi-unit clusters could not be identified as isolated single units, waveform shape, amplitude, and response properties were consistent over the duration of recording.

4.7 Data analysis

Post-experiment data analysis was performed in Matlab. Peri-stimulus time histograms (PSTHs), response maps and rate-level functions were generated from single and multi-unit activity from both DCN and A1. The effect of Sp5 stimulation on the firing rate in response to BF tones during and after bimodal stimulation was assessed using percent difference in firing rates. Any increase in firing rate was described as “facilitation,” while any decrease in firing rate interpreted as “suppression.” The *immediate* bimodal effect on the response to sound was calculated as follows: $100 \times (FR_b - FR_{u1}) / FR_{u1}$, where FR_b is the average firing rate in response to bimodal stimulation (Sp5 + tone) and FR_{u1} is the average firing rate in response to unimodal stimulation (tone) presented before bimodal stimulation. The *long-lasting* bimodal effect on the response to sound was calculated as follows: $100 \times (FR_{u2} - FR_{u1}) / FR_{u1}$ where FR_{u2} is the average firing rate in response to unimodal stimulation 5–10 minutes after bimodal stimulation. Average firing rates of unimodal responses were computed from 50–300 trials of a 50ms tone stimulation window. Average firing rates of bimodal responses were computed from 500 trials of bimodal stimulation.

4.8 Statistics

Statistical analysis was conducted for the raw changes in firing rates from both DCN and A1 in both immediate (bimodal-response) and long-lasting (bimodal-plasticity) bimodal responses. To evaluate the immediate responses, a one-way analysis of covariance (ANCOVA) was performed on the raw average firing rates to unimodal stimulation and compared to bimodal rates at each pairing interval (10, 20, and 40ms; SPSS 19.0; SPSS Inc.). To evaluate long-lasting effects, the unimodal responses were compared before and after bimodal stimulation. The dependent variable tested was either the response to bimodal stimulation (immediate) or response to sound following bimodal stimulation (long-lasting) with the independent variables being the pairing interval and brain region (DCN or A1). The covariant factor was the pre-test spiking (unimodal sound response) before each test condition.

4.9 Histology

The locations of the recording electrodes in DCN and A1 and the stimulating electrode in SP5 were verified post mortem. To mark the electrode tracks, the recording and stimulating electrodes were dipped in fluorogold (2%) before being inserted into the brain. At the end of each experiment the animal was sacrificed and the brain was removed from the skull and immersed in 4% paraformaldehyde for 48 hours followed by immersion in a 20% sucrose solution (Zhou & Shore, 2006). The following day, the brain was blocked by removing the

frontal lobe and cerebellum, mounted posteriorly on the chuck in mounting medium and cryosectioned at 40–60µm in the coronal plane through the entorhinal fissure to ensure consistent sectioning between animals. Sections were placed on slides and examined under epifluorescence to document recording and stimulating locations (Fig. 1A).

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Abbreviations

A1	primary auditory cortex
BF	best frequency
DCN	dorsal cochlear nucleus
PSTH	peri-stimulus time histogram
Sp5	spinal trigeminal nucleus

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Figure 1.

A. Magnified histologic region of A1 demonstrating fluorogold labeling and tract tracing confirming Michigan Probe electrode insertion in A1 (left; A1, primary auditory cortex; ERF, entorhinal fissure). B. Schematic demonstrating electrode array placement in the dorsal cochlear nucleus (DCN) in a rostral-caudal dorsal-ventral plane from the surface of the DCN and stimulating current probe placement within the spinal trigeminal nucleus (Sp5) for somatosensory stimulation. C. Surgical preparation showing simultaneous placement of probes in DCN (left; ipsilateral to sound source), Sp5 (middle; ipsilateral to sound source) and A1 (right; contralateral to sound source).

Figure 2.

Pairing protocol used to ascertain responses to immediate bimodal stimulation and long-lasting (5–10 minutes after pairing), persistent responses to sound following pairing. Responses to single level tones (Tone) were recorded during and after bimodal stimulation (pairing protocol). Recording blocks were randomly performed with varied pairing intervals between Sp5 always preceding tone (Pairing Intervals = 10, 20 or 40ms).

Figure 3.

The immediate effect of bimodal stimulation on DCN(A) and A1(B). The far left hand column in both DCN and A1 demonstrates peri-stimulus time histograms (PSTH) showing the immediate effects of somatosensory-auditory stimulation (bimodal; red) as compared to sound alone (blue; middle column) at various pairing intervals (40, 20 and 10ms from top to bottom). Histograms in the far-right hand column of both groups reveal the corresponding quantified bimodal effect presented as a percentage of units with values right of zero (vertical line) being facilitated and those left of zero being suppressed at each pairing interval. Black bar under PSTHs indicates tone duration.

Figure 4.

Bimodal stimulation results in long-lasting changes in DCN (A, B) and A1 (C, D). Upper panels (A and C) are peri-stimulus time histograms (PSTHs) demonstrating the long-lasting effects of somatosensory-auditory stimulation. Pre-bimodal responses are shown in blue; post-pairing responses to sound alone are shown in red. (Pairing interval = 10ms). Histograms for DCN (B) and A1 (D) demonstrate the long-term changes in firing rates following bimodal stimulation at a pairing interval of 10ms. Responses to the right of zero are facilitated, while those left of zero are suppressed. Black bar under PSTHs indicates tone duration.

Figure 5. Peri-stimulus time histograms (PSTH) of responses from single and multi-unit analysis from DCN and A1 ((DCN-SU; A1-SU; DCN-MU; A1-MU). Long-term effects (Bimodal Plasticity; right column) of somatosensory-auditory stimulation are compared to sound alone (Pre-Tone; left column) for a 20ms pairing interval. Note the similarities in PSTH profiles between multi- and single-unit responses from both brain regions. Black bars under PSTHs indicate tone durations.

Figure 6.

Mean effects of immediate (A; bimodal response) and long-lasting (B; bimodal plasticity) bimodal stimulation in DCN and A1. The percent change in firing rate after bimodal stimulation is plotted at each bimodal pairing interval. Values above zero represent facilitation; values below zero represent suppression. Single-unit responses are plotted for DCN (open circles) and A1 (filled-circles). Similar responses occur for single- and multi-units at all pairing-intervals in both brain stations with the exception of maximal suppression at 20ms in both bimodal response and plasticity.

Figure 7.

Scatter plot demonstrating the immediate (Bimodal response; top) and long-lasting (Bimodal plasticity, bottom) effects of bimodal stimulation in A1 as a function of cortical depth at each pairing interval. The percent of units that are facilitated or suppressed are shown across cortical depths from shallow to deep. No significant trend is seen for facilitated or suppressed units across cortical depth. Single-units (filled triangles); Multiunits (circles).

Figure 8.

Anatomic diagram demonstrating the “already-processed” lemniscal auditory pathway and Sp5 innervation to the DCN. A secondary/”alternate” somatosensory pathway includes Sp5 projections to thalamus to somatosensory cortices and the auditory cortex belt region.