

Synaptic Input Variability Drives Functional Heterogeneity for Sound Encoding in Globular Bushy Cells

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Table of Contents

Summary	1
Zusammenfassung	3
Abbreviations	5
Figures.....	7
1. Introduction.....	9
1.1 Auditory system overview	9
1.2 GBCs and excitatory convergence	14
1.2.1 Morphology and synaptic specializations of GBCs.....	15
1.2.2 Electrophysiological signatures and identification	17
1.2.3 Convergence and heterogeneity of endbulb inputs	17
1.2.4 Implications for temporal coding.....	18
1.3 Inhibitory control at GBCs.....	19
1.3.1 Sources of inhibition	19
1.3.2 Kinetics and short-term dynamics	21
1.3.3 Functional effects on temporal coding	21
1.4 Research gap and approach	22
2. Aims	24
3. Materials and Methods	25
3.1 Key resources table	25
3.2 Animal.....	25
3.3 Acute brain slice preparation	26
3.4 Electrophysiology	26
3.5 Stimulus Template	27

3.5.1 Simulating auditory nerve fiber input	27
3.5.2 Simulating inhibitory input.....	28
3.5.3 Modeling synaptic input variation	29
3.5.4 Generating the synaptic conductance templates.....	29
3.5.5 Conductance Clamp Implementation	32
3.6 Post-hoc identification of GBCs	33
3.7 GBC Model	33
3.7.1 Globular bushy cell model	33
3.7.2 Model parameters and validation	34
3.8 Experimental design and data analysis	36
3.8.1 Data analysis.....	36
3.8.2 Statistical analysis	36
4. Results.....	38
4.1 Validation of Conductance Clamp Implementation	38
4.2 Impact of Excitatory Input Variation on GBC Encoding.....	41
4.2.1 Synaptic Input Variation Increases GBC Firing Rate but Reduces Temporal Precision	41
4.2.2 Higher Input Variation Shortens AP Latency but Increases Temporal Jitter ..	43
4.2.3 Input Variation Enhances Firing Rate Tracking to Amplitude-modulated Sounds	45
4.2.4 Impact of Input Variation is Consistent with Morphological Plausible Input Sizes	49
4.2.5 Effects of input variation are well reproduced in a coincidence-counting model	52
4.3 Inhibitory Modulation of GBC Encoding under Excitatory Input Variation	54

4.3.1 Establishing Excitation-Inhibition Balance by Titrating Inhibitory Strength....	54
4.3.2 Synaptic Input Variation Preserves Its Temporal Tradeoff Effects Under Balanced Inhibition	57
4.3.3 Inhibition Modulates the Effects of Input Variation on GBC Onset Responses	60
4.3.4 Inhibitory Inputs Enhance Temporal Precision but Reduce Rate-Based Envelope Tracking in GBCs	63
5. Discussion	68
5.1 Input heterogeneity shapes GBC encoding strategy	68
5.2 Role of Inhibition in Regulating GBC Encoding Strategies	71
5.3 Experimental Limitations and Physiological Relevance	74
Supplementary.....	76
Publication	84
Acknowledgements.....	85
Versicherung.....	97

Summary

Most neurons in the central nervous system receive convergent input and thereby integrate activity from many other neurons. In sensory pathways, particularly the auditory brainstem, such convergence can enhance sensitivity or sharpen temporal precision when individual inputs are subthreshold and spikes are generated only by multiple coincident inputs (coincidence detection). Within the cochlear nucleus (CN), globular bushy cells (GBCs) receive large axosomatic synapses—the endbulbs of Held—from auditory nerve fibers (ANFs) and transmit their activity to binaural targets with high temporal fidelity. Each GBC is contacted by multiple endbulbs that vary in size, reaching up to a ten-fold difference between the smallest and largest input. To date, it remains unclear how this morphological heterogeneity contributes to function, namely the encoding of sound information.

This thesis investigates how variability in the strength of converging endbulb inputs shapes GBCs' output, and how inhibition contributes to auditory encoding. To address this, I performed conductance-clamp recordings in acute brain slices of Mongolian gerbils. Excitatory input patterns were constructed to span anatomically informed ranges of input-strength variation, and inhibitory drive was included to test its regulatory role. Responses were assessed for simple pure-tone and naturalistic amplitude-modulated (AM) sounds.

Three consistent results emerged. First, greater variability among excitatory inputs increased overall firing and improved tracking of stimulus envelopes. Second, the same variability reduced spike-timing precision, revealing a trade-off between rate-based envelope coding and faithful temporal coding. Third, inhibition acted primarily as gain control: it decreased sustained spiking and sharpened timing without eliminating reliable onset responses. Together, these findings support a framework in which anatomical heterogeneity positions GBCs along a functional continuum, from precise temporal transfer to improved envelope coding. Inhibitory inputs primarily regulate gain and refine timing, improving onset precision for pure tone and enhancing locking to AM.

By combining conductance-clamp recordings with a coincidence-counting cell model (developed in collaboration), this work defines how input-strength variability and inhibition jointly set the timing-rate trade-off in GBCs. The data explain how the operation mode can

shift depending on the information provided to the downstream binaural pathways, advancing a mechanistic understanding of the structure-function principles that support robust hearing in natural acoustic environments.

Zusammenfassung

Die meisten Neuronen im Zentralnervensystem erhalten konvergierende Eingänge und integrieren die Aktivität vieler anderer Neuronen. In sensorischen Bahnen, insbesondere im auditorischen Hirnstamm, kann eine solche Konvergenz die Sensitivität oder die zeitliche Präzision erhöhen, wenn einzelne Eingänge unterschwellig sind und Aktionspotenziale nur dann entstehen, wenn mehrere Eingänge innerhalb eines engen Zeitfensters nahezu gleichzeitig eintreffen (Koinzidenzdetektion). Globuläre Buschzellen (GBCs) im Cochleariskern (CN) erhalten große axosomatische Synapsen—die Held'schen Endknöpfe—von Hörnervenfaser (ANFs) und leiten deren Aktivität mit hoher zeitlicher Präzision an binaurale Zielstrukturen im auditiven Hirnstamm weiter. Jede GBC wird von mehreren Endknöpfen kontaktiert, die in ihrer Größe stark variieren und Unterschiede von bis zum Zehnfachen zwischen dem kleinsten und dem größten Eingang aufweisen. Bislang wurde nicht erforscht, wie diese Größenheterogenität die Kodierung von Schallinformation beeinflusst.

Diese Arbeit untersucht, wie Variationen der Eingangsstärken konvergierender Endknöpfe die Schallkodierung von GBCs definieren und wie synaptische Hemmung die Schallkodierung verbessert. Hierzu habe ich Conductance-Clamp-Ableitungen in akuten Hirnschnitten der Mongolischen Rennmaus durchgeführt. Erregende Eingabemuster wurden so konstruiert, dass sie die morphologische Variabilität der Eingangsstärke abdeckten. Die Implementierung der Inhibition verdeutlichte deren spezifische Rolle bei der Kodierung von Reintönen und amplitudenmoduliertem (AM) Schall.

Die Studie liefert drei konsistente Erkenntnisse. Erstens erhöhte eine größere Variabilität unter den erregenden Eingängen die Gesamtfeuerrate und verbesserte die Abbildung der Hüllkurve des auditorischen Stimulus. Zweitens verringerte dieselbe Variabilität die Präzision des Spike-Timings und offenbarte einen Kompromiss (trade-off) zwischen ratenbasierter Hüllkurvenkodierung und präziser zeitlicher Kodierung. Drittens wirkte Hemmung vor allem als Verstärkungsregelung: Sie reduzierte anhaltendes Feuern und schärfte das Timing, ohne die Onset-Antworten zu verringern. Zusammengefasst zeigen diese Ergebnisse, dass GBCs entlang eines funktionellen Kontinuums operieren: von präziser zeitlicher Übertragung bis hin zu ausgeprägter Hüllkurvenkodierung. Die

Inhibition übernimmt dabei vor allem zwei Funktionen: sie reguliert die Verstärkung (gain) und verfeinert das Timing in dem sie die Onset-Präzision bei Reintönen verbessert und die Phasenbindung an AM verstärkt.

Durch die Kombination von Conductance-Clamp-Ableitungen mit einem Coincidence-Counting-Zellmodell (entwickelt in Kooperation) zeigt diese Arbeit, wie Variabilität der Eingangsstärke und Hemmung gemeinsam den Funktionsmodus zwischen Timing- und Rate-Kodierung in GBCs festlegen. Somit wird der Ausgang dieses Neurontyps an die Verarbeitung in den Nachgeschalteten binauralen Neuronen des Hirnstamms angepasst. Die Studie liefert einen wichtigen Beitrag zum mechanistischen Verständnis jener Struktur-Funktion-Prinzipien, die die Grundlage für robustes Hören in natürlichen Umgebungen bilden.

Abbreviations

aCSF	Artificial cerebrospinal fluid
AN	Auditory nerve
ANF	Auditory nerve fiber
AP	Action potential
AVCN	Anteroventral cochlear nucleus
BC	Bushy cell
CF	Characteristic frequency
CI	Correlation index
CN	Cochlear nucleus
DCN	Dorsal cochlear nucleus
DNLL	Dorsal nucleus of the lateral lemniscus
EI	Entrainment index
EPSC	Excitatory postsynaptic current
EPSP	Excitatory postsynaptic potential
GBC	Globular bushy cell
IC	Inferior colliculus
ILD	Interaural level differences
INLL	Intermediate nucleus of the lateral lemniscus
IPSC	Inhibitory postsynaptic current
ITD	Interaural time differences
LSO	Lateral superior olive
MGB	Medial geniculate body
MNTB	Medial nucleus of the trapezoid body
MSO	Medial superior olive
NLL	Nuclei of the lateral lemniscus
PSTH	Peristimulus time histogram
PVCN	Posteroventral cochlear nucleus
SAM	Sinusoidally amplitude modulated
SBC	Spherical bushy cell
SD	Standard deviation
SGN	Spiral ganglion neuron
SOC	Superior olivary complex

VCN	Ventral cochlear nucleus
VNLL	Ventral nucleus of the lateral lemniscus
VS	Vector strength

Figures

Figure 1. Outer, middle, and inner ear anatomy.

Figure 2. Auditory ascending pathway.

Figure 3. The two primary circuits of binaural interaction.

Figure 4. Para-sagittal slice of CN.

Figure 5. Synaptic organization of a GBC.

Figure 6. Response patterns of BCs.

Figure 7. 3D reconstructions of endbulb of Held terminals on a mouse GBC.

Figure 8. Schematic representation of the convergence of ANF (SGC) inputs onto BCs and local inhibitory interneurons in the cochlear nucleus.

Figure 9. Schematic of conductance clamp stimulation paradigm.

Figure 10. Synaptic input strength distributions derived from anatomical reconstructions and model conditions.

Figure 11. Validation of the conductance clamp system used for conductance injection in BCs.

Figure 12. Quantifying the temporal offset between experimental and theoretical current traces in a GBC.

Figure 13. Increased synaptic input variability enhances firing rate but reduces temporal precision in GBCs.

Figure 14. Increased synaptic input variability elevates sustained firing rates but reduces the precision of onset responses in GBCs.

Figure 15. Variability in synaptic inputs improves GBC responses to amplitude-modulated stimuli.

Figure 16. Morphological synaptic input variability underlies functional heterogeneity in GBC responses to pure tones.

Figure 17. Morphologically defined input variability shapes GBC coding of SAM sounds.

Figure 18. An adaptive coincidence-detection model accounts for GBC responses to synaptic input variability.

Figure 19. Constant inhibitory conductance modulates GBC firing rate and reveals frequency-dependent excitation-inhibition balance.

Figure 20. Synaptic input variation preserves its temporal tradeoff effects under balanced inhibition.

Figure 21. Inhibition does not affect temporal precision under pure-tone stimulation.

Figure 22. Influence of combined synaptic input variability and inhibition on GBC onset response properties.

Figure 23. Inhibition prolongs the latency of onset responses but decreases the jitter without affecting onset spike probability.

Figure 24. Effects of excitatory input variability on GBC encoding of AM stimuli under inhibitory conductance.

Figure 25. Inhibition specifically enhances temporal precision but decreases rate-coding of AM sounds.

Figure S1. Onset response of GBC model.

Figure S2. GBC model responses to SAM stimuli

Figure S3. Morphological input variation reproduces the pure-tone timing-rate trade-off in the GBC model.

Figure S4. Morphology-derived input variation reproduces the SAM timing-rate trade-off in the GBC model.

Figure S5. Reduction of APs under pure-tone stimulus with inhibitory conductance.

Figure S6. SAM-evoked responses under the excitation and inhibition (exc+inh) condition.

Figure S7. Fitting rate reduction induced by inhibition at different AM modulation frequencies.

1. Introduction

Hearing depends on the brain's encoding of temporal, spectral, and intensity features of sound. To support accurate perception and sound localization, these features must be transmitted with high fidelity from the periphery to the central auditory system. The auditory brainstem plays a critical role in this process, as its circuits preserve and refine timing cues with sub-millisecond precision (Joris & van der Heijden, 2019; Keine & Englitz, 2025).

The auditory system relies particularly on precise temporal information because acoustic signals unfold over time, and many perceptual tasks, such as sound localization and speech perception, depend on sub-millisecond spike timing. In the cochlear nucleus (CN), the first station of the central auditory system, globular bushy cells (GBCs) transmit activity from auditory nerve fibers (ANFs) to the medial nucleus of the trapezoid body (MNTB), which provides precisely timed inhibition to binaural nuclei (Smith et al., 1998; Joris & Trussell, 2018). GBCs receive multiple large axosomatic terminals from ANFs, i.e., endbulbs of Held, the number and size of which vary substantially (Spirou et al., 2023). However, the functional consequences of this input heterogeneity for GBCs' output are not well defined. Therefore, this study examined how heterogeneity in the distribution of excitatory input strengths affects GBC encoding and how inhibitory mechanisms modulate this process.

1.1 Auditory system overview

Natural sounds vary over multiple time scales in both amplitude (envelope) and spectral content, and they span a wide dynamic range (Wen et al., 2009). Acoustic signals can be described at two temporal scales: the rapid fine structure, corresponding to individual cycles of the waveform, and the slower amplitude envelope, which reflects fluctuations in sound intensity over time. The auditory system transforms these features into action potentials (APs) conveyed through parallel brainstem pathways. Among these features, temporal precision is crucial: spatial hearing relies on interaural time differences of only a few hundred microseconds (Grothe et al., 2010; Brughera et al., 2013), and speech perception depends on accurate encoding of rapid fluctuations in the sound waveform (Shannon et al., 1995; Rosen, 1997). Thus, preserving fine temporal information in the auditory brainstem is

fundamental in complex acoustic environments.

From the periphery, sound is funneled by the pinna and ear canal to the tympanic membrane (Pickles, 1982). These pressure oscillations are amplified by the middle-ear ossicles (impedance matching) to the oval window of the inner ear (Fig. 1). Movement of the stapes footplate at the oval window drives a pressure wave in the cochlear fluid, which is released at the round window. Hair cells convert these mechanical vibrations into receptor potential, leading to glutamate release at their synapses, which activates the spiral ganglion neurons (SGNs) (Hudspeth, 2014). Then the ANFs (axons of SGNs) transmit these signals to the CN. In vivo recordings from ANFs reveal phase locking, in which spike times recur at similar phase positions within each cycle of the stimulus. This synchronization is robust at low-mid frequencies (up to a few kilohertz in small mammals; <3 kHz in gerbils) while at higher frequencies, where fine-structure locking is lost, synchronization persists to the slower amplitude envelope (typically tens to a few hundred hertz) (Palmer & Russell, 1986; Joris & Yin, 1992; Versteegh et al., 2011; Heeringa et al., 2020).

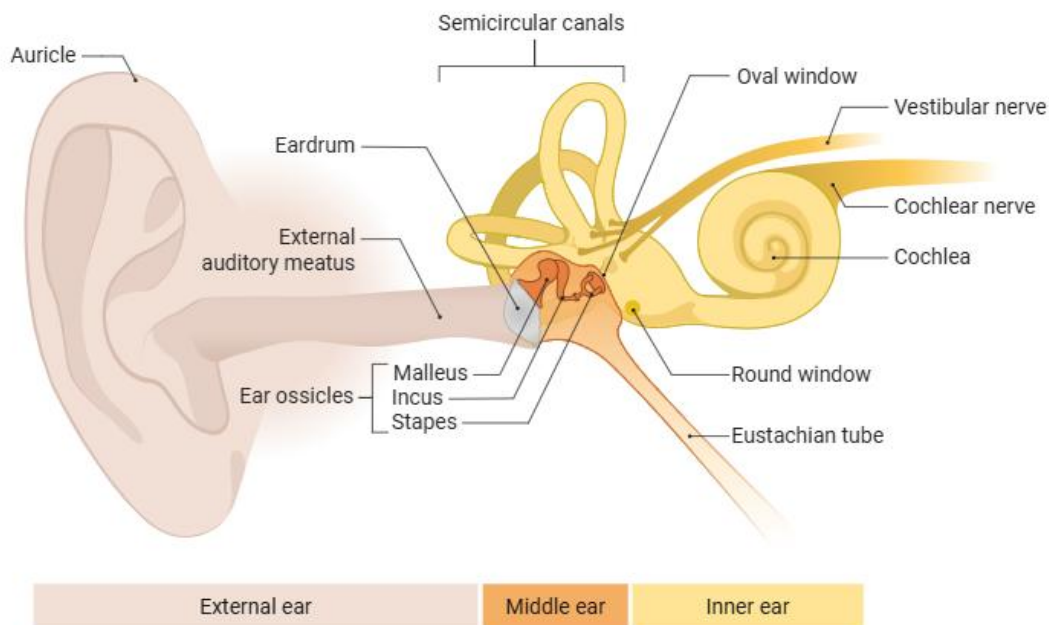


Figure 1. Outer, middle, and inner ear anatomy. The outer ear (pinna, ear canal) funnels sound to the tympanic membrane. The middle ear ossicles (malleus, incus, stapes) transmit vibrations to the oval window. In the inner ear, the cochlea converts these vibrations into neural signals via hair cells and spiral ganglion neurons. Created with BioRender.com.

On entering the brainstem, ANFs bifurcate in the CN to form ascending and descending branches, distributing their synaptic terminals across the CN subdivisions. The ascending branch mainly projects to the anteroventral cochlear nucleus (AVCN), whereas the descending branch contacts the posteroventral cochlear nucleus (PVCN) and the dorsal cochlear nucleus (DCN) (Fekete et al., 1984). In the ventral cochlear nucleus (VCN), several neuron classes receive direct ANF input; among them, bushy cells (BCs), which represent a principal class specialized for temporal precision (Cao & Oertel, 2010; Campagnola & Manis, 2014; Joris & Trussell, 2018; Ashida et al., 2019). Their short dendrites and low input resistance minimize electrotonic filtering and shorten the membrane time constant, which together with large endbulbs of Held inputs enable rapid membrane responses. These mechanisms promote reliable spike initiation with low-jitter and preserve peripheral timing information (Osen, 1969; Joris et al., 1994; Cao & Oertel, 2010; Joris & Trussell, 2018).

BCs' axons provide input to the binaural nuclei in the brainstem involved in sound localization (Smith et al., 1998; Joris & Trussell, 2018). These nuclei are part of the superior olivary complex (SOC), which comprises the medial superior olive (MSO), lateral superior olive (LSO), MNTB, and superior paraolivary nucleus (SPN). Two processes underlie sound localization in the azimuthal plane: the computation of interaural time differences (ITDs) and interaural level differences (ILDs). ILDs are computed mainly in LSO by integrating ipsilateral excitation with contralateral inhibition conveyed via MNTB (Yin, 2002; Grothe et al., 2010). ITDs are computed in MSO, which receives fast excitation from both CN together with temporally precise inhibition from the MNTB and the lateral nucleus of the trapezoid body (Hassfurth et al., 2010). ITD and ILD must be estimated with sub-millisecond resolution and remain reliable even in noisy environments (Klumpp & Eady, 1956; Grantham, 1984; Zwicker & Zwicker, 1984; Keine & Englitz, 2025). Downstream nuclei in the midbrain (inferior colliculus (IC)), thalamus (medial geniculate body (MGB)), and the auditory cortex integrate these signals to generate perception and guide behavior (Fig. 2).

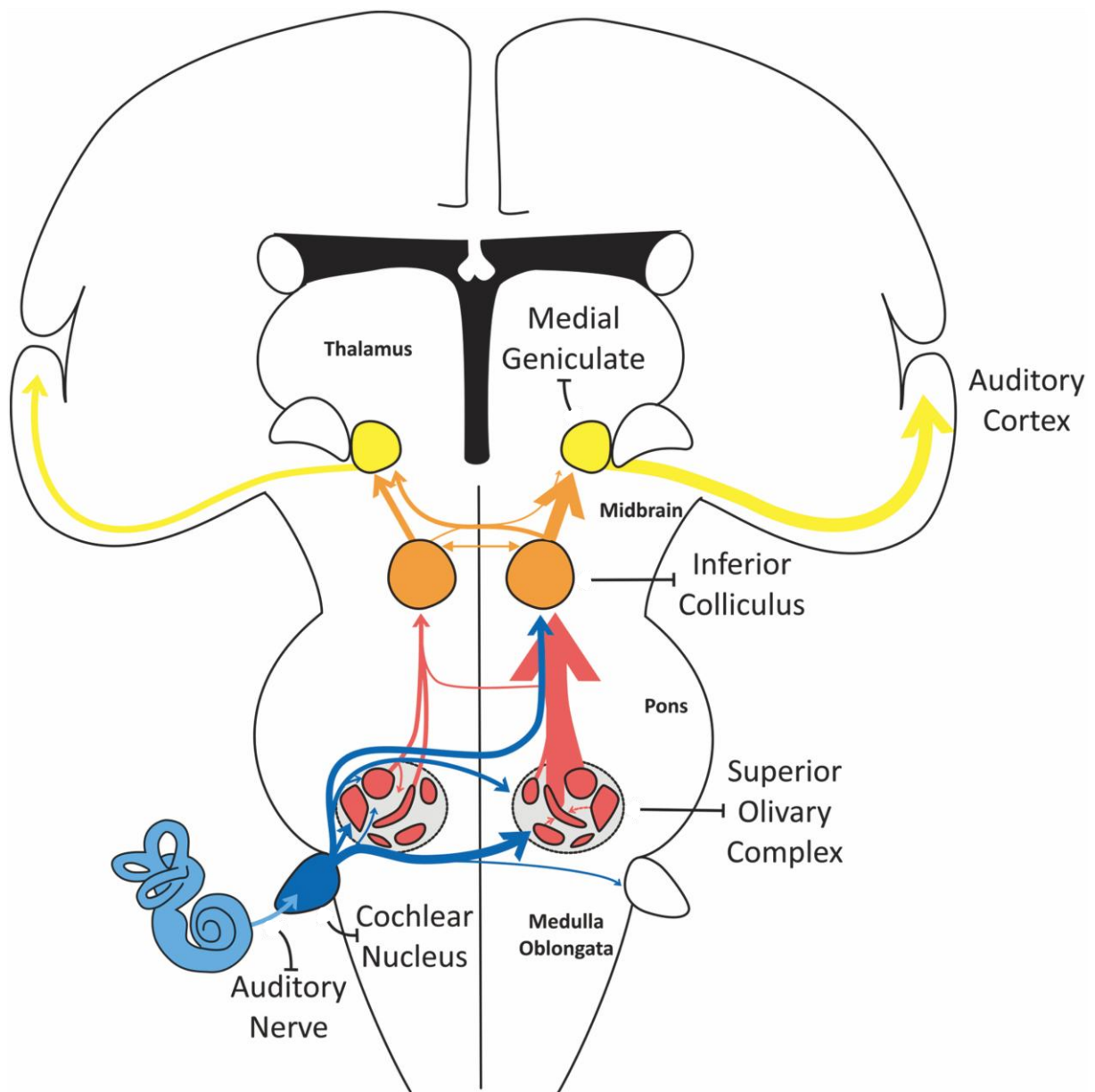


Figure 2. Auditory ascending pathway. Peripheral input arises from spiral ganglion neurons (SGNs) whose axons form the auditory nerve fibers (ANFs). The ANFs innervate subdivisions of the cochlear nucleus. The cochlear nucleus projects bilaterally to the nuclei of the superior olivary complex and to the contralateral inferior colliculus. The inferior colliculus projects to the medial geniculate body, which in turn projects to the auditory cortex. Adapted from Smith et al., 2019.

BCs in the VCN comprise two principal classes—GBCs and spherical bushy cells (SBCs). Both receive axosomatic endbulbs of Held from ANFs, but their input complement differs in number and morphology: GBCs receive a larger number of “modified” endbulbs that are typically smaller, whereas SBCs are contacted by a few large “classical” endbulbs (Spirou et al., 2005; Yu & Goodrich, 2014; Spirou et al., 2023). GBCs project predominantly to the

contralateral MNTB, where they form calyces of Held (giant axosomatic terminals on principal MNTB neurons specialized for rapid, reliable transmission) (Spirou et al., 1990; Smith et al., 1998; Felmy & Schneggenburger, 2004). This pathway provides the primary excitatory drive to the MNTB, which delivers precisely timed feedforward glycinergic inhibition to MSO, LSO, SPN, and ventral nucleus of the lateral lemniscus (VNLL) (Spangler et al., 1985; Kuwabara & Zook, 1992; Smith et al., 1998; Rajaram et al., 2020). SBCs preserve the timing of ANF spikes and provide temporally precise excitatory drive to binaural nuclei such as the MSO and LSO (Fig. 3). Through the MNTB, GBC output provides the precisely timed inhibitory counterpart to the SBC excitation, and thus the fidelity of GBC output critically constrains binaural computations that operate on sub-millisecond timescales (Joris et al., 1994; P. X. Joris et al., 1994; Yu & Goodrich, 2014; Keine & Englitz, 2025).

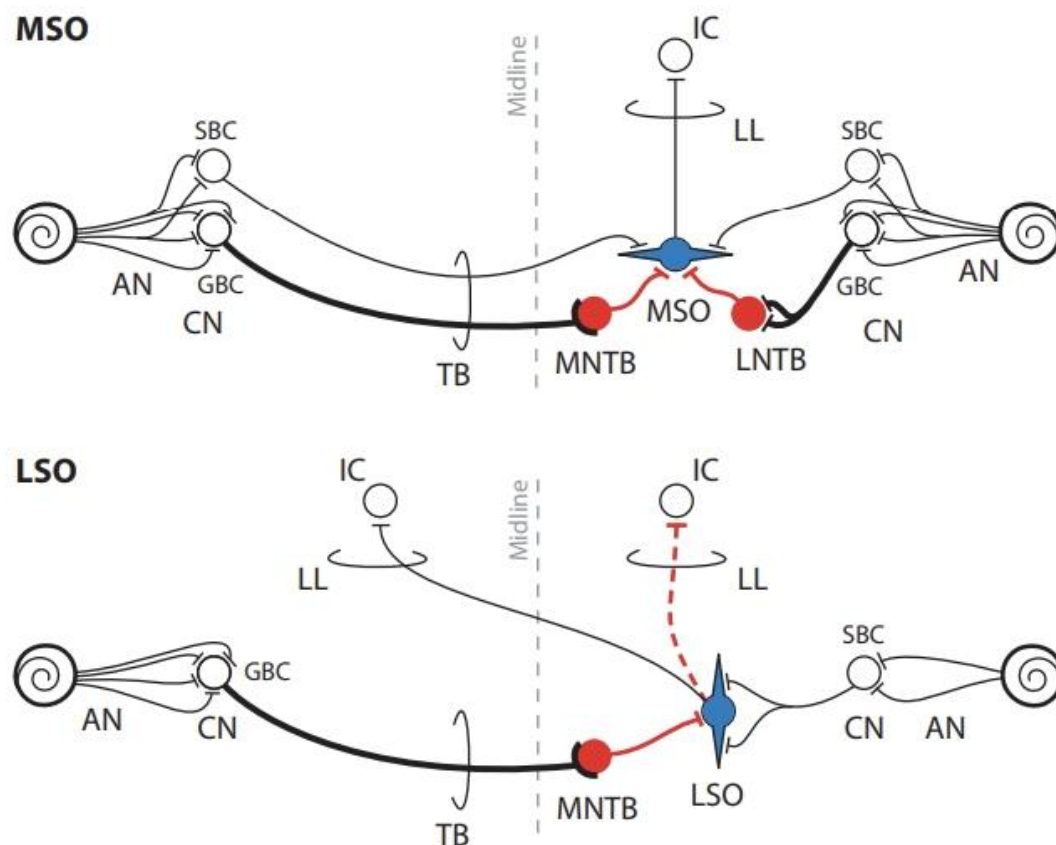


Figure 3. The two primary circuits of binaural interaction. Trapezoid body (TB) and lateral lemniscus (LL) are fiber tracts, with inhibitory projections (red) and excitatory projections (black) indicated. GBCs in the CN provide excitatory drive to the MNTB, which conveys precise inhibition to the MSO and LSO. Adapted from Joris & van der Heijden, 2019.

The endbulbs are excitatory, glutamatergic synapses eliciting large postsynaptic currents in GBCs, dominated by fast AMPA receptors (GluA3/GluA4), with comparatively small NMDA contribution in mature animals (Bellingham et al., 1998; Wang et al., 1998; Rubio et al., 2017). These properties enable GBCs to preserve temporal fidelity in their output, supporting the precise encoding of timing cues. Consistent with this requirement, heterogeneity in the number and size of converging endbulbs alters effective synaptic strength and convergence, potentially shifting the balance between spike-timing precision and rate/envelope coding. I therefore examined how the distribution of excitatory input strengths to GBCs shapes this balance at the first central synapse.

1.2 GBCs and excitatory convergence

In the VCN, the two major principal classes are BCs (SBCs and GBCs) and stellate cells. GBCs are located in the caudal AVCN near the ANF root and along the border with the posteroventral division. Figure 4 shows the locations of the major cell types in the cat CN and provides anatomical landmarks for identifying GBCs because the overall arrangement is broadly conserved across mammals (Ostapoff & Morest, 1991; Gómez-Nieto & Rubio, 2011; Godfrey et al., 2016).

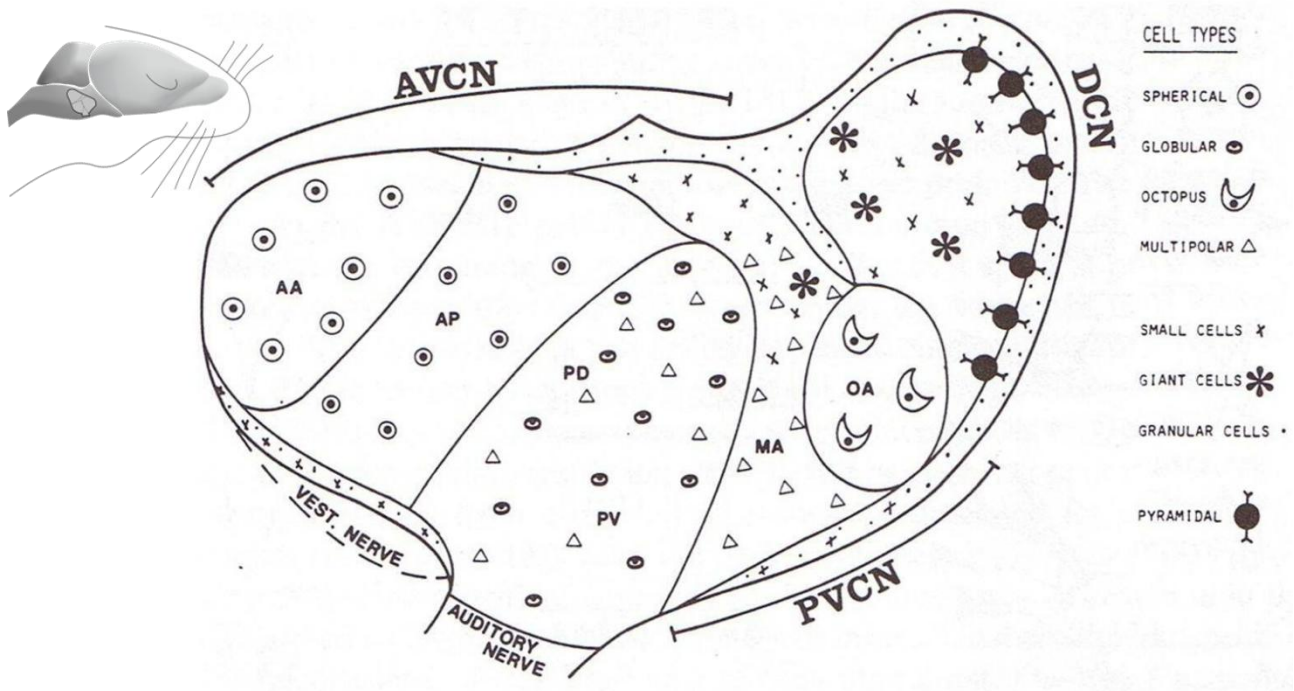


Figure 4. Para-sagittal slice of CN. Scheme of the CN with its subdivisions (lateral view, cat). AA: anterior part of the anterior division of AVCN; AP: posterior part of the anterior division of AVCN; AVCN: anteroventral cochlear nucleus; DCN: dorsal cochlear nucleus; MA: multipolar cell area; OA: octopus cell area; PD: dorsal part of the posterior division of AVCN; PV: ventral part of the posterior division of AVCN; PVCN: posteroventral cochlear nucleus, modified from Osen, 1969; Bahmer, 2007.

1.2.1 Morphology and synaptic specializations of GBCs

GBCs show features associated with precise temporal transmission (Ryugo & Fekete, 1982; Spirou et al., 2005). BCs in general have low input resistances (30-100 MΩ) and short membrane time constants (0.5-2 ms) (Cao et al., 2007); together with their compact dendrites, these properties shorten EPSPs and limit temporal summation, supporting precise spike timing. Compared to SBCs, GBCs exhibit significantly lower input resistance and shorter time constants (Jing et al., 2025). Excitatory input is dominated by endbulbs of Held (Fig. 5, blue), which contain multiple presynaptic active zones with closely apposed postsynaptic densities at the somatic membrane (Nicol & Walmsley, 2002). This arrangement enables large, fast, and reliable excitatory currents with minimal electrotonic filtering, supporting high-fidelity spike initiation in GBCs (Nicol & Walmsley, 2002; Cao et al., 2007; Cao & Oertel, 2010).

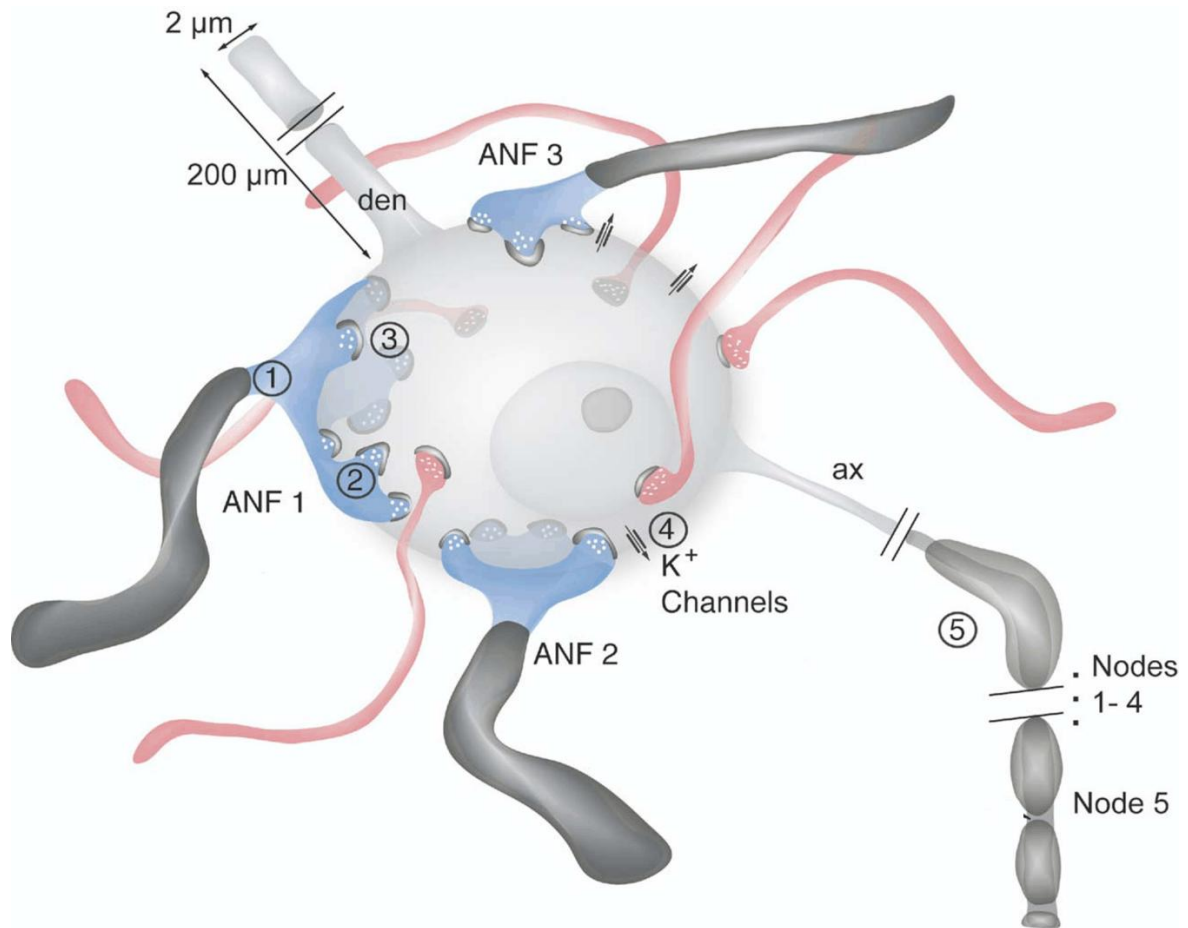


Figure 5. Synaptic organization of a GBC. Multiple ANFs converge on the soma via endbulbs of Held (blue; examples ANF 1-3). Terminals differ in size (1) and in the number of active zones (2); each synapse engages a compact postsynaptic receptor assembly (3). A short dendrite (den) and the axon (ax) are indicated. Small red boutons denote inhibitory contacts discussed later. Modified from Joris & van der Heijden, 2019.

The endbulb-GBC synapses provide powerful and synchronous excitation, while the low input resistance and short membrane time constant of GBCs result in a short effective integration window that promotes highly precise spike timing (Wang & Manis, 2006; Cao et al., 2007). Based on their morphological and biophysical properties, GBCs achieve higher temporal precision than SBCs under comparable conditions (Joris et al., 1994; Ashida et al., 2019; Jing et al., 2025). In response to sound, GBCs generate short-latency, low-jitter spikes. At low to mid frequencies, their discharges phase lock to the fine structure of the waveform. At higher carrier frequencies, where fine-structure locking deteriorates, GBCs instead synchronize to the slower amplitude envelope, which represents fluctuations in sound intensity over time. In both cases, this precise temporal coding provides an early timing signal to downstream binaural circuits (Joris et al., 1994; Cao et al., 2007; Joris & Trussell,

2018).

1.2.2 Electrophysiological signatures and identification

The morphological and synaptic specializations of GBCs are matched by distinctive electrophysiological signatures. In current-clamp, GBCs fire one or a few APs (Fig. 6B) at the onset of suprathreshold depolarizing current injections (whereas SBCs usually fire a single AP) and show a pronounced voltage sag during hyperpolarizing steps mediated by I_h conductance (Cao et al., 2007; Cao & Oertel, 2011). In vivo, GBCs typically show a primary-like-with-notch peristimulus time histogram (PSTHs) pattern, whereas SBCs show primary-like PSTHs (Smith et al., 1991; Typlt et al., 2012) (Fig. 6A).

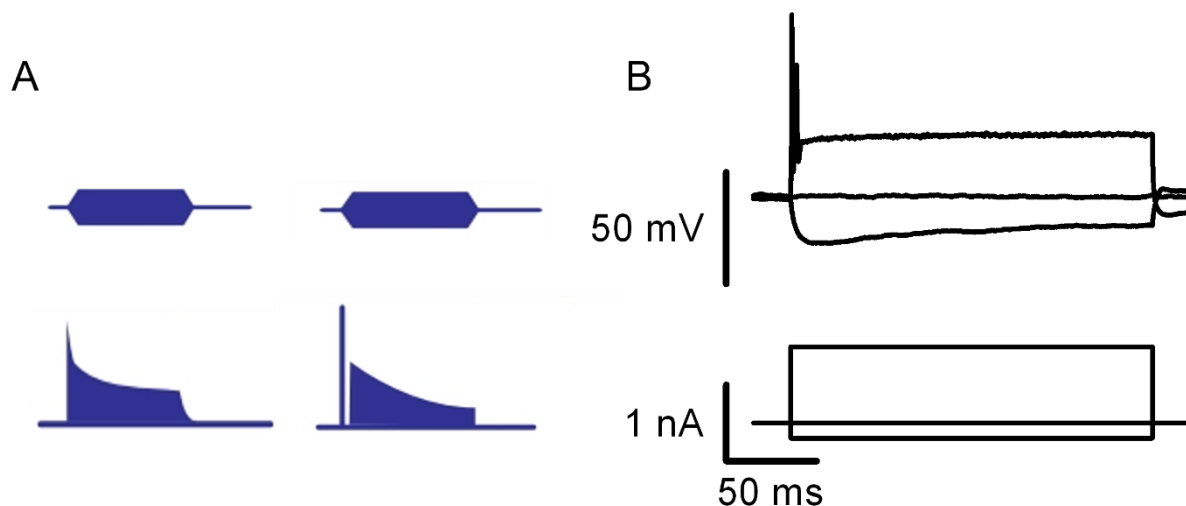


Figure 6. Response patterns of BCs. (A) Top: schematic of a tone stimulus. Bottom left: SBCs typically exhibit a primary-like PSTH pattern. Bottom right: GBCs often display a primary-like-with-notch PSTH pattern. Modified from (Squire et al., 2013). (B) Electrophysiological characteristics. Top: response to suprathreshold depolarizing current injection, showing one or a few action potentials at stimulus onset and a voltage sag elicited by a hyperpolarizing current injection. Bottom: Stimulus current.

1.2.3 Convergence and heterogeneity of endbulb inputs

GBCs receive input from multiple endbulbs that differ in size and number (around 20 in cat and 5-12 in mouse, Fig. 7) (Spirou et al., 2005; Spirou et al., 2023). Since larger terminals typically contain more active zones and larger pools of readily releasable vesicles, endbulb size differences translate into different EPSC amplitudes at the same postsynaptic neuron

(Nicol & Walmsley, 2002; Sätzler et al., 2002; Wong & Xu-Friedman, 2022; Spirou et al., 2023). Consequently, whether a GBC reaches threshold depends on both the input coincidence and on the strength of inputs at a given time point: several subthreshold inputs arriving together can drive a spike, but one or two strong terminals may also suffice even when the other inputs are weak or poorly aligned. This heterogeneity is therefore critical to consider when analyzing synaptic integration in GBCs: treating input strength as a distribution rather than a single mean value captures how the combination of strong and weak inputs determines spike timing and reliability.

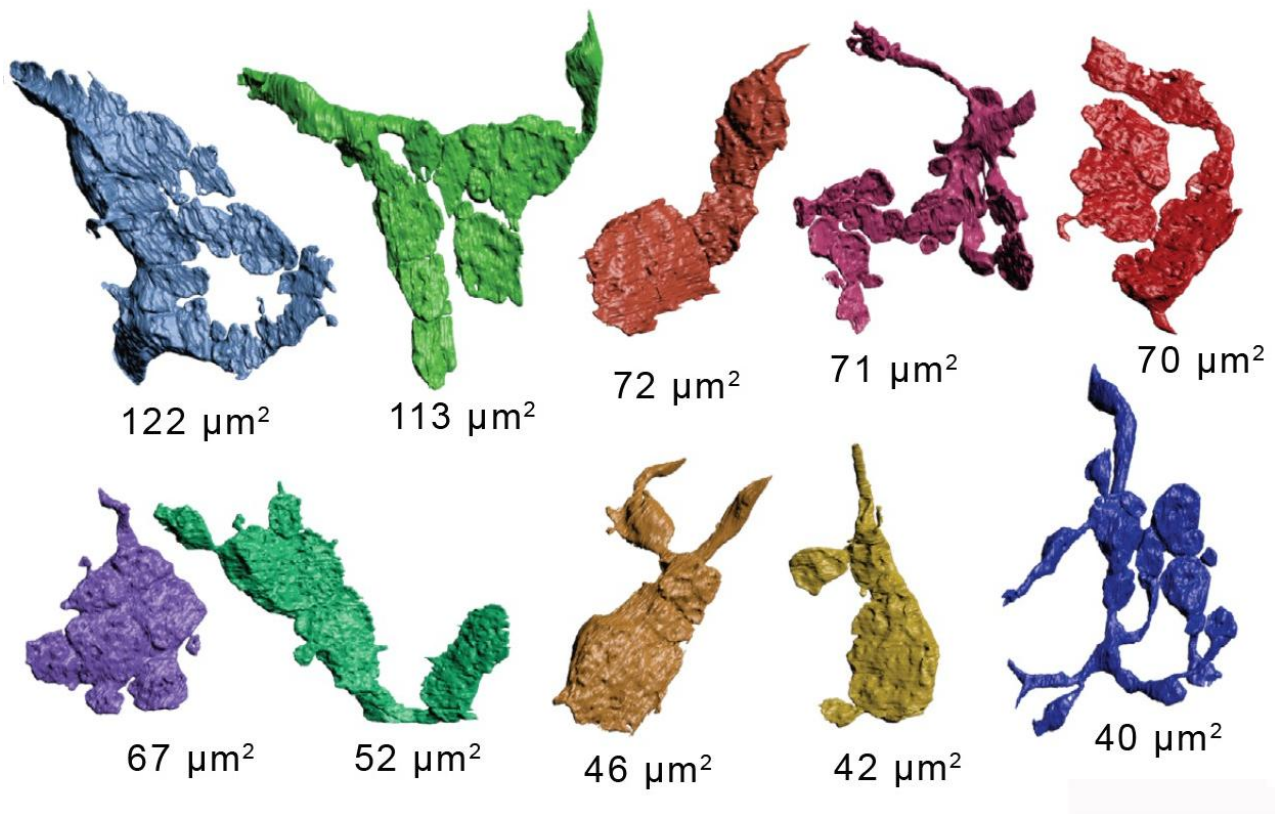


Figure 7. 3D reconstructions of endbulb of Held terminals on a mouse GBC. Multiple somatic inputs contact the same cell and vary markedly in size. Reconstructions are color-coded and labeled by apposed surface area (ASA, μm^2). Modified from Spirou et al., 2023.

1.2.4 Implications for temporal coding

For temporal coding, prior work suggests that variability in endbulb strength, together with activity-dependent depression, could place GBCs along a continuum between precise spike timing and rate/envelope readout (Wang & Manis, 2008; Yang & Xu-Friedman, 2009; Spirou

et al., 2023). When multiple subthreshold inputs arrive within a short temporal window, GBCs can spike with sub-millisecond precision, preserving the fine temporal structure required for subsequent binaural comparisons (Joris et al., 1994; Louage et al., 2005). Increased variability in synaptic strength does not change the postsynaptic spike threshold; rather, one or two stronger endbulbs permit threshold crossings without exact coincidence, shifting the neuron from pure coincidence detection toward a mixed mode in which spikes can be driven either by coincident summation of multiple subthreshold inputs or by single suprathreshold inputs (Spirou et al., 2023). As the dependence on near-coincident input decreases, spikes are evoked over a broader range of input-arrival times, causing greater spike-time dispersion. In contrast, more uniform inputs facilitate coincidence detection and temporal precision, but at a reduced firing rate during prolonged stimulation (Joris et al., 1994; Yang & Xu-Friedman, 2009).

These considerations motivate focusing on how excitatory drive is distributed across the converging endbulbs onto a single GBC. Different distributions imply different requirements for spike initiation. Quantifying the effects of input-strength variability is therefore essential for understanding how temporal information is preserved or transformed at the endbulb-GBC synapse.

1.3 Inhibitory control at GBCs

1.3.1 Sources of inhibition

In addition to strong excitatory drive from ANF endbulbs, GBCs receive inhibitory input from within the CN (Fig. 5) (Xie & Manis, 2013b; Campagnola & Manis, 2014; Ngodup et al., 2020). D-stellate cells are wide-band glycinergic neurons, broadly tuned in frequency and providing cross-frequency inhibition onto GBCs in the ventral division (Fig. 8). Another recently identified glycinergic interneuron class, the L-stellate cell, provides local inhibition within the AVCN; however, their contribution to BC inhibition remains less well established (Campagnola & Manis, 2014; Ngodup et al., 2020). Projections from the DCN, classically attributed to vertical (tuberculoventral) cells, are another potential inhibitory source (Manis & Campagnola, 2018). Fast inhibition in the adult AVCN and SOC is predominantly

glycinergic; GABAergic-mediated currents contribute only a minor fraction (Xie & Manis, 2013b, 2013a; Nerlich et al., 2014). In BCs, however, glycinergic IPSCs often decay more slowly than in other principal neurons, indicating that the inhibitory time course is determined by receptor and synaptic properties rather than by transmitter type (Xie & Manis, 2013b). Extrinsic inhibitory inputs to the VCN have been reported in some species (Ostapoff et al., 1997; Xie & Manis, 2014). Their prevalence and postsynaptic targets vary, and they are not further considered here.

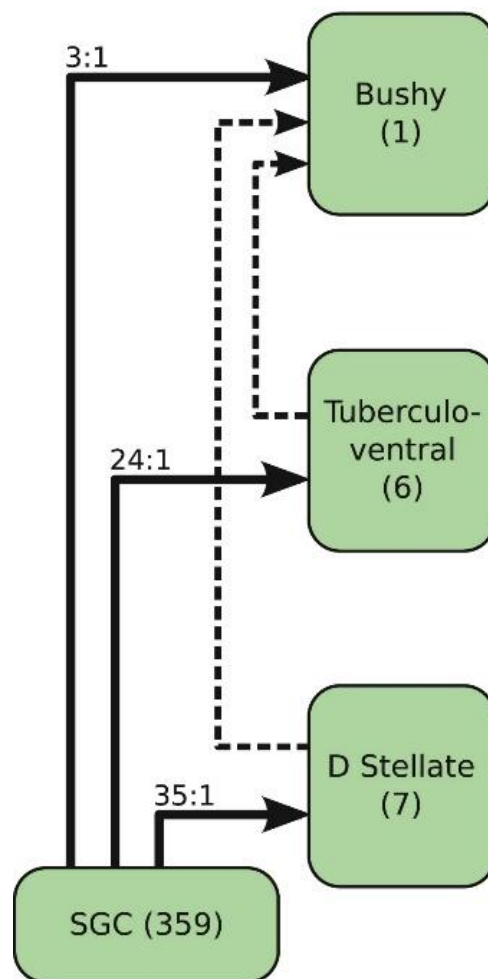


Figure 8. Schematic representation of the convergence of ANF (SGC) inputs onto BCs and local inhibitory interneurons in the cochlear nucleus. Each BC receives direct excitatory input from a small number of SGCs, while D-stellate cells and tuberculoventral cells receive convergent input from a larger number of SGCs and provide inhibitory projections onto BCs. Modified from the computational model of Manis & Campagnola, 2018.

1.3.2 Kinetics and short-term dynamics

Evoked inhibitory currents in the VCN are slower than endbulb-evoked excitation, particularly evident in SBCs (Xie & Manis, 2013b). Research on mice showed that evoked glycinergic IPSCs have target-specific kinetics: BCs receive slowly decaying inhibition, whereas T-stellate cells receive faster inhibition, implying a postsynaptic, target-dependent specialization (Xie & Manis, 2013b).

Consistent with this, measurements in mouse VCN report spontaneous IPSCs in BCs that outlast spontaneous EPSCs by an order of magnitude, reinforcing the notion that inhibition shapes excitability over a wider temporal window than the brief excitatory integration period (Campagnola & Manis, 2014).

In gerbil SBCs, the slow decay of IPSCs leads to temporal inhibitory summation in both in vivo and in vitro recordings (Kuenzel et al., 2011; Nerlich et al., 2014). For GBCs, direct measurements of inhibitory kinetics and short-term dynamics are not available. In this study, I recorded evoked IPSCs from identified GBCs and quantified IPSC kinetics, which I then implemented in conductance-clamp experiments to examine how inhibition interacts with excitation.

1.3.3 Functional effects on temporal coding

Studies on SBCs demonstrated that inhibition enhances temporal precision by reducing spike-time jitter and increases trial-to-trial reproducibility and sparsity in complex acoustic scenes (Keine & Rübsamen, 2015; Keine et al., 2016). Modeling and juxtacellular recordings in SBCs indicate that inhibition elevates the effective spike threshold and constrains rate-level functions. Under these conditions, spikes are preferentially triggered by large, well-timed EPSPs (Kuenzel et al., 2011).

In summary, BCs integrate fast axosomatic excitation with slowly decaying inhibition, providing a narrow integration window to suppress poorly timed inputs. Still, the onset spikes remain low-jitter, providing a precise output for sub-millisecond binaural computations (Joris et al., 1994; Grothe et al., 2010; Joris & Trussell, 2018). Clarifying how heterogeneity in the strengths of converging endbulb inputs is integrated with inhibition is essential for

understanding the GBC output.

1.4 Research gap and approach

Several studies on the ANF-BC processing have represented excitatory drive as homogeneous, for example, by using multiple identically weighted inputs or summarizing excitation with a single mean parameter to reproduce PSTH classes and high entrainment (Rothman et al., 1993; Rudnicki & Hemmert, 2017; Ashida et al., 2019). However, anatomical reconstructions show substantial heterogeneity in the size of endbulb inputs converging on a single cell (Spirou et al., 2023), underscoring the need to assess how this heterogeneity interacts with inhibition to shape temporal coding in GBCs.

This thesis addresses the knowledge gap with three guiding questions:

Excitatory heterogeneity. How does the variation of excitatory input strength affect the sound-driven firing of GBCs across different acoustic stimuli, particularly in terms of onset precision, first-spike reliability, and envelope following—features that are critical for temporal sound coding?

Morphology-based validation. Do the conclusions hold when excitatory input strengths are based on morphology-derived endbulb size distributions (from volumetric reconstructions) rather than on parametric assumptions?

Effect of inhibition. When combined with endbulb-driven excitation, how does inhibition shape temporal coding in the sound-driven responses of GBCs?

To answer these questions, I used whole-cell conductance clamp recordings in identified GBCs. Excitatory and inhibitory inputs were delivered as synaptic conductances based on EPSC and IPSC waveforms, respectively. This approach was appropriate because it allows the injection of realistic, voltage-dependent synaptic conductances rather than fixed currents, thereby closely mimicking natural synaptic input. The primary manipulation was the distribution of excitatory input strengths, including morphology-informed distributions derived from endbulb size, while synaptic kinetics and reversal potentials were held constant. I then added inhibition with physiologically realistic amplitude and fixed kinetics, keeping

spontaneous inhibitory activity low to minimize tonic build-up before stimulus onset. Coding was quantified using three complementary readouts: (i) onset responses (first-spike probability, latency, jitter), (ii) sustained firing, and (iii) envelope following. Temporal precision and rate coding were assessed by VS, CI, and CorrNorm in within-cell comparisons.

As a complementary approach, Dr. Go Ashida ran an established adaptive coincidence-detection model using the same input-strength distributions and stimuli. Concordance between model and recordings was used to assess whether a short integration window interacting with input heterogeneity is sufficient to reproduce the observed coding behavior.

Together, this design maps the input-output relation of GBCs by investigating how heterogeneity in converging excitatory inputs shapes coding ability and how inhibition modifies that mapping.

2. Aims

Synaptic convergence and the diversity of input strength are fundamental features that shape the computational strategies of neurons in sensory circuits. In the mammalian auditory brainstem, GBCs in the CN receive multiple endbulb inputs from ANFs, which vary considerably in number and size even among inputs converging onto a single cell. This structural heterogeneity raises questions of how differences in synaptic input strength affect GBC encoding, particularly temporal precision and sensitivity to the stimulus envelope, which are critical for accurate sound localization and robust speech perception in complex acoustic environments.

This question was addressed in two interconnected experimental parts:

The first part focuses on quantifying the impact of the excitatory input variation on GBC sound encoding. Using in vitro whole-cell conductance-clamp recordings and computational modeling (developed in collaboration) in young, adult Mongolian gerbils, I systematically manipulated the degree of input strength variation across simulated ANFs and examined GBC responses to both pure tone and sinusoidally amplitude-modulated (SAM) stimuli.

In the second part, an experimentally determined inhibitory conductance was incorporated, and the excitatory stimulus conditions were repeated to assess how inhibition modulates GBC responses.

Together, these two experimental parts aimed to elucidate how variation in the strength of converging excitatory inputs and their interaction with inhibitory inputs shape the encoding strategies of GBCs.

3. Materials and Methods

3.1 Key resources table

Table 1. List of key resources

Reagent type (species) or resource	Designation	Source or reference	Identifiers	Additional information
Chemical compound, drug	cyTM5-conjugated Streptavidin	Jackson ImmunoResearch Laboratories	Cat#016-170-084;	5mg/mL
Chemical compound, drug	Biocytin	Sigma-Aldrich	Cat#B4261	0.2%
Software, algorithm	Matlab	The Mathworks	RRID:SCR_001622	Version R2021b
Software, algorithm	GraphPad Prism	GraphPad Software	RRID:SCR_002798	Version 8
Software, algorithm	Igor Pro	Wavemetrics	RRID:SCR_000325	Version 8
Software, algorithm	Fiji	https://fiji.sc/	RRID:SCR_002285	
Software, algorithm	pClamp	Molecular Devices	RRID:SCR_011323	Version 11.2.2
Software, algorithm	MultiClamp 700B Commander	Molecular Devices	RRID:SCR_018455	Version 2.2.2

3.2 Animal

All animal procedures were conducted in accordance with the German Animal Welfare Act (TSchG §4) and the European Union Directive 2010/63/EU. Experiments were performed at the Department of Human Medicine, University of Oldenburg. Mongolian gerbils (*Meriones*

unguiculatus) of both sexes between postnatal days P20 and P25 were used. Animals were group-housed under a standard 12-hour light/dark cycle with unrestricted access to food and water. All measures were taken to minimize the number of animals used and to alleviate any potential pain or distress.

3.3 Acute brain slice preparation

Following rapid decapitation, acute parasagittal slices (200 μ m) containing the CN were made with a vibrating microtome (Leica VT1200 S) in ice-cold, low-calcium artificial cerebrospinal fluid (aCSF) containing (in mM): 125 NaCl, 2.5 KCl, 0.1 CaCl₂, 3 MgCl₂, 1.25 NaH₂PO₄, 25 NaHCO₃, 10 glucose, 3 myo-inositol, 2 sodium pyruvate, 0.4 ascorbic acid, continuously bubbled with 5% CO₂ and 95% O₂, and maintained at pH 7.4. Slices were then incubated in standard recording solution (same aCSF as for slicing, except CaCl₂ and MgCl₂ were changed to 1.2 mM and 1 mM, respectively) for 30-45 min at 37 °C and transferred to a recording chamber with the same aCSF at physiological temperature (~37°C) for recordings. All solutions were prepared in ultrapure water (0.055 μ S/cm).

3.4 Electrophysiology

For electrophysiology experiments, slices were transferred to a recording chamber with recording aCSF at physiological temperature (~37°C) and then continuously perfused with recording solution (aCSF) bubbled with 95% O₂ and 5% CO₂ at a rate of 1mL/min. Both the recording chamber and perfusion inlet were temperature-controlled with a dual automatic temperature controller (TC-344B, Warner Instruments), and the bath temperature was monitored throughout the experiment. Neurons were visualized with an upright microscope (Axioskop 2 FS plus, Zeiss), the microscope was equipped with a CCD camera (IR-1000E, DAGE-MIT), 10x and 63x water-immersion objectives (Objective W Plan-Apochromat 10x/1.0, Objective W Plan-Apochromat 63x/1.0, Zeiss).

Borosilicate glass capillaries (GB150F-8P, OD 1.5mm; Science Products) were pulled using a universal electrode puller (DMZ, Zeitz-Instruments) to a tip resistance of 3-5 M Ω . For

whole-cell voltage clamp, whole-cell current clamp, and conductance clamp experiments when filled with an intracellular solution containing (in mM): 120 K-Gluconate, 20 KCl, 5 ethylene glycol tetraacetic acid, 10 HEPES, 0.05 CaCl₂, 5 Na₂phosphocreatine, 4 MgATP, 0.3 NaGTP, and 0.2% biocytin. IPSCs were recorded with a Cs-Gluconate-based intracellular solution containing (in mM): 115.5 Cs-Gluconate, 14.5 CsCl, 5 ethylene glycol tetraacetic acid, 10 HEPES, 20 tetraethylammonium chloride (TEA-Cl), 5 Na₂phosphocreatine, 4 MgATP, 0.3 NaGTP, 6 QX-314, and 0.2% biocytin. In addition, CNQX (10 μ M) or NBQX (10 μ M) and AP5 (50 μ M) were included in the recording solution (aCSF) to block AMPA/kainate and NMDA receptor-mediated excitatory synaptic transmission, respectively.

Recordings were performed using an Axon Multiclamp 700B amplifier (Molecular Devices). Data acquisition was performed at a low-pass filter at 10 kHz and digitized at a 100 kHz sampling rate with a Digidata 1440A interface (Molecular Devices). For both current-clamp and conductance-clamp recordings, the cell's resting membrane potential was maintained around -60 mV between trials by applying a small holding current (<140 pA). Bridge balance and pipette capacitance neutralization were checked and adjusted regularly to ensure accurate injection and membrane potential measurements during experiments. All recordings were carried out at physiological temperatures (36-37°C).

Recordings were targeted to GBCs, which were identified in acute slices by (i) onset spiking with a pronounced voltage sag in whole-cell current clamp, (ii) somatic location near the AN entry zone in caudal AVCN, and (iii) bushy-like dendritic morphology.

3.5 Stimulus Template

3.5.1 Simulating auditory nerve fiber input

To simulate ANFs spiking activity, I employed the 2018 version of the auditory periphery model developed by Bruce et al. (2018). Given that gerbil AN responses closely resemble those observed in cats (Versteegh et al., 2011; Huet et al., 2016; Heeringa et al., 2023), I utilized the model configured specifically for the "cat" auditory system parameters.

Based on previous findings indicating that GBCs predominantly receive input from high-

spontaneous rate (type Ia) SGNs (Lieberman, 1991; Wang et al., 2021; Wong et al., 2025), I selected an SR of 70 spikes/s for my modeled ANFs. Refractory periods were fixed at the midpoint of the model's default parameter range, representing typical physiological values (absolute: 0.45 ms, relative: 0.5125 ms).

Anatomical evidence indicates variability in the number of ANFs converging onto single GBCs, ranging from approximately 5-12 fibers in mice to around 20 fibers in cats (Spirou et al., 2005; Spirou et al., 2023). Taking this variability into consideration, I chose an intermediate convergence level, simulating each GBC with inputs from 10 ANFs.

Given the narrow frequency selectivity of GBCs, which closely aligns with the tuning properties of ANFs (Rhode & Smith, 1986), all modeled AN inputs were assigned the same characteristic frequency (CF). Specifically, for pure-tone stimuli, the CF of the ANFs matched the stimulus tone frequency, and for amplitude-modulated (AM) sound stimuli, the CF corresponded to the carrier frequency.

3.5.2 Simulating inhibitory input

Inhibitory inputs to GBCs were simulated considering that neurons providing inhibitory input to GBCs also receive direct excitatory input from ANFs. Therefore, inhibitory spike trains were generated from the same ANF model driving the inhibitory pathway. This simplifying assumption is based on the slow kinetics of IPSCs and the convergence of numerous inhibitory inputs. Under these conditions, inhibition effectively acts as a tonic conductance, so the fine timing of spike trains has little impact on its steady level. The results are interpreted considering this caveat. Anatomical and physiological evidence in mice indicates that each BC typically receives inhibitory inputs from multiple sources, with a combined estimate of approximately 13 inhibitory synapses (Manis & Campagnola, 2018). Given the slow kinetics of inhibition at the BC synapse (Xie & Manis, 2013b; Nerlich et al., 2014), inhibitory inputs tend to summate into a tonic conductance over time, reducing the impact of precise variations in input number. Thus, I selected a representative value of 10 inhibitory inputs per GBC, consistent with the number chosen for excitatory inputs, facilitating direct comparison between excitatory and inhibitory pathways.

To minimize tonic inhibitory build-up prior to stimulus onset, which could artificially influence experimental results, I assigned inhibitory input fibers a SR near the lower boundary of type-I SGNs, specifically 18 spikes/s (Liberman, 1991).

3.5.3 Modeling synaptic input variation

Synaptic input variation (v) is defined as the standard deviation (s) of the 10 ANF synaptic input amplitudes divided by their mean (m ; $v = \frac{s}{m}$). To systematically vary the input variation, input strengths were drawn from a Gamma distribution defined by a shape ($a = \frac{1}{v^2}$) and scale parameter ($b = mv^2$). By using these settings, I generated sets of 10 ANF synaptic input amplitudes across 3 levels of input variation v (no variation, $v = 0$; medium variation, $v = 0.4$; high variation, $v = 1$). The resulting distribution of these input amplitudes qualitatively matches the histological observation and spans the range reported from morphological estimates (Spirou et al., 2023). To confirm the biological relevance of my findings, I also implemented synaptic strength distributions based on the morphological data (Spirou et al., 2023).

3.5.4 Generating the synaptic conductance templates

Stimulus-to-conductance paradigm is illustrated in Fig. 9. Acoustic stimuli, pure tones or SAM tones, as indicated in each experiment, were passed to the auditory-periphery model to generate spike trains from 10 ANFs.

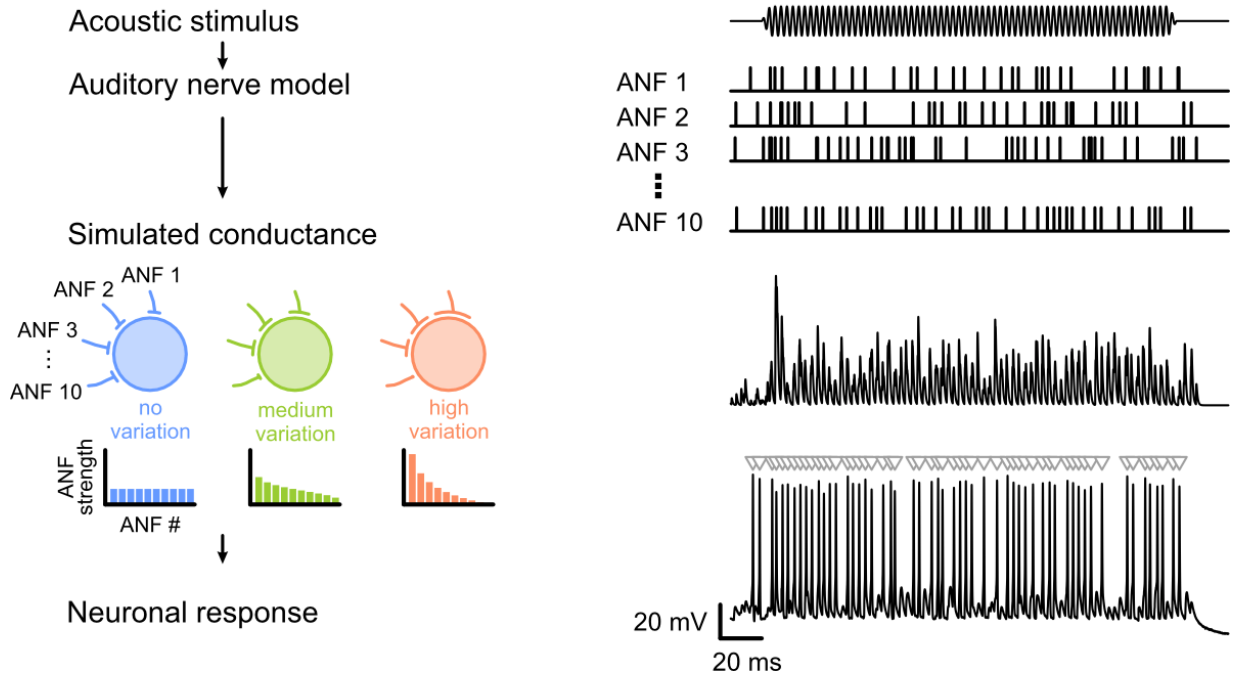


Figure 9. Schematic of conductance clamp stimulation paradigm. Acoustic stimuli were processed through an ANF model to simulate spike trains from 10 ANFs. These trains were convolved with EPSC templates to generate synaptic conductance waveforms with different degrees of input variability: no (blue), medium (green), and high variation (orange). The GBC response to each condition was recorded. Raster plots and voltage traces illustrate representative outputs, with gray triangles marking detected spikes. Panels illustrate the pure-tone case; SAM stimuli followed the same procedure.

For the excitatory component, synaptic conductance template traces ($G_{syn_{exc}}$) were generated by convolving the simulated ANF spike trains with a stereotypical EPSC waveform, which was modeled with an alpha function with a time constant of 0.2 ms. To manipulate input heterogeneity, amplitude distributions were assigned to the predefined input distributions: no variation ($v=0$, all equal), medium variation ($v=0.4$), and high variation ($v=1$). The total $G_{syn_{exc}}$ represented the linear summation of all active ANFs with the average input strength kept constant between input variation conditions.

For the inhibitory component, synaptic conductance template traces ($G_{syn_{inh}}$) were generated by convolving the simulated inhibitory input trains with a stereotypical IPSC waveform, which was modeled with a beta function with a τ_{rise} of 0.3 ms and a τ_{decay} of 20.5 ms obtained from my recordings, and amplitude distributions according to the

predefined no variation ($v=0$, all of the inputs are uniform). The total $G_{syn_{inh}}$ represented the linear summation of all active inhibitory inputs.

To further evaluate the biological plausibility of my model, I incorporated synaptic strength distributions based on anatomical reconstructions (Spirou et al., 2023). Two representative GBCs were selected from the dataset: GBC21, previously associated with a coincidence detection mechanism (coefficient of variation, CV = 0.33, similar to my “medium variation” condition), and GBC18L, characterized by mixed-mode synaptic integration (CV = 0.79, representing my “high variation” condition) (Fig. 10). To allow direct comparison between these anatomically motivated input configurations, the number of converging ANF inputs was standardized to eight for both conditions.

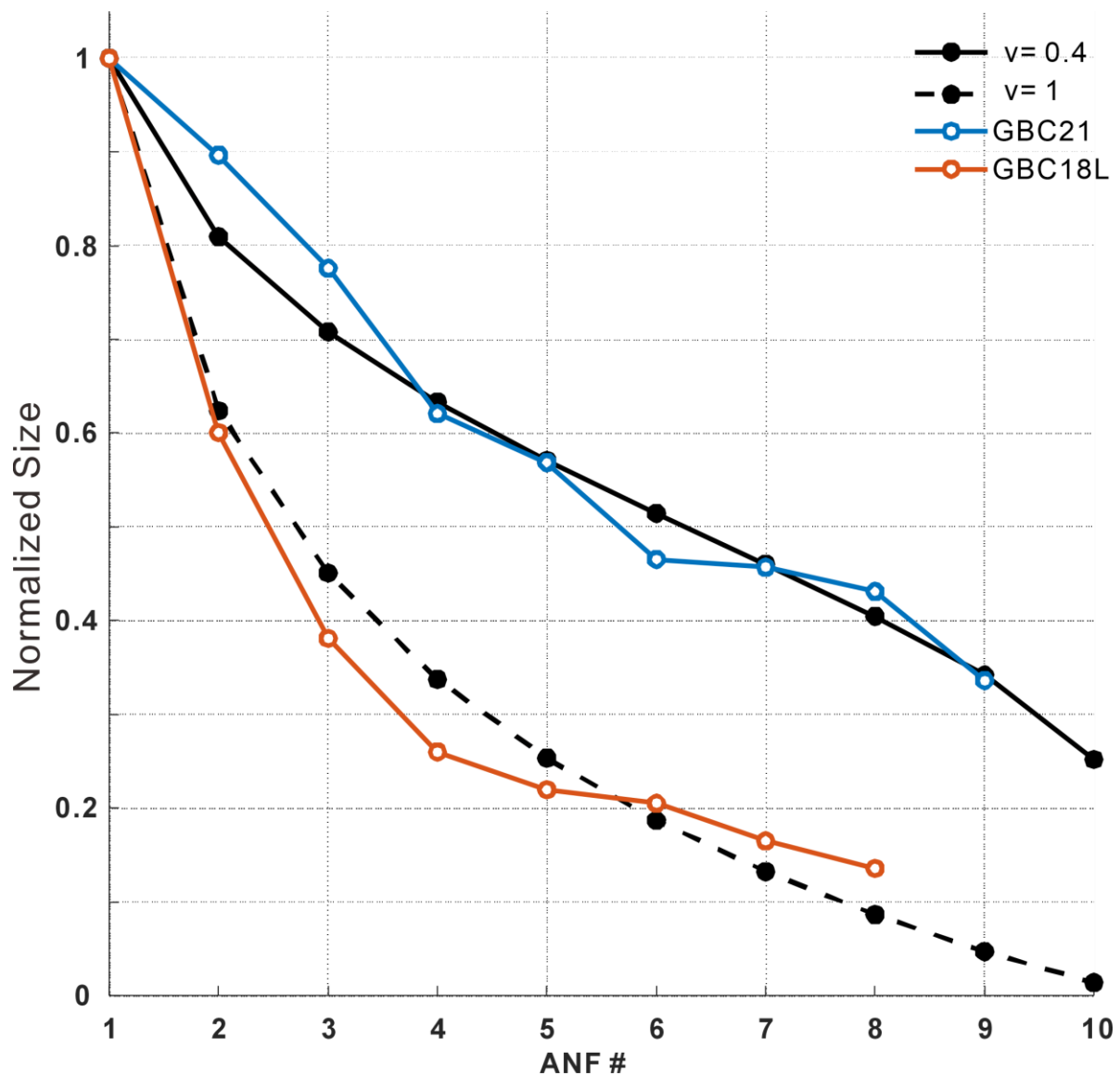


Figure 10. Synaptic input strength distributions derived from anatomical reconstructions and model conditions. Normalized synaptic strength profiles are shown for two anatomically reconstructed GBCs from Spirou et al. (2023): GBC21 (blue) exhibits relatively uniform input strengths (CV = 0.33), representative of moderate input variation and consistent with coincidence detection. In contrast, GBC18L (orange) shows a steeper decay in input strength (CV = 0.79), reflecting higher input heterogeneity and mixed-mode integration. For comparison, model-based distributions are overlaid: the solid black line represents a low-variation condition ($v = 0.4$), and the dashed black line represents a high-variation condition ($v = 1.0$). Two conditions were normalized to eight converging ANF inputs for consistent comparison.

In this approach, synaptic input amplitudes were derived directly from morphological measurements of endbulb apposed surface area (ASA), providing a biologically grounded alternative to the predefined Gamma-distributed amplitude variations. The relative ASA of the endbulbs was normalized to generate input-specific scaling factors, which were applied to a stereotypical EPSC waveform. Synaptic conductances ($G_{syn_{exc}}$) were then computed by the procedure described above for the excitatory component. This configuration preserved anatomical variability while maintaining consistency with the modeling framework established for the excitatory component.

3.5.5 Conductance Clamp Implementation

Conductance clamp experiments were carried out using a master-slave setup based on the approach described by Yang et al. (2015). Specifically, I used custom software (mafDC, provided by Xu-Friedman), running on IGOR Pro 8 (WaveMetrics, Lake Oswego, OR) on a dedicated slave computer. The slave computer continuously monitored and digitized real-time membrane potential data of recorded GBC from a PCIe-6361 data acquisition card (National Instruments) connected via a BNC-2120 interface (National Instruments).

Pre-generated synaptic conductance waveforms (excitatory ($G_{syn_{exc}}$) and inhibitory ($G_{syn_{inh}}$)) were created on a master computer and transmitted to the slave computer. Real-time synaptic currents (excitatory ($I_{syn_{exc}}$) and inhibitory ($I_{syn_{inh}}$)) were then computed from the recorded membrane potential (V_m) and the corresponding synaptic reversal potentials (excitatory ($V_{rev_{exc}}$) and inhibitory ($V_{rev_{inh}}$)) according to Ohm's law:

$$I_{syn} = I_{syn_{exc}} + I_{syn_{inh}} = G_{syn_{exc}}(V_m - V_{rev_{exc}}) + G_{syn_{inh}}(V_m - V_{rev_{inh}})$$

With $V_{rev_{exc}} = 0 \text{ mV}$ (Kuenzel et al., 2015) and $V_{rev_{inh}} = -75 \text{ mV}$ (Milenković et al., 2007; Kuenzel et al., 2015). For experiments using only excitatory inputs, the inhibitory term was omitted. The calculated currents were injected back into the GBCs through the same patch-clamp electrode used for recording.

3.6 Post-hoc identification of GBCs

To identify biocytin-filled GBCs after recordings, slices were promptly transferred to a culture dish and fixed in 4% paraformaldehyde (PFA) overnight at 4°C. Following fixation, slices were rinsed six times for 5 minutes each in PBS and another six times rinsed (5 min each) in 0.3% Triton X-100 in PBS (PBST) to permeabilize the tissue. For labeling, slices were incubated with Cy5-conjugated streptavidin (5 µg/mL, 016-170-084, Jackson ImmunoResearch) at room temperature for 2.5 hours, diluted in the PBST. After incubation, slices were rinsed again (6 × 5 min in PBST, 2 × 5 min in PBS, and 2 × 5 min in ultrapure water). All incubation and wash steps were carried out in the dark on a gentle shaker to avoid bleaching. Finally, slices were mounted on microscope slides using Aqua-Poly/Mount medium (Cat#18606, Polysciences, Inc.) and imaged with a Leica SP8 confocal microscope using a 10× (0.3 NA) dry and a 63× (1.4 NA) oil-immersion objective, with excitation wavelength at 647 nm.

3.7 GBC Model

This model, originally implemented by Dr. Go Ashida, simulates a coincidence detection mechanism based on temporally aligned excitatory inputs.

3.7.1 Globular bushy cell model

Computational modeling of GBC responses was performed using the "adaptive coincidence-counting model" described previously (Ashida et al., 2019). In this model, each incoming

spike from a simulated ANF evoked a simplified rectangular postsynaptic response (Fig. 13A). The duration (W) of this rectangle remained constant for all inputs, whereas its amplitude (A) varied across the 10 inputs based on the specified input variation condition. These individual postsynaptic responses were linearly summed to produce the total integrated input $v(t)$. The model generated an output spike when $v(t)$ exceeded an adaptive threshold $\theta(t)$. Following an output spike, the model entered an absolute refractory period (R) during which no further spikes could be triggered.

The adaptive threshold consisted of static and dynamic components: $\theta(t) = \theta_s + \theta_D(t)$. The static component θ_s was fixed at 1, so that postsynaptic response amplitudes (A) were normalized to this static input threshold. The dynamic component $T(d\theta_D/dt)$ changed according to the total input amplitude: $T(d\theta_D/dt) = -\theta_D(t) + S \cdot (u(t))^2$, where T is the time constant and S is the strength of adaptation (Ashida et al., 2019). The quadratic exponent of $u(t)$ was designed to mimic, in a simplified manner, the nonlinear activation of various adaptive mechanisms in BC, such as low-threshold K^+ channels and inhibitory feedback from other CN neurons. This adaptive threshold mechanism allowed the model to reject poorly synchronized inputs, preventing them from reaching the threshold (Fig. 13A). The spike generation mechanism implemented in the GBC model is deterministic and does not produce spike time jitter. To simulate this intrinsic variability, a random number, drawn from a uniform distribution of 0.2-0.6 ms, was added to the output spike timing. This modification was based on the spike latency data from pure-tone stimulation (Fig. 14C2 and 14C3) and resulted in a mean increase in response latency of 0.4 ms (SD = 0.116 ms).

3.7.2 Model parameters and validation

In addition to the input variation described above, the GBC model used in this study had five free parameters (Table 2). The default values (Table 2, center column) were initially chosen such that the model's responses to pure-tone stimuli resembled those observed in my conductance-clamp experiments. To confirm that the modeling results were robust and did not critically depend on this specific parameter set, Dr. Go Ashida systematically varied these parameter values around the default values (Table 2, right column). Each "model instance" with a unique parameter combination was tested with pure-tone stimulation, and

only those instances exhibiting GBC-like responses, as defined by the criteria below, were adopted for further testing with AM sound stimulation.

Table 2: Model parameters. The total number of parameter combinations was $14 \times 8 \times 8 \times 8 \times 8 = 57,344$. The default parameter values were used for Figs. 13-17.

Parameter	Default value	Ranges tested
(mean) Input amplitude A	0.36	0.26 – 0.52 (step 0.02)
Input duration (ms) W	0.7	0.2 – 1.6 (step 0.2)
Refractory period (ms) R	1.7	0.8 – 2.2 (step 0.2)
Adaptation strength S	0.9	0.2 – 1.6 (step 0.2)
Adaptation time constant (ms) T	1.1	0.3 – 2.4 (step 0.3)

The adoption criteria were based on my experimental observations (Figs. 13-14): (1) spontaneous rate of 10-60 (spikes/s); (2) steady-state driven rate >150 (spikes/s) and vector strength >0.8 for 350 Hz tonal stimulation; (3) steady-state driven rate of 60-140 (spikes/s) and onset spike probability >0.95 for 3500 Hz tonal stimulation. In addition, the PSTH shape near the onset of the 3500 Hz tone was examined to ensure a primary-like-with-notch (PLN) spiking pattern, characterized by a sharp peak and a succeeding notch (pause of spiking activity), followed by a smooth recovery towards the steady-state spiking response (Fig. 14A2/D1). Specific criteria for PLN responses were as follows: (4) the notch appears within 3 ms after the onset peak; (5) the notch amplitude (minimum rate in PSTH) is below 50% of the steady-state spike rate; (6) a second peak in the PSTH should be absent or, if present, be below 600 spikes/s; (7) the average spike rate at 9-13 ms after the stimulus onset is $>90\%$ of the steady-state spike rate; and (8) a second notch should be absent, or if present, be higher than 50% of the steady-state spike rate. The last criterion was used to distinguish PLN-type PSTHs from chopper-type PSTHs.

3.8 Experimental design and data analysis

3.8.1 Data analysis

All data analyses were performed using custom-written MATLAB scripts (MATLAB 2021a, MathWorks). APs were identified by applying a voltage threshold criterion of -20 mV. Each detected event was subsequently verified through manual visual inspection to ensure accuracy and remove potential artifacts. Spike timing was defined using the peak voltage time point of each AP. Firing rates were calculated separately for stimulus-evoked and spontaneous conditions. Stimulus-evoked firing rates were computed within predefined analysis windows aligned to each stimulus (200 ms for pure tone recordings, 150 ms for PSTH recordings, and 400 ms for SAM recordings), while spontaneous activity rates were determined from periods without auditory stimulation (150 ms). To quantify response reliability, onset probabilities were evaluated as the proportion of trials exhibiting at least one spike within a fixed window of 1.5 ms following stimulus onset. PSTHs were constructed by aggregating spike occurrences across 100-200 repetitions of identical auditory stimuli, using a bin width of 0.5 ms. The neuron's ability to phase-lock to periodic auditory stimuli, including pure tones and SAM signals, was measured using VS, calculated according to Goldberg and Brown (1969). Additionally, spike timing precision independent of the stimulus waveform was assessed via the correlation index (CI), derived from the normalized peak height of shuffled auto-correlograms across stimulus repetitions, as described by Joris et al. (2006). For SAM stimuli, neuronal encoding of the stimulus envelope was analyzed using the normalized cross-correlation measure (CorrNorm). Specifically, CorrNorm was computed between the stimulus envelope and the corresponding neuronal response PSTH, following adjustment for the neuron's latency, based on methods described earlier (Tolnai et al., 2008; Keine et al., 2016). CorrNorm values range from 0 to 1, where a value of approximately 0.82 indicates an unmodulated neuronal response. Higher CorrNorm values (>0.9) indicate that firing rate tracks the stimulus envelope.

3.8.2 Statistical analysis

Individual GBC recordings were considered independent biological samples. Data are summarized and presented as mean $\pm$ standard deviation (SD) unless specified otherwise.

Statistical comparisons involved repeated measures from individual neurons to assess the effects of stimulus conditions and input variability. Consequently, statistical analysis was carried out using two-way repeated-measures ANOVA in GraphPad Prism 8 (GraphPad Software). Post-hoc pairwise comparisons were adjusted for multiple testing using the Bonferroni correction (Bonferroni, 1936). A significance threshold of $p < 0.05$ was applied throughout. Sample sizes were informed by variability and effect sizes reported in prior relevant studies (Milenković et al., 2007; Kuenzel et al., 2015). No statistical methods were used to pre-determine sample sizes prior to data collection.

4. Results

4.1 Validation of Conductance Clamp Implementation

To investigate how synaptic input variation affects the encoding properties of GBCs, I employed a conductance-clamp approach that allows controlled injection of excitatory and inhibitory synaptic conductance templates. Conductance clamp provides a more realistic approximation of synaptic inputs compared to current injection, as it dynamically adjusts the injected current based on the cell's membrane potential and the input conductance profile.

As illustrated in Fig. 11A, the master system was used for whole-cell patch-clamp recording and stimulus control, while the slave system executed the conductance clamp algorithm in real time. The membrane potential (V_m) was recorded by an Axon Multiclamp 700B amplifier (Molecular Devices). Given the dynamic nature of this process, it was critical to validate the temporal precision and stability of the conductance clamp system before proceeding to hypothesis-driven experiments.

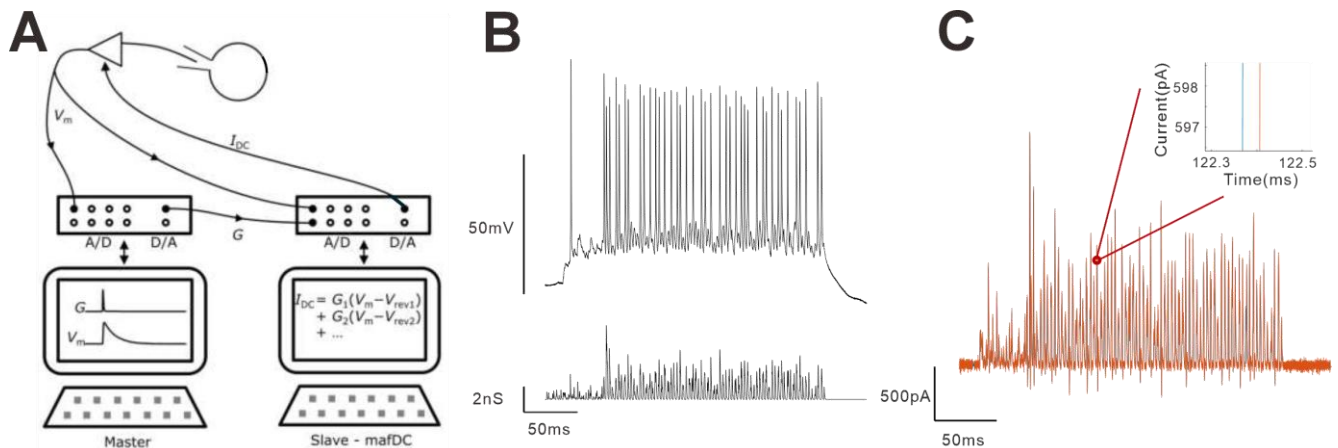


Figure 11. Validation of the conductance clamp system used for conductance injection in BCs. (A) Schematic of a conductance clamp setup. Whole-cell patch-clamp recording is performed through the master setup, responsible for monitoring the membrane potential (V_m) and sending it to the slave setup via an analog-to-digital (A/D) interface. The slave computer continuously calculates the injected current (I_{DC}) in real time based on predefined synaptic conductance templates and Ohm's law, using the instantaneous V_m . The resulting current is then converted through a digital-to-analog (D/A) interface and injected back into the neuron via the same electrode, closing the feedback loop. All membrane potential measurements and current

injections are performed using a single patch pipette. A/D: analog-to-digital; D/A: digital-to-analog; V_m : membrane potential; I_{DC} : dynamic clamp current. Modified from Yang et al., 2015. (B) Example recording from a BC during conductance injection (bottom) and resulting action potentials (top). (C) Comparison of the experimentally injected current (orange) and the theoretical current (blue) calculated from the recorded V_m and known conductance. The overall waveforms closely match, validating system fidelity. Inset: Zoomed-in view of a representative segment highlights a small temporal offset between the two traces.

To assess system fidelity, I used conductance templates identical to those later applied in my synaptic variation experiments (see Materials and Methods), injected them into GBCs, and recorded both the neuronal responses (APs) and the injected current ($I_{experimental}$) simultaneously. A representative example of the GBC's spike train and injected conductance waveform is shown in Fig. 11B.

Then I calculated a theoretical current trace ($I_{theoretical}$) by applying the recorded membrane potential to the known conductance template using Ohm's law. Using this calculation, I obtained the current predicted by Ohm's law, $I_{theoretical}(t) = g(t)\{V_m - E_{rev}\}$, with $E_{rev} = 0\text{ mV}$, allowing a direct comparison with measured current. Overlaying $I_{theoretical}$ and $I_{experimental}$ in Fig. 11C showed a similar overall waveform shape, which means that the system precisely calculated the current based on the conductance template and membrane potential. However, closer inspection at higher temporal resolution showed a slight temporal offset between the two traces (Fig. 11C inset).

To quantify this offset, I systematically analyzed the alignment between the experimental current trace and the theoretical current trace. Specifically, I incrementally shifted one trace relative to the other in steps of 0.01 ms (one data point at 100 kHz sampling rate) and computed the correlation coefficient between these two traces. The difference between these two traces ($I_{diff} = I_{experimental} - I_{theoretical}$) was used to calculate the SD of their pointwise difference. Among these shifts, a lag of 4 data points (0.04 ms) produced the highest correlation coefficient (0.9942) and the lowest SD (0.0789), showing the best temporal alignment between the two traces (Fig. 12).

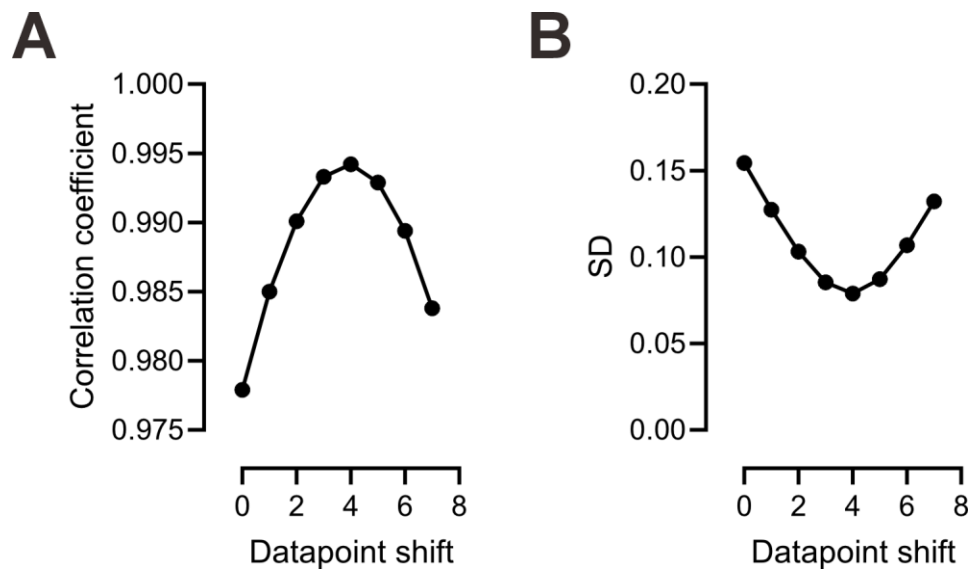


Figure 12. Quantifying the temporal offset between experimental and theoretical current traces in a GBC.

(A) Correlation coefficient between the experimental current trace and the theoretical current trace as a function of the relative shift (datapoint shift). One trace was shifted in 0.01 ms steps (100 kHz sampling; 1 data point per step). (B) Standard deviation (SD) of the pointwise difference across the same shifts. A lag of 4 data points (0.04 ms) yielded the highest correlation ($r = 0.9942$) and the lowest SD (0.0789), indicating the best temporal alignment between the two traces.

To further assess the consistency of this observed delay, I repeated this analysis across nine different synaptic stimulus conditions (high, medium, and no variation at frequencies of 350 Hz, 1 kHz, and 3.5 kHz) in two additional GBCs. In all nine conditions tested, the optimal alignment consistently occurred at a shift of 0.04 ms, characterized by maximal correlation coefficients and minimal SD (Table 3, supplementary).

Importantly, the observed system latency of approximately 0.04 ms constitutes only a small fraction ($<10\%$) of the membrane time constant typically reported for BCs in rodents, ≥ 0.5 ms (Cao et al., 2007). Therefore, this minimal delay is unlikely to significantly affect synaptic integration or spike timing precision in my conductance clamp experiments. The calculated and the measured current closely matched apart from this small offset (Fig. 11C), indicating faithful conductance-to-current conversion. Taken together, these analyses confirm that the conductance clamp system reliably and accurately reproduced synaptic-like conductance waveforms with negligible temporal lag.

4.2 Impact of Excitatory Input Variation on GBC Encoding

4.2.1 Synaptic Input Variation Increases GBC Firing Rate but Reduces Temporal Precision

To investigate how variations in synaptic input affect their output properties, GBCs were selected for recording according to established anatomical criteria and characteristic electrophysiological features (Fig. 13A1-A2). GBCs typically respond to suprathreshold current injections with only a single or a few action potentials at the onset (Fig. 6B).

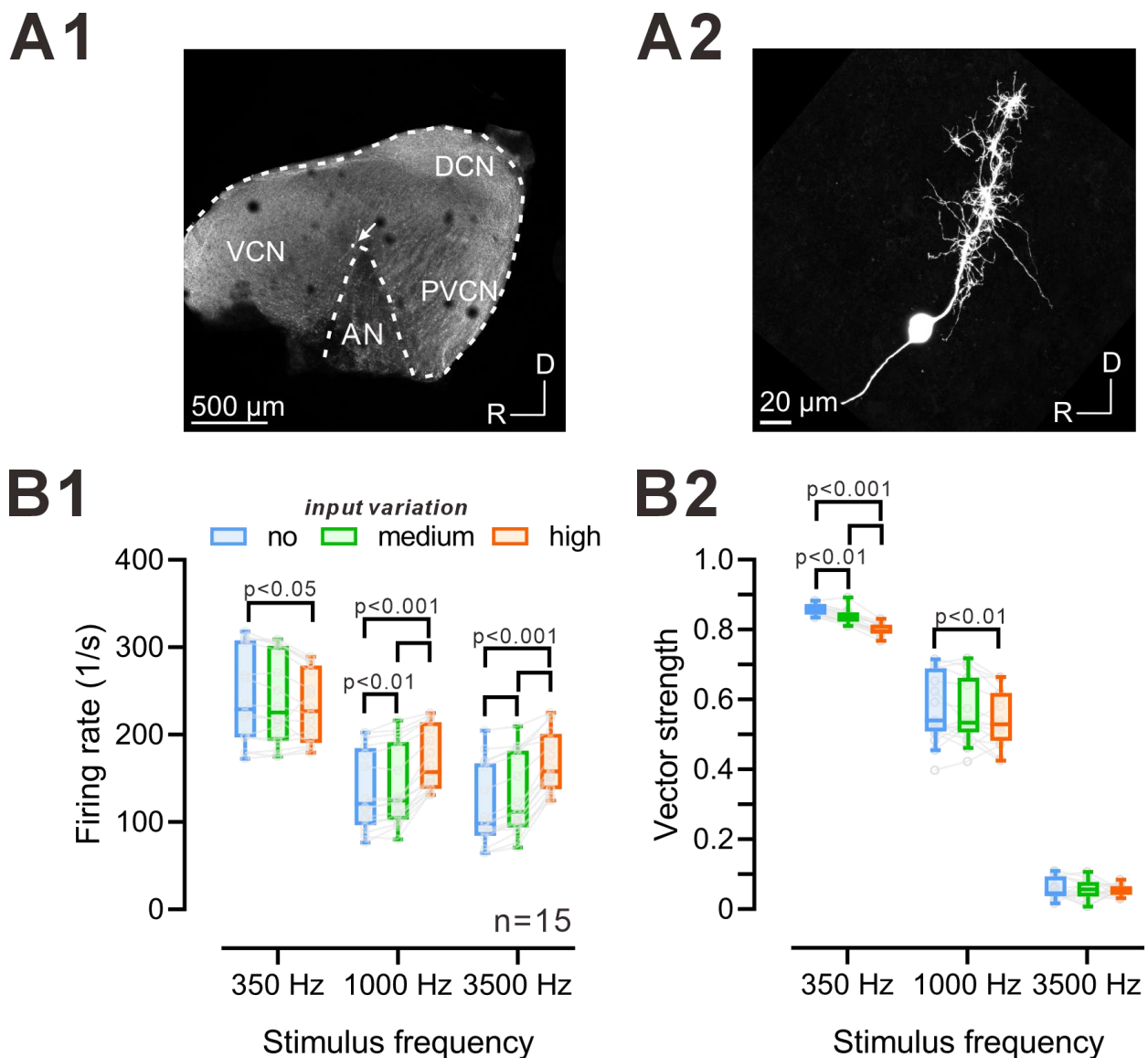


Figure 13. Increased synaptic input variability enhances firing rate but reduces temporal precision in GBCs.

(A) GBC identification based on morphological properties. A1: Low-magnification image of a parasagittal CN slice showing a biocytin-labeled GBC near the AN root. A2: High-magnification view of the same cell reveals the typical GBC morphology with an oval soma and short dendrites. (B1) Summary of GBC firing rate across

conditions. Population data ($n = 15$) show that higher synaptic variability significantly increased firing rates at 1000 and 3500 Hz. Boxplots represent medians and interquartile ranges; whiskers extend to $1.5 \times \text{IQR}$. (B2) Input variability reduces phase-locking precision. VS analysis indicates a decrease in temporal precision with increasing synaptic variability, especially at lower frequencies. Gray dots indicate individual cell data. Statistical analysis was conducted using repeated-measures two-way ANOVA with Bonferroni correction.

I first generated spike trains from 10 converging ANFs in response to 200-ms pure-tone stimuli (10 repetitions per frequency) at 350 Hz, 1 kHz, and 3.5 kHz using an established auditory periphery model (Bruce et al., 2018). These frequencies were selected to include ranges where ANFs exhibit robust phase-locking (350 Hz and 1 kHz) as well as a frequency (3.5 kHz) beyond the typical upper limit of phase-locking observed in rodents (Wei et al., 2017). Subsequently, the ANF spike trains were convolved with experimentally derived EPSC waveforms to create synaptic conductance templates (see Methods). These conductance templates were then directly injected into recorded GBCs to measure the membrane potential. To systematically assess the influence of synaptic input variation, I manipulated the distribution of synaptic strengths among the 10 simulated ANFs, generating three distinct conditions: ‘no variation’ (all inputs equal; $v = 0$), ‘medium variation’ ($v = 0.4$), and ‘high variation’ ($v = 1$) (Fig. 9).

First, it was analyzed how variations in synaptic input strength affected the average firing rates of GBCs. Increased input variation significantly enhanced the firing rate of GBCs in response to pure-tone stimuli at 1 kHz and 3.5 kHz (1 kHz: no variation = 131 ± 43 Hz vs. high variation = 168 ± 35 Hz, $p < 0.001$, $n = 15$). In contrast, at 350 Hz, the highest input variation ($v = 1$) led to a reduction in average firing rate (no variation = 239 ± 53 Hz vs. high variation = 229 ± 39 Hz, $p = 0.01$, Fig. 13B1).

Next, I examined how input variation influenced the temporal precision of GBC firing, quantified as vector strength (VS), a commonly used measure of neuronal phase-locking to periodic stimuli (Goldberg & Brown, 1969). The results demonstrated robust phase-locking at stimulus frequencies of 350 Hz and 1 kHz, but not at 3.5 kHz. Increasing the degree of synaptic input variation significantly reduced the phase-locking accuracy at both 350 Hz and 1 kHz (350 Hz: no variation = 0.86 ± 0.02 vs. high variation = 0.8 ± 0.02 , $p < 0.001$, Fig. 13B2), indicating a decrease in temporal precision. Hence, higher input variation increased firing

rates but reduced temporal precision. Together, these findings suggest that synaptic input variation mediates a trade-off between enhancing neuronal firing rates and reducing temporal precision, highlighting its potential role in modulating auditory temporal coding under different acoustic conditions.

4.2.2 Higher Input Variation Shortens AP Latency but Increases Temporal Jitter

To further quantify how input variability affects different phases of neuronal firing (onset and sustained firing), spike templates were generated from simulated ANFs in response to a 3.5 kHz pure-tone stimulus (200 repetitions), predicted to elicit minimal phase-locking (Wei et al., 2017). PSTHs were then constructed from both the simulated AN inputs and the experimentally recorded GBC outputs (Fig. 14A), showing a primary-like PSTH in the AN input and a primary-like-with-notch PSTH in GBCs. Both have a prominent onset peak, but only the GBC PSTH includes an early trough (notch) prior to lower sustained rate (Pfeiffer, 1966; Rhode & Smith, 1986).

The results revealed that increasing synaptic input variation caused a significant reduction in spike probability during the onset phase (no variation = 0.97 ± 0.02 vs. high variation = 0.94 ± 0.02 , $p < 0.001$, $n = 19$, Fig. 14B1). In contrast, both sustained and spontaneous firing rates were significantly increased (sustained: no variation = 84 ± 28 Hz vs. high variation = 138 ± 30 Hz, $p < 0.001$; spontaneous: no variation = 28 ± 15 Hz vs. high variation = 65 ± 15 Hz, $p < 0.001$, Fig. 14B2-B3).

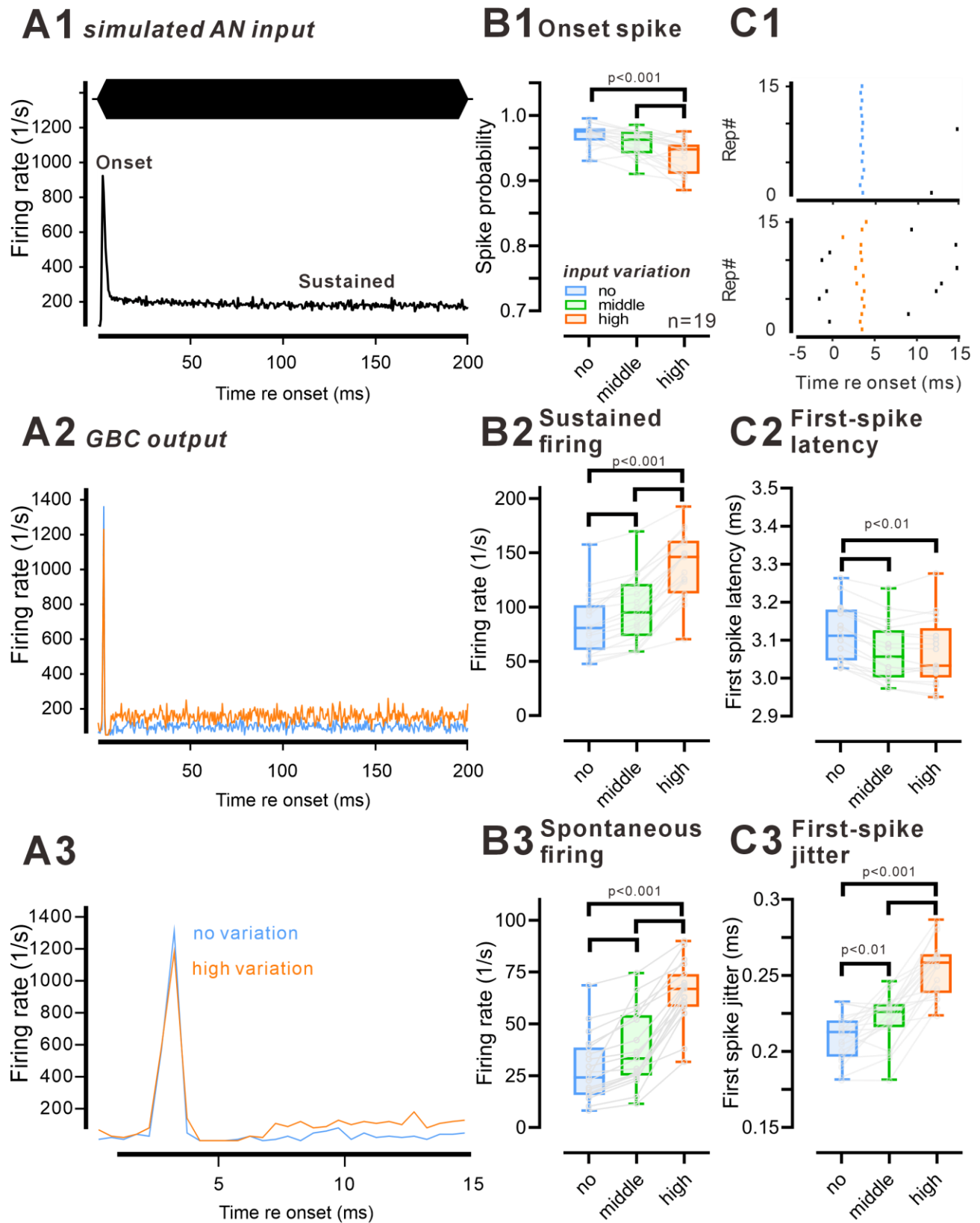


Figure 14. Increased synaptic input variability elevates sustained firing rates but reduces the precision of onset responses in GBCs. (A) Example responses of AN input and corresponding GBC output. A1: Representative PSTH of simulated AN input responding to a 3500 Hz pure-tone, showing a prominent onset response followed by adaptation to a lower sustained firing level. A2: Corresponding GBC responses under

conditions of low (blue) and high (orange) input variability. Increased variability reduces the sharpness of the initial onset response (A3) but enhances the sustained firing level. (B) Population analysis of GBC firing properties (n=19 cells). B1: The probability of generating an onset spike significantly decreases as input variability increases. B2-B3: Increased synaptic input variability enhances both sustained firing rates (50–200 ms post-onset; B2) and spontaneous firing rates (B3). (C) Impact of input variability on spike timing precision. C1: Raster plots showing first-spike timing across 15 repetitions for low (top, blue) and high (bottom, orange) input variability. Increased variability leads to earlier but less consistent first spikes. C2: Average first-spike latency is shorter at higher variability levels. C3: First-spike jitter (standard deviation of latencies) significantly increases with greater input variability, indicating reduced temporal precision. Boxplots (panels B, C) show medians and interquartile ranges (IQR), with whiskers extending to $1.5 \times \text{IQR}$; gray circles denote individual cell measurements. Statistical tests employed two-way repeated measures ANOVA with Bonferroni correction.

As shown in Fig. 14C1, higher input variation shortens first-spike latencies but disrupts trial-to-trial alignment compared with the no-variation condition. This effect was quantified by measuring first-spike latency and first-spike jitter (latency SD). Increased synaptic input variation reduced the first-spike latency (no variation = 3.12 ± 0.08 ms vs. high variation = 3.06 ± 0.09 ms, $p < 0.01$, Fig. 14C2) while increasing the first-spike jitter (no variation = 0.21 ± 0.02 ms vs. high variation = 0.25 ± 0.02 ms, $p < 0.001$, Fig. 14C3).

Taken together, these findings indicate that increasing synaptic input variability will decrease onset response probability and enhance sustained as well as spontaneous firing rates. Moreover, input variability reduces onset response latency but compromises spike timing precision and reliability.

4.2.3 Input Variation Enhances Firing Rate Tracking to Amplitude-modulated Sounds

The results from pure-tone stimulation showed that low input variation favors high temporal precision, whereas high input variation enhances firing rate. Next, AM stimuli were used to more closely reflect the structure of natural acoustic signals. AM stimuli, characterized by temporal envelope fluctuations, are essential for speech perception and the detection of environmental cues (Van Tasell et al., 1987; Drullman et al., 1996; Joris et al., 2004). In both ANFs and CN neurons, envelope coding plays an important role in tracking temporal patterns and conveying information about complex sounds (Joris et al., 2004). GBCs are

important contributors to envelope encoding, owing to their precise onset responses and strong temporal fidelity (Joris & Yin, 1992). Therefore, to assess whether synaptic input variability affects encoding strategies under naturalistic stimuli, GBC responses to AM tones across multiple modulation frequencies were acquired.

I first generated simulated ANF spike trains driven by AM tone stimuli. Specifically, the ANF model (Bruce et al., 2018) was used to generate spike trains from 10 converging ANFs responding to SAM tones. Stimuli consisted of a 13 kHz carrier frequency at 25 dB SPL, modulated by envelope frequencies of 200 Hz, 600 Hz, and 2000 Hz. The 13 kHz carrier frequency was selected as a balance between the optimal frequency range for robust envelope phase-locking, typically occurring above 10-20 kHz in high-characteristic-frequency fibers (Joris & Yin, 1992; Rhode & Greenberg, 1994), and practical limitations imposed by the 100 kHz simulation sampling rate. Furthermore, since phase-locking to AM envelopes typically declines at higher sound levels (Joris & Yin, 1992), a moderate level of 25 dB SPL was selected to ensure precise temporal encoding. Lastly, modulation frequencies of 200, 600, and 2000 Hz were specifically chosen to span the physiological range of envelope phase-locking abilities previously characterized in ANFs (Frisina et al., 1990; Rhode & Greenberg, 1994), thus providing comprehensive insight into GBC envelope processing capabilities across a broad frequency range.

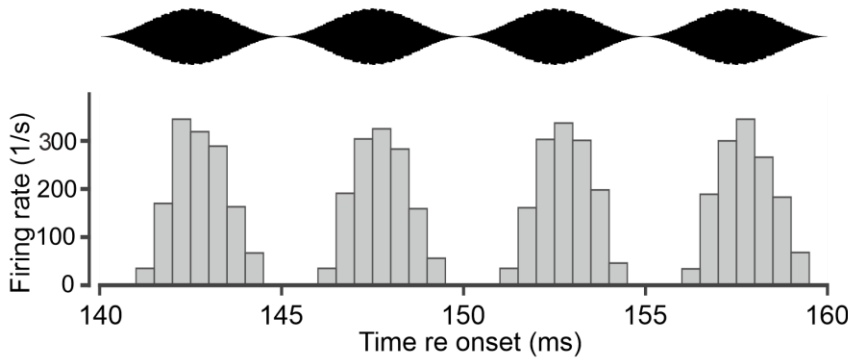
Using these designed stimuli, this study systematically varied synaptic input strength distributions to test how input variability affects firing rate tracking and temporal coding. The firing rates of simulated ANFs showed clear modulation corresponding to the stimulus envelope (Fig. 15A1), consistent with in vivo findings (Smith & Brachman, 1980; Joris & Yin, 1992). Injection of these conductance templates into GBCs under different variation conditions (no, medium, and high) revealed distinctly modulated firing rate responses (Fig. 15A2). The PSTHs in Fig. 15A2 show narrow, envelope-locked peaks for no variation condition, indicating higher temporal precision. By contrast, the peaks are broader and less tightly aligned to the envelope in high variation conditions.

Increasing synaptic input variation significantly elevated the overall firing rates across all tested modulation frequencies (600 Hz: no variation = 82 ± 46 Hz vs. high variation = 128 ± 33 Hz, $p < 0.001$, $n = 15$, Fig. 15B1). Conversely, the temporal fidelity assessed by VS showed

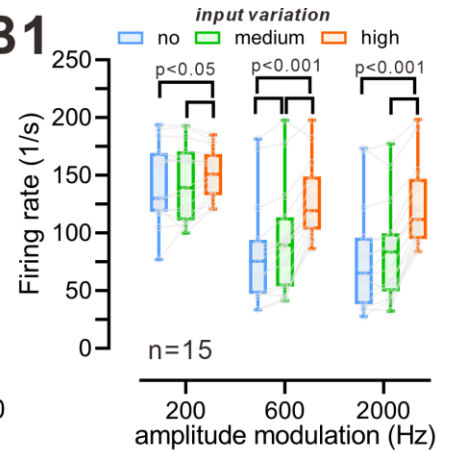
a significant decrease (600 Hz: no variation = 0.47 ± 0.07 vs. high variation = 0.35 ± 0.03 , $p < 0.001$, Fig. 15B2), indicating reduced temporal precision. Similarly, the correlation index (CI), which quantifies the consistency of spike timing across repeated stimulus presentations, decreased with higher input variation (600 Hz: no variation = 1.68 ± 0.27 vs. high variation = 1.28 ± 0.05 , $p < 0.001$, Fig. 15B3). However, the entrainment index (EI), measuring the alignment of inter-spike intervals with the stimulus modulation period (Joris et al., 1994), was significantly enhanced, especially at lower modulation frequencies (200Hz: no variation = 0.69 ± 0.18 vs. high variation = 0.73 ± 0.1 , $p < 0.05$, Fig. 15B4). To evaluate how GBC firing rates tracked the stimulus envelope, I calculated the normalized cross-correlation (CorrNorm) between the stimulus envelope and GBC firing rates. Notably, CorrNorm significantly improved with increasing input variation, particularly at the lowest modulation frequency (200 Hz: no variation = 0.84 ± 0.02 vs. high variation = 0.92 ± 0.01 , $p < 0.001$, Fig. 15B5). This highlights that higher input variation enhances the rate-coding capacity of GBCs for AM envelope representation.

Taken together, higher synaptic input variability was associated with reduced spike-timing precision and stronger tracking of envelope fluctuations in GBCs, consistent with a rate-timing trade-off. These findings suggest that different patterns of convergent ANFs onto GBCs may distinctly drive auditory processing, balancing the trade-off between temporal precision for encoding fine structure and improved representation of envelope dynamics through higher firing rates.

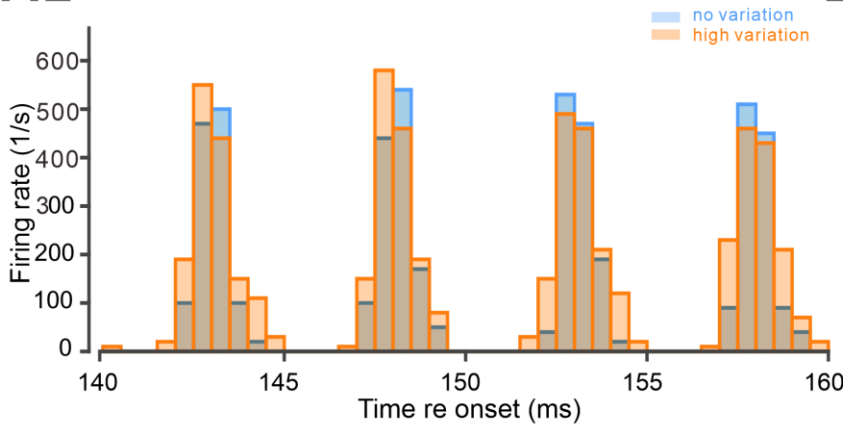
A1 AN response to 200Hz AM



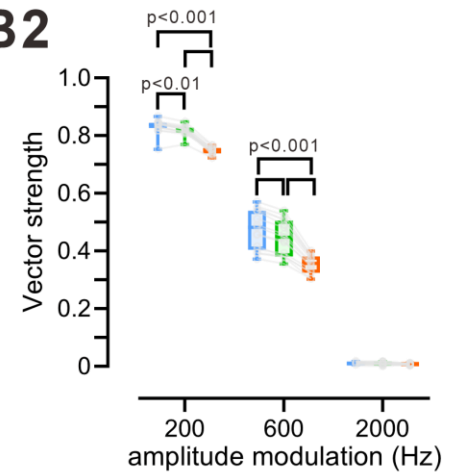
B1



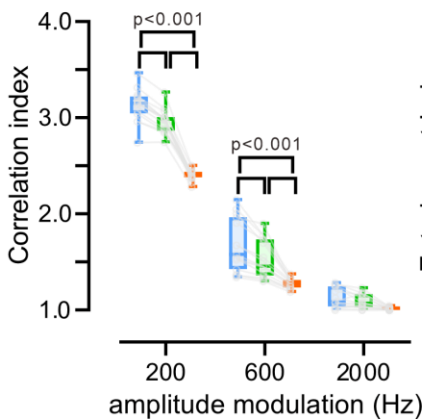
A2



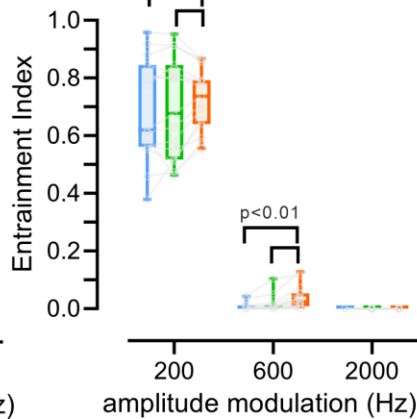
B2



B3



B4



B5

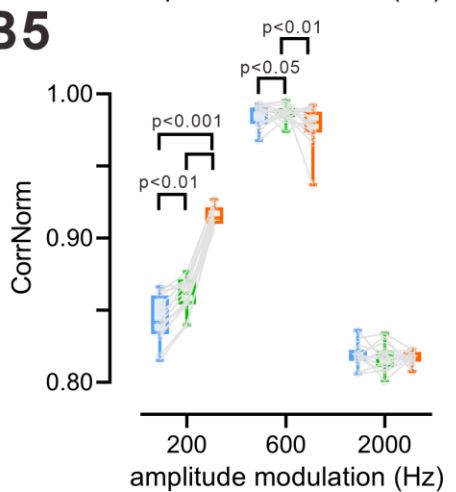


Figure 15. Variability in synaptic inputs improves GBC responses to amplitude-modulated stimuli. (A)

Example responses to amplitude modulation (AM). A1: Simulated ANF activity in response to a 200 Hz AM stimulus following the modulation envelope. A2: PSTH from a representative GBC recorded under conditions of low input variability (blue) and high variability (orange). Increased variability broadened response peaks. (B) Population analysis of GBC responses ($n = 15$) across three modulation frequencies (200, 600, 2000 Hz). B1: Firing rates increased significantly with synaptic input variability. B2-B3: Temporal encoding precision, measured as VS and CI, decreased with higher input variability. B4-B5: Conversely, EI and CorrNorm improved at lower modulation frequencies (200, 600 Hz) with increased variability and showed no significant effect at

higher modulation frequency (2000 Hz). Boxplots represent medians, interquartile ranges (IQR), and whiskers ($1.5 \times \text{IQR}$), with gray circles indicating individual cells. Statistical significance was evaluated using two-way RM ANOVA with Bonferroni post-hoc tests.

4.2.4 Impact of Input Variation is Consistent with Morphological Plausible Input Sizes

To test whether the effects of input variation observed with a gamma distribution are robust under physiological conditions, I implemented endbulb of Held size distributions measured from mouse ANFs converging onto GBCs (Spirou et al., 2023). Synaptic input variation from morphological data was used to create templates. Representative endbulb size distributions were selected to approximately match previously tested “medium” and “high” variation conditions (GBC21 ($v \approx 0.33$) and GBC18L ($v \approx 0.79$), respectively; see Methods). By employing these two distributions, I constrained synaptic input variability within anatomically realistic limits and examined the resulting coding capabilities of GBCs.

The anatomically synaptic variations produced similar effects on GBC responses to pure-tone stimuli as gamma-distributed variability. The high variation condition showed increased firing rates at 1 kHz and 3.5 kHz compared to the medium variation condition (1 kHz: medium variation = 96 ± 28 Hz vs. high variation = 121 ± 31 Hz, $p < 0.001$, $n = 18$, Fig. 16A). The VS was significantly decreased under the high variation condition for 350 Hz and 1 kHz stimuli (350 Hz: medium variation = 0.86 ± 0.01 vs. high variation = 0.82 ± 0.01 , $p < 0.001$, Fig. 16B). Analysis of onset responses further revealed a reduction in first-spike latency (1 kHz: medium variation = 4 ± 0.17 ms vs. high variation = 3.88 ± 0.21 ms, $p < 0.001$, Fig. 16C) with increasing first-spike jitter under high input variation (1 kHz: medium variation = 0.25 ± 0.05 ms vs. high variation = 0.31 ± 0.03 ms, $p < 0.001$, Fig. 16D). These results suggest that the capacity of input variation to shape GBC output is a direct functional consequence of the morphological heterogeneity among synaptic inputs.

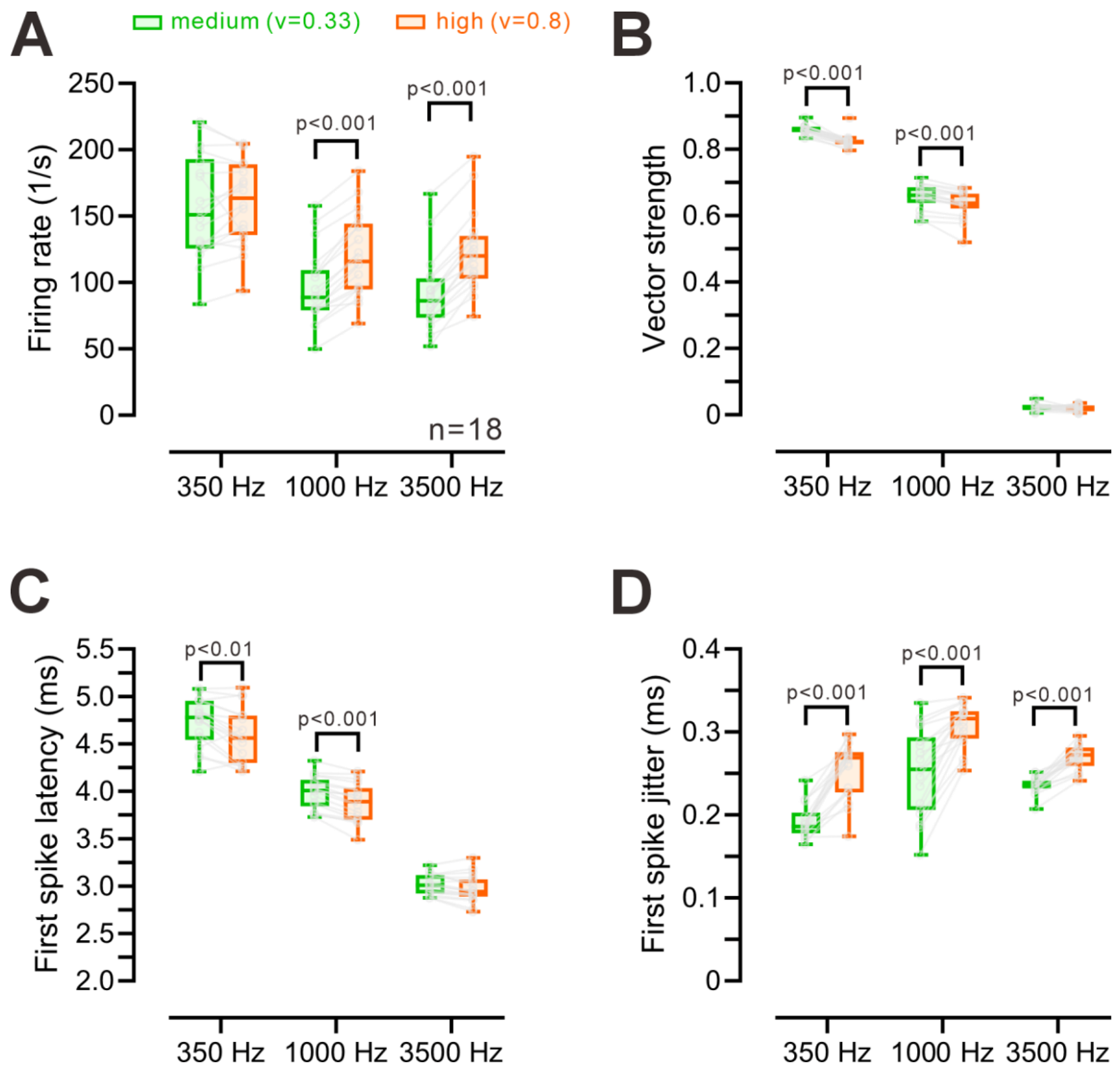


Figure 16. Morphological synaptic input variability underlies functional heterogeneity in GBC responses to pure tones. Population data (n = 18) comparing two morphologically derived variability conditions: medium (CV $\approx$ 0.33, green) vs. high variability (CV $\approx$ 0.8, orange) from previous reconstructions (Spirou et al., 2023). (A) GBC firing rates were significantly enhanced under high variability at higher stimulus frequencies (1000 and 3500 Hz), while responses to 350 Hz tones were unaffected. (B) VS analysis showed a decrease in temporal precision with higher input variability, particularly at lower frequencies. (C-D) Analysis of onset spike timing revealed shortened first-spike latencies (C) but increased jitter (D) under high variability conditions. Boxplots indicate median values, IQR, and whiskers extending to $1.5 \times$ IQR. Gray circles show individual data points. Statistical comparisons used two-way RM ANOVA with Bonferroni corrections.

Finally, I assessed how synaptic input variation derived from actual morphological ratios influenced GBC encoding of AM stimuli. Using the same two anatomically constrained conditions, the AM experiments were repeated. The results were consistent with

observations using gamma-distributed input variations, demonstrating that higher input variation substantially increased GBC firing rates (600 Hz: medium variation = 95 ± 19 Hz vs. high variation = 124 ± 35 Hz, $p < 0.001$, $n = 19$, Fig. 17A). However, the temporal precision was reduced (VS: 600Hz: medium variation = 0.46 ± 0.04 vs. high variation = 0.37 ± 0.02 , $p < 0.001$; CI: 600 Hz: medium variation = 1.46 ± 0.09 vs. high variation = 1.27 ± 0.03 , $p < 0.001$, Fig. 17B-C; VS and CI). Furthermore, rate-coding fidelity (CorrNorm) at the 200 Hz modulation frequency improved significantly under high variation (200 Hz: medium variation = 0.87 ± 0.01 vs. high variation = 0.98 ± 0.01 , $p < 0.001$, Fig. 17D).

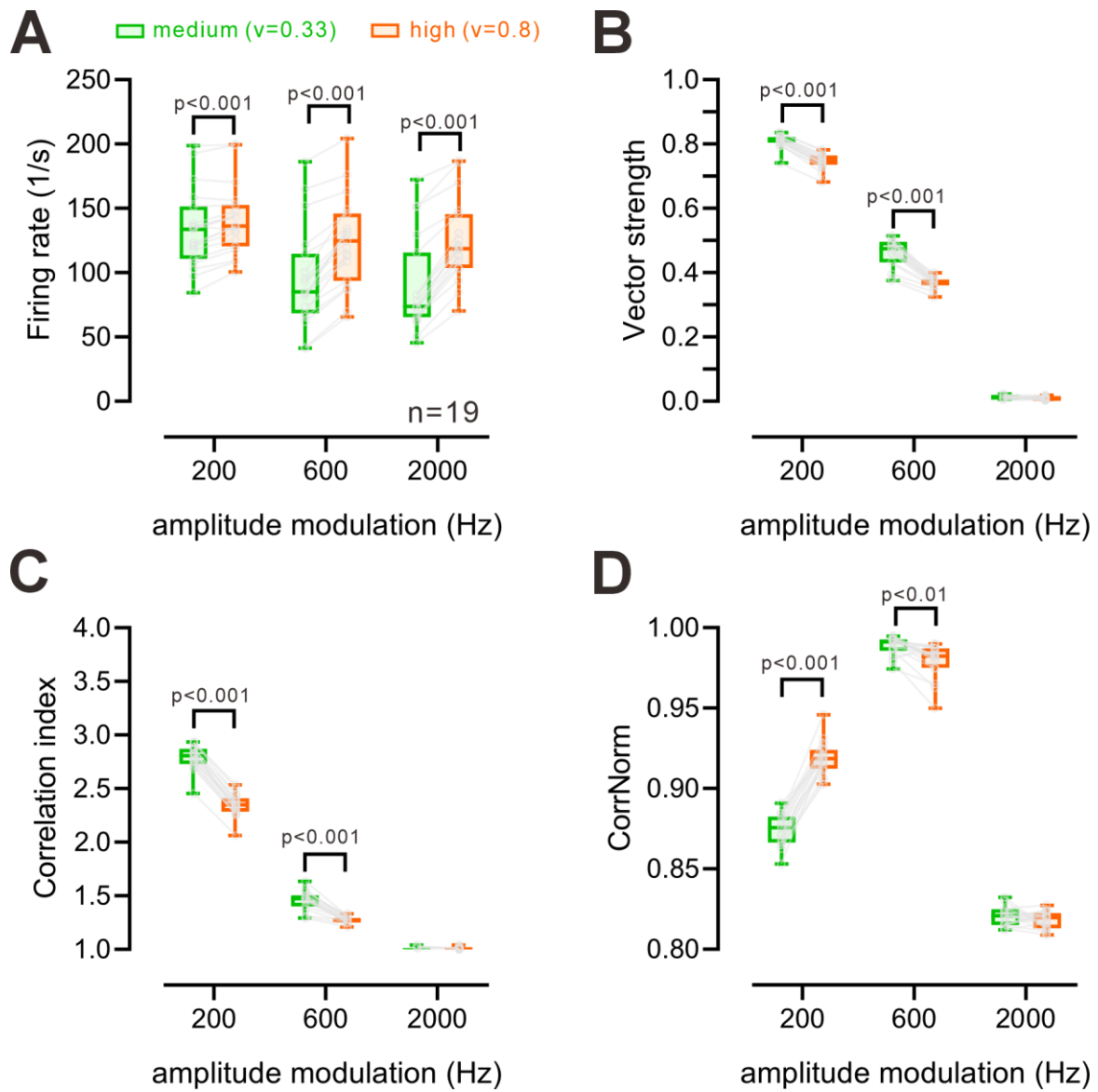


Figure 17. Morphologically defined input variability shapes GBC coding of SAM sounds. Population data ($n = 19$) comparing GBC responses to SAM under morphologically constrained variability conditions: medium

(CV $\approx$ 0.33, green) and high variability (CV $\approx$ 0.8, orange), based on morphological reconstruction data (Spirou et al., 2023). (A) Elevated firing rates were observed in GBCs receiving high variability inputs across modulation frequencies (200, 600, 2000 Hz). (B-C) High input variability significantly reduced temporal precision, measured as VS (B) and Clx (C). (D) Despite reduced temporal precision, encoding of the stimulus envelope (CorrNorm) improved significantly at higher variability, particularly at lower modulation frequencies. Boxplots represent median, interquartile ranges, whiskers ($1.5 \times$ IQR), and individual cells (gray circles). Statistical analysis employed two-way RM ANOVA with Bonferroni post-hoc testing.

In summary, these anatomically informed results confirmed the conclusion that heterogeneity in GBC endbulb inputs critically shapes the balance between temporal precision and rate coding, underlying the encoding strategies for both simple and complex auditory stimuli.

4.2.5 Effects of input variation are well reproduced in a coincidence-counting model

To investigate whether these experimental observations could be generalized and to ascertain if they arise primarily from the input statistics, Dr. Go Ashida employed a computational model for GBC, which was first developed for cats and adapted for gerbils in the present study (Ashida et al., 2019). This model generates an output spike when the summed inputs exceed an adaptive threshold. The model was driven with simulated ANF trains identical to those used in the in vitro experiments, incorporating ‘no’, ‘medium’, and ‘high’ input variations across a continuous scale (Fig. 18A). Notably, the model reproduced the key experimental findings, such as increased firing rates (Fig. 18B) and reduced temporal precision with higher input variation (Fig. 18C). This suggests that the interaction between the synaptic input statistics and a simplified cellular integration mechanism is sufficient to explain the experimentally observed phenomena. All simulations were performed by Dr. Go Ashida. Additional modeling results are provided in the dissertation’s Supplementary chapter.

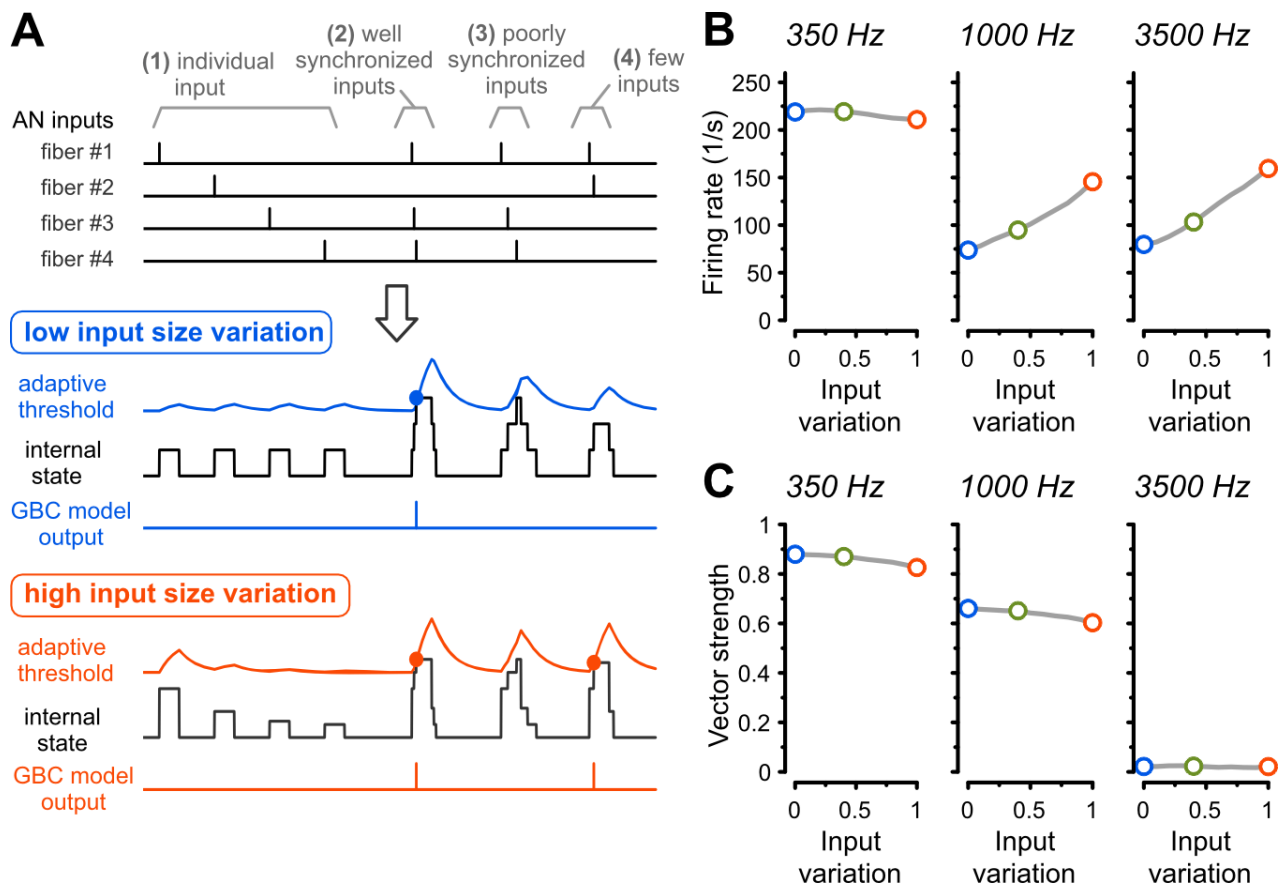


Figure 18. An adaptive coincidence-detection model accounts for GBC responses to synaptic input variability. (A) Schematic of the model framework. Top: Example spike trains from four individual ANFs, illustrating differences in synchrony among inputs. Middle: The model processes these inputs under two conditions- low (blue) and high (orange) input size variability. An internal state (black trace) integrates incoming EPSCs, while the adaptive threshold (colored trace) adjusts based on recent input activity. A spike is triggered when the internal state surpasses the threshold (highlighted dots). Under high variability, the model can fire even with fewer or desynchronized inputs, due to the stronger impact of large EPSCs. (B) Model output firing rate. Simulated GBC responses to pure-tone stimulation at 350 Hz, 1000 Hz, and 3500 Hz show that firing rate increases with input variability at 1000 and 3500 Hz, consistent with experimental observations. Colored dots indicate the same input variability levels used in slice experiments (no, blue; medium, green; high, orange). (C) Temporal precision declines with increasing input variability. VS, a measure of phase locking, decreases slightly as input variation increases, especially at lower frequencies.

4.3 Inhibitory Modulation of GBC Encoding under Excitatory Input Variation

In SBCs, *in vivo* studies showed that acoustically evoked glycinergic inhibition can suppress spikes in a stimulus-dependent manner, reduce the EPSP rising slope, and prolong the EPSP-to-AP transition. By suppressing poorly timed EPSPs, this inhibition also improves phase locking, effectively providing gain control (Kuenzel et al., 2011; Keine & Rübsamen, 2015; Keine et al., 2016). Direct functional evidence for inhibitory regulation in GBCs is comparatively sparse: BCs, including GBCs, receive glycinergic inputs from VCN D-stellate and dorsal CN tuberculoventral neurons, IPSC kinetics in BCs are slow in slices, and anatomical reconstructions report dense inhibitory contacts (Xie & Manis, 2013b, 2013a; Spirou et al., 2023).

Because both GBCs and SBCs receive large axosomatic endbulb inputs from ANFs and support sub-millisecond timing, I asked whether inhibition in GBCs affects timing and firing rate, analogous to SBCs. To address this question, I characterized evoked IPSCs in GBCs by analyzing the amplitude and temporal dynamics. Subsequently, these parameters were used to generate inhibitory conductance templates and integrate them into the conductance-clamp paradigm. This enabled to determine how the interaction between excitatory synaptic input variability and inhibition modulates GBC encoding strategies for both simple and complex auditory stimuli.

4.3.1 Establishing Excitation-Inhibition Balance by Titrating Inhibitory Strength

To assess how inhibition shapes GBC responses, I first calibrated the inhibitory conductance strength to reproduce failure rates comparable to *in vivo* observations. In previous studies of SBCs, inhibitory inputs were shown to substantially modulate excitatory drive, with 49% of sound-evoked stimuli (IQR 0.41-0.59) failing to elicit an AP during ongoing stimulation (Nerlich et al., 2014; Keine et al., 2016). Although these findings were reported in SBCs, they provide a reasonable reference for estimating effective excitatory-inhibitory balance in early auditory processing.

Earlier *in-vivo* and *in-vitro* recordings from SBCs have demonstrated slow temporal

dynamics of inhibition (Xie & Manis, 2013b; Kuenzel et al., 2015). In identified GBCs, I recorded evoked IPSCs with slow $\tau_{decay} = 20.5$ ms, which falls within the SBC range reported in rodents (~9 ms in mouse (Xie & Manis, 2013b) and ~24 ms in gerbil (Nerlich et al., 2014)), suggesting that inhibitory inputs to GBCs may also integrate over time to shape their output (Xie & Manis, 2013b; Kuenzel et al., 2015).

Initially, excitatory synaptic conductance templates were paired with an inhibitory step conductance of variable amplitude to find the balance strength between the excitatory and inhibitory templates that causes the same AP failure rate (Fig. 19A).

This approach allowed me to assess how static levels of inhibition suppress spiking activity across conditions of varying excitatory input. The rationale was that if a given level of constant inhibition could suppress APs under typical excitatory input, a dynamically generated inhibitory template with the same charge would likely achieve a comparable effect while preserving temporal fidelity. This enabled setting the inhibitory input parameters before assessing how excitatory input variation interacts with inhibition to shape GBC firing.

The excitatory conductance templates from pure-tone stimulation (350 Hz, 1 kHz, and 3.5 kHz) were used under both no and high synaptic input variation conditions. The protocol was as in other experiments, extended by independent conductance channel delivering a step-like inhibitory input (Fig. 19A). By gradually increasing the strength of the inhibitory step conductance, GBC firing rates decreased across all frequencies and input variation levels (Fig. 19C). To determine the balanced inhibition level for each condition, I measured the inhibitory conductance strength (as a percentage of excitatory conductance) required to achieve the reduction.

Under high input variation, a stronger inhibitory conductance was required to achieve the same level of suppression as in the no variation condition across all three frequencies (Fig. 19C). One plausible explanation for these findings is that under high input variation, a small number of strong excitatory inputs can dominate the postsynaptic response and drive spiking even in the presence of inhibitory conductance. Because these potent inputs are more likely to reach threshold on their own, stronger inhibition is needed to suppress their effect. In contrast, in the no variation condition, inputs are uniformly weighted, and thus the entire excitatory drive is more easily attenuated by inhibition.

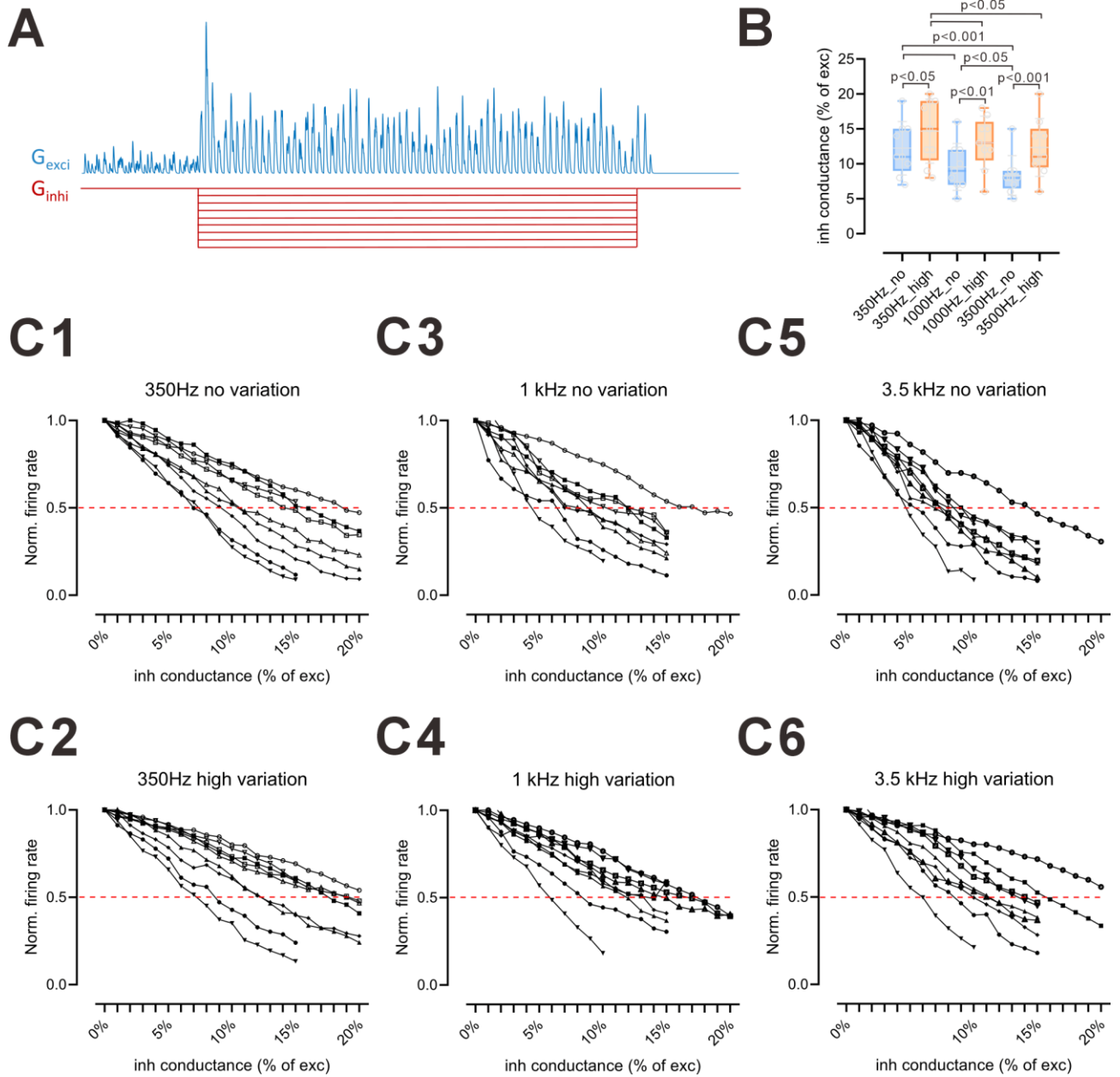


Figure 19. Constant inhibitory conductance modulates GBC firing rate and reveals frequency-dependent excitation-inhibition balance. (A) Schematic illustrating the simplified inhibition protocol. Excitatory synaptic conductance (blue) derived from pure-tone stimuli was combined with a tonic, step-like inhibitory conductance (red). The amplitude of inhibition was stepwise increased to systematically suppress GBC firing. (B) Quantification of inhibitory conductance required for 50% firing rate suppression. High input variation required significantly stronger inhibitory input than no variation conditions across all frequencies. Additionally, the inhibitory-to-excitatory conductance ratio necessary for balanced suppression was higher at lower stimulus frequencies (350 Hz) compared to higher frequencies (1000 Hz and 3500 Hz), indicating frequency-dependent differences in excitatory-inhibitory integration. (C1-C6) Representative data from GBCs showing a firing rate decline with increasing inhibition across six conditions: three stimulus frequencies (350, 1000, and

3500 Hz) under two input variation levels (no variation and high variation). The red dashed line represents a 50% threshold. Each subplot illustrates firing rate as a function of inhibitory strength for one frequency–variation combination. Each line represents one cell. Boxplots depict medians, interquartile ranges (IQR), whiskers extending to $1.5 \times \text{IQR}$, with individual cells shown as gray circles. Statistical analysis employed a paired t-test.

Furthermore, the 350 Hz stimulation required a higher inhibitory-to-excitatory ratio to suppress firing by 50% compared to 3.5 kHz, indicating a frequency-dependent difference in the balance between excitation and inhibition.

The frequency-dependent difference may be attributed to the enhanced temporal synchrony of excitatory inputs at lower frequencies. At 350 Hz, GBCs exhibit stronger phase-locking (Joris et al., 1994), which promotes temporal summation of excitatory postsynaptic potentials (EPSPs), increasing the possibility of spike generation within narrow time windows. To counteract this synchronized excitatory drive, stronger tonic inhibition is required. At 3.5 kHz, reduced phase-locking and dispersed EPSPs limit summation and increase relatively impact of inhibition. These findings suggest that the efficacy of inhibitory modulation depends not only on the strength of excitation but also on its temporal structure, shaped by both input variability and stimulus frequency.

Based on the titration curves (Fig. 19B, C), the inhibitory-to-excitatory conductance ratio required to reduce firing by ~50% was varied with frequency and input variability (5-20% across conditions). To avoid introducing condition-dependent parameters, I fixed the inhibitory setting to $I/E = 0.1$ for subsequent experiments. This value reduced firing ~20-70% across cells, providing a constant operating point for comparing how input variation and frequency affect GBC responses.

4.3.2 Synaptic Input Variation Preserves Its Temporal Tradeoff Effects Under Balanced Inhibition

To investigate how variations in synaptic input influence GBC response properties under balanced inhibitory conditions, I employed the same conductance templates with 100 repetitions.

Consistent with previous excitatory-only findings, increased synaptic input variability integrated with inhibition elevated the firing rates at all three frequencies (1 kHz: no variation = 60 ± 29 Hz vs. high variation = 100 ± 35 Hz, $p < 0.001$, $n = 17$, Fig. 20A), whereas temporal precision (quantified as VS) decreased at 350 Hz and 1 kHz (350 Hz: no variation = 0.89 ± 0.02 vs. high variation = 0.83 ± 0.01 , $p < 0.001$, Fig. 20B).

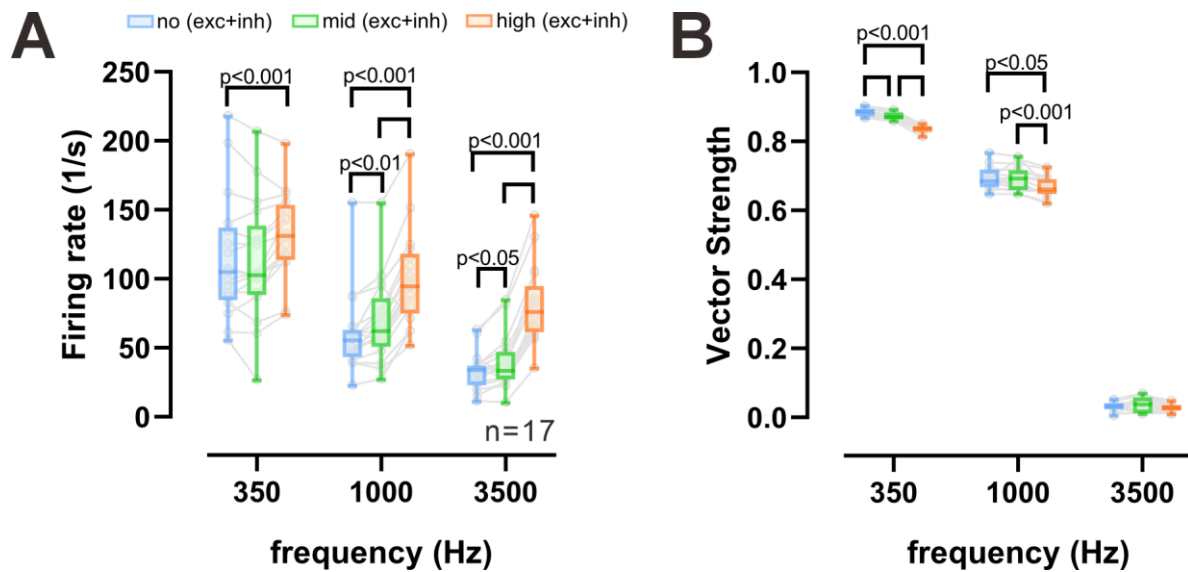


Figure 20. Synaptic input variation preserves its temporal tradeoff effects under balanced inhibition. (A) Effects of synaptic input variability on GBC firing rate and temporal precision under simultaneous excitatory and inhibitory conductance application (exc+inh). (A) Population data ($n = 17$) showing firing rates across three levels of synaptic input variability (no, medium, and high) at 350 Hz, 1000 Hz, and 3500 Hz pure-tone stimulation. Firing rate increased with greater input variability, particularly at 1 kHz and 3.5 kHz. (B) Temporal precision measured as VS decreased with increasing input variability at 350 Hz and 1 kHz. Notably, inhibition did not alter this trend. Box plots show median, interquartile range (IQR), and $1.5 \times$ IQR whiskers; individual data points represent single cells. Statistical analysis: repeated-measures two-way ANOVA with Bonferroni correction.

A direct comparison between no-variation and medium-variation conditions under excitatory-only (exc) and exc+inh conditions demonstrated that inhibition reduced overall spike counts by 20-80% (Fig. S5A-B). Additionally, inhibition reduced spiking between 10% and 70% under the high-variation condition (Fig. S5C).

Additional analysis was performed to evaluate whether the inhibition affects temporal

precision in addition to spike reduction. Specifically, I artificially reduced spike counts in exc trials (simulated_inh) by randomly deleting the same number of spikes as under exc+inh for the same cell and condition. Hence, the effect of spike-rate reduction was isolated from the effect of inhibition. Comparisons among exc, exc+inh, and simulated_inh revealed that excitatory input variation-induced trends regarding firing rate and temporal precision were maintained in the presence of inhibition. Notably, inhibition did not significantly alter the temporal VS when compared to either exc or simulated_inh conditions at corresponding variation levels (350 Hz: no variation: exc = 0.89 ± 0.01 vs. exc+inh = 0.89 ± 0.02 , Fig. 21A-C). These results indicate that inhibitory conductance primarily reduces firing without altering the temporal coding under the pure-tone stimulus. A plausible explanation is that, under pure-tone stimulation, GBCs receive multiple endbulb inputs, generating well-synchronized, phase-locked events per cycle. Therefore, even with inhibition suppressing weaker or poorly synchronized events, the remaining synchronous events still drive APs at a similar phase of the cycle, so temporal precision (VS) remains unchanged.

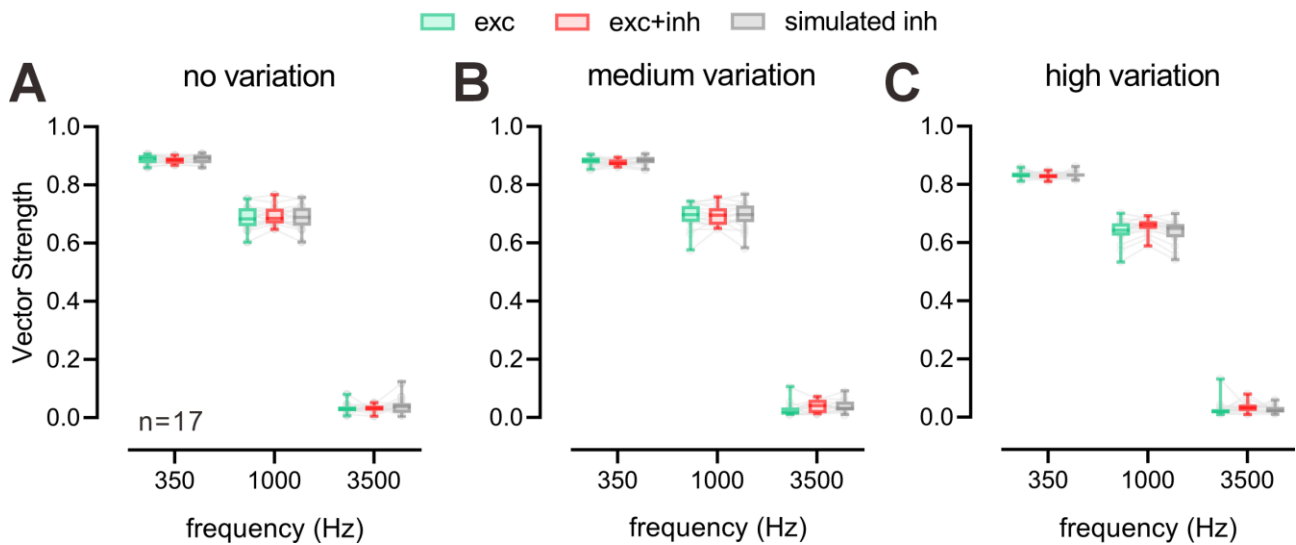


Figure 21. Inhibition does not affect temporal precision under pure-tone stimulation. Population data (n = 17) comparison among excitatory-only (exc), combined excitatory and inhibitory (exc+inh), and simulated combined excitatory and inhibitory conditions by randomly decreasing half of the APs in the excitatory-only condition (simulated_inh). (A-B) The same recording group as Fig. S5A-B, (C) same recording group as Fig. S5C. In simulated_inh, spikes were randomly removed to match the same number of spikes generated under exc+inh. Across all frequencies and variation levels, difference in VS was found neither between exc and

simulated_inh, nor between simulated_inh and exc+inh.

4.3.3 Inhibition Modulates the Effects of Input Variation on GBC Onset Responses

To investigate how synaptic-input variation affects GBC onset responses in the presence of inhibition, I used conductance templates for excitation and inhibition as above.

First, the combined excitatory-inhibitory conditions (exc+inh) were compared across the three input variability levels. Similar to results under excitatory-only (exc) condition, increasing synaptic input variability reduced onset spike probability (1 kHz: no variation = 0.97 ± 0.04 vs. high variation = 0.94 ± 0.05 , $p < 0.001$, $n = 17$, Fig. 22A) and first-spike temporal precision (350 Hz, first-spike latency decreased: no variation = 4.6 ± 0.05 ms vs. high variation = 4.56 ± 0.07 ms, $p < 0.05$ while jitter increased: no variation = 0.20 ± 0.02 ms vs. high variation = 0.29 ± 0.05 ms, $p < 0.001$; at 1 kHz, latency remained stable: no variation = 4.01 ± 0.05 ms vs. high variation = 4.00 ± 0.07 ms while jitter increased: no variation = 0.33 ± 0.04 ms vs. high variation = 0.37 ± 0.02 ms, $p < 0.01$; and at 3.5 kHz, both latency and jitter increased: first-spike latency: no variation = 2.97 ± 0.05 ms vs. high variation = 3.02 ± 0.06 ms, $p < 0.001$; first-spike jitter: no variation = 0.20 ± 0.01 ms vs. high variation = 0.24 ± 0.02 ms, $p < 0.001$, Fig. 22B-C).

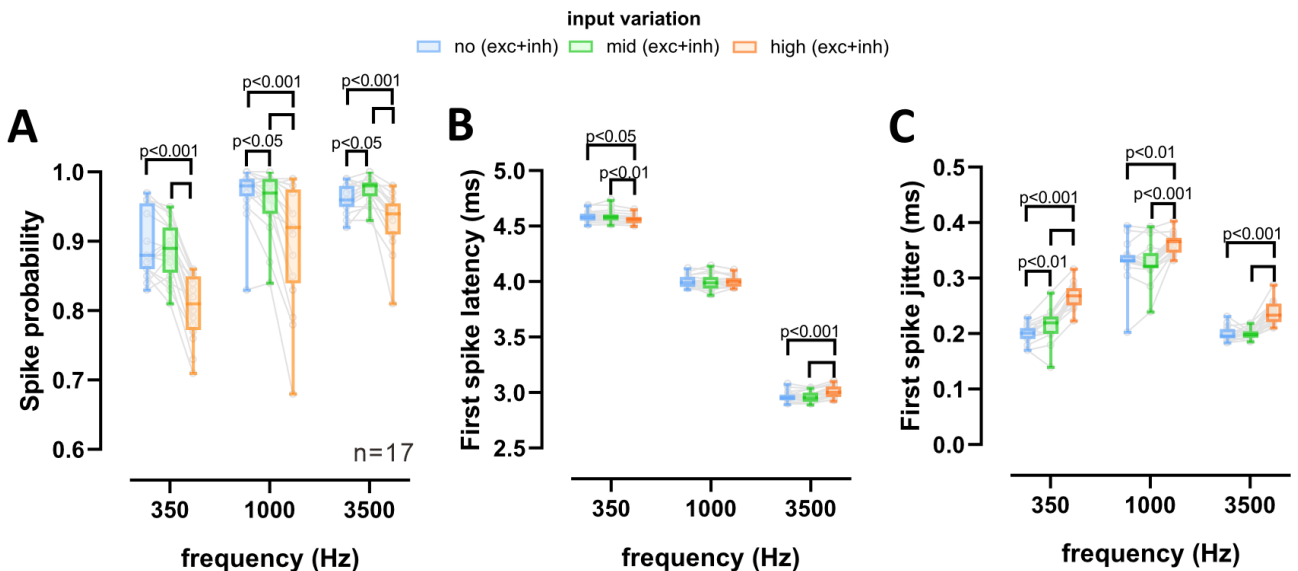


Figure 22. Influence of combined synaptic input variability and inhibition on GBC onset response properties.

(A) First-spike probability across three levels of input variability (no, medium, high) and three stimulation frequencies (350 Hz, 1000 Hz, 3500 Hz) under simultaneous excitatory and inhibitory (exc+inh) conditions.

Spike probability decreased with increasing input variability under combined excitatory-inhibitory conditions. (B) Increased synaptic input variability decreased first-spike latency at 350 Hz, but increased latency at 3500 Hz. (C) Higher input variability consistently elevated first-spike jitter, reflecting decreased temporal precision. Box plots indicate median, interquartile range, and whiskers represent $1.5 \times \text{IQR}$. Gray dots represent individual cell data ($n=17$). Statistical comparisons were performed using repeated-measures two-way ANOVA with Bonferroni correction for multiple comparisons.

To confirm that the observed changes were specifically due to inhibitory modulation rather than merely reduced spike counts, I used the same simulated_inh control as described above. First-spike probability decreased in the simulated_inh control because spikes were randomly removed; when the deleted event happened to be the first spike of a trial, first-spike probability decreased (Fig. 23A). In contrast, inhibition produced no additional decrease compared with the excitatory-only group (1000 Hz, no variation: $\text{exc} = 0.96 \pm 0.05$ vs. $\text{exc+inh} = 0.97 \pm 0.04$), indicating that inhibition does not abolish the onset spike.

Analysis of first-spike latency revealed that inhibition prolonged latencies at no and medium variability across nearly all tested frequencies, while having minimal influence under high variability (no variation: 350 Hz: $\text{exc} = 4.59 \pm 0.06$ ms vs. $\text{exc+inh} = 4.60 \pm 0.06$ ms, $p < 0.05$, 1 kHz: $\text{exc} = 3.99 \pm 0.06$ ms vs. $\text{exc+inh} = 4.00 \pm 0.07$ ms, $p < 0.05$, 3500 Hz: $\text{exc} = 2.95 \pm 0.04$ ms vs. $\text{exc+inh} = 2.97 \pm 0.05$ ms, $p < 0.01$; high variation: 350 Hz: $\text{exc} = 4.56 \pm 0.07$ ms vs. $\text{exc+inh} = 4.56 \pm 0.07$ ms, 1 kHz: $\text{exc} = 4.00 \pm 0.09$ ms vs. $\text{exc+inh} = 4.00 \pm 0.08$ ms, 3500 Hz: $\text{exc} = 3.00 \pm 0.06$ ms vs. $\text{exc+inh} = 3.02 \pm 0.06$ ms, $p < 0.001$, Fig. 23B). Analysis of first-spike jitter showed that inhibition reduced jitter in both no and medium variability conditions, particularly noticeable at 350 Hz and 1 kHz (no variation: 1 kHz: $\text{exc} = 0.35 \pm 0.04$ ms vs. $\text{exc+inh} = 0.33 \pm 0.05$ ms, $p < 0.001$, Fig. 23C). This observation aligns with earlier studies on SBCs, suggesting that inhibitory conductance sharpens temporal precision by selectively suppressing weaker or poorly synchronized inputs under no/medium-variation conditions, thereby stabilizing spike timing (Keine & Rübsamen, 2015; Keine et al., 2016).

A plausible explanation is that, at the time of first spike generation, inhibitory conductance had already accumulated due to the time-aligning excitation and inhibition. This produced hyperpolarization that raised the effective threshold, thereby prolonging spike latency while

reducing jitter in the no- and medium-variation conditions. Under high-variability conditions, stronger (suprathreshold) inputs can overcome inhibitory effects, thereby minimizing their impact.

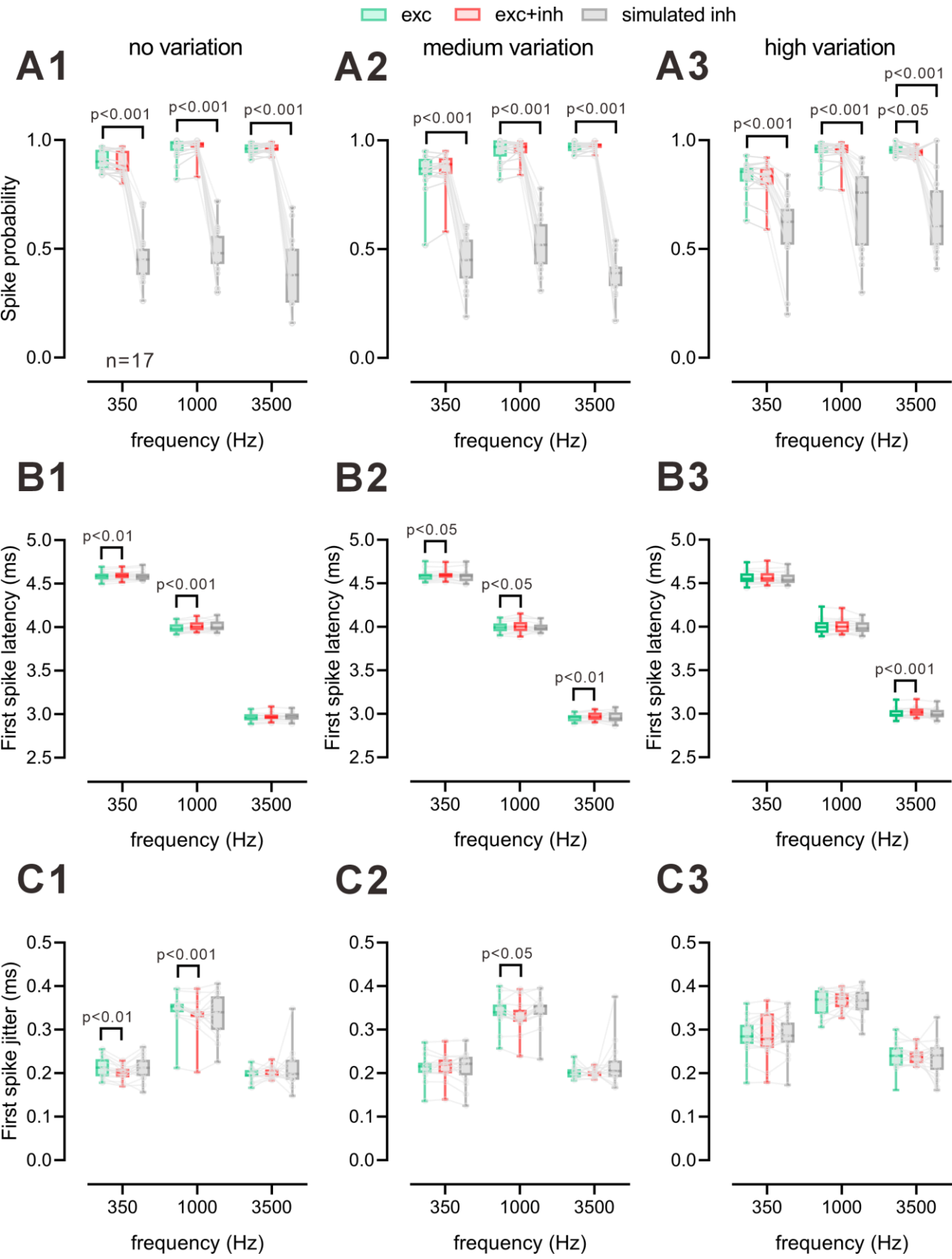


Figure 23. Inhibition prolongs the latency of onset responses but decreases the jitter without affecting onset spike probability. (A) Comparison of first-spike probability among the three experimental conditions. Spikes were randomly removed in the simulated_inh condition to match the firing observed under exc+inh. Simulated_inh showed reduced first-spike probabilities relative to exc, confirming the validity of the control manipulation. The inhibition (exc+inh) did not alter first-spike probability compared to exc. (B) Inhibitory conductance prolonged first-spike latency under no and medium variability conditions at 350 and 1000 Hz, and prolonged first-spike latency under high variability only at 3500 Hz. No difference between exc and simulated_in. (C) Jitter was reduced by inhibitory conductance relative to exc and simulated_inh, particularly under no and medium variability conditions at lower frequencies. Box plots display median, interquartile range, and whiskers represent $1.5 \times \text{IQR}$. Individual data points represent single cells ($n=17$). No and medium variations recordings are the same recording group as Fig. S5A-B; high variation recordings are the same recording group as Fig. S5C. Statistical tests were conducted using repeated-measures two-way ANOVA followed by Bonferroni post hoc tests.

Comparison between the exc and simulated_inh conditions showed no significant difference in first-spike latency and jitter. Therefore, the changes in first-spike latency and jitter are due to inhibitory conductance. Taken together, these observations reinforce the notion that synaptic input variability fundamentally governs onset coding properties in GBCs, consistently decreasing spike-time precision and increasing sustained firing rates. The inhibition modulates these properties subtly and selectively by prolonging first-spike latency, especially at lower-to-medium input variations, and reducing first-spike jitter.

4.3.4 Inhibitory Inputs Enhance Temporal Precision but Reduce Rate-Based Envelope Tracking in GBCs

To further investigate how synaptic input variability shapes GBC encoding under inhibitory conditions, I extended the analysis to AM sound stimuli. The same excitatory conductance templates were derived from ANF responses to AM tones at modulation frequencies of 200 Hz, 600 Hz, and 2000 Hz and applied with no, medium, and high input variability, along with inhibitory conductance.

Across all tested AM frequencies, the fundamental influence of synaptic input variation persisted under combined excitatory and inhibitory (exc+inh) conditions. Consistent with

earlier observations, increased input variation generally elevated firing rates (600 Hz: no variation = 27 ± 18 Hz vs. high variation = 79 ± 34 Hz, $p < 0.001$, $n = 23$, Fig. 24A) and decreased temporal precision (reduced VS and CI values, VS: 600 Hz: no variation = 0.63 ± 0.03 vs. high variation = 0.43 ± 0.02 , $p < 0.001$; CI: 600 Hz: no variation = 1.94 ± 0.23 vs. high variation = 1.39 ± 0.05 , $p < 0.001$, Fig. 24B-C). Moreover, envelope tracking fidelity, measured by CorrNorm and EI, was improved (CorrNorm 200 Hz: no variation = 0.83 ± 0.01 vs. high variation = 0.99 ± 0.01 , $p < 0.001$, Fig. 24D; EI 200 Hz: no variation = 0.41 ± 0.15 vs. high variation = 0.53 ± 0.1 , $p < 0.001$, Fig. S6A). To validate the magnitude of inhibitory modulation, I recorded an additional excitatory-only (exc) condition at 200 Hz (high variation) and observed a ~70% reduction in firing rates (Fig. S6B), confirming the robustness of inhibitory effects.

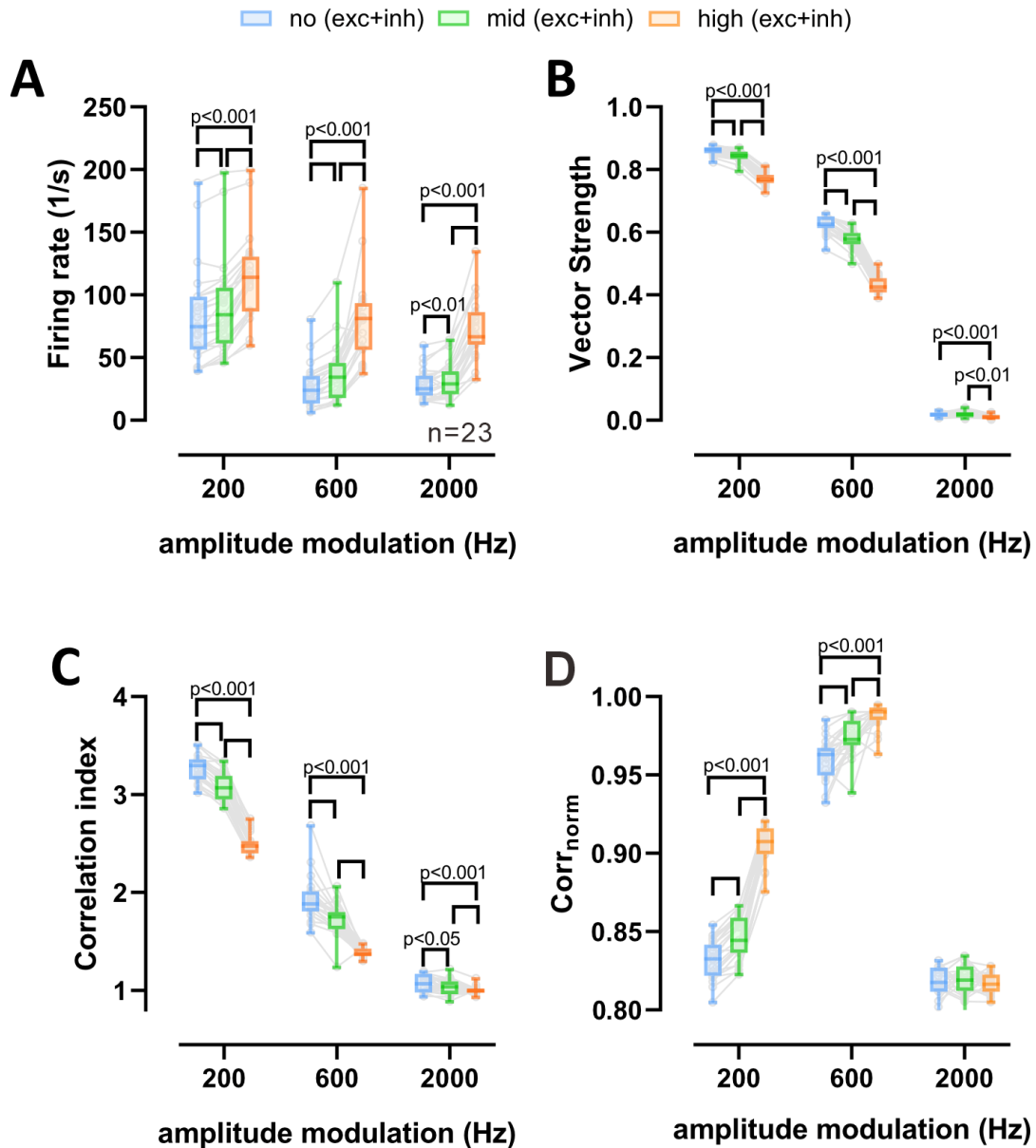


Figure 24. Effects of excitatory input variability on GBC encoding of AM stimuli under inhibitory conductance. (A) Firing rates were measured under three different AM frequencies (200 Hz, 600 Hz, 2000 Hz) and three excitatory input variability conditions (no, medium, high). Population data ($n = 23$) showed consistent increases in firing rates, with higher input variability. (B-C) Temporal precision quantified by VS (B) and CI (C) was reduced with input variability. (D) Envelope-tracking fidelity measured by CorrNorm increased with input variability. Data are presented as box plots (median, interquartile range [IQR], whiskers $1.5 \times \text{IQR}$); individual cells are represented by circles. Statistical analysis performed via repeated-measures two-way ANOVA with Bonferroni correction.

At 2000 Hz modulation frequency, the low VS, CI, and CorrNorm values indicated minimal envelope tracking capability, reflecting GBCs' inherent physiological limitations at such high modulation frequencies. This is consistent with the limited phase-locking capacity of GBCs when the temporal structure of the envelope becomes too rapid for reliable tracking by individual spikes (Johnson, 1980; Joris & Yin, 1992). Thus, subsequent analyses focused on 200 Hz and 600 Hz, which are in the phase-locking range of BCs.

To determine whether observed changes resulted specifically from inhibitory modulation or simply from reduction of APs, I implemented a simulated inhibitory control (simulated_inh) group with randomly removed spikes to match the firing under exc+inh. Comparative analyses demonstrated that inhibitory conductance significantly increased temporal precision (increased VS and CI, VS: no variation: 600 Hz: exc = 0.56 ± 0.05 vs. exc+inh = 0.62 ± 0.03 , $p < 0.001$; CI: no variation: 600 Hz: exc = 1.67 ± 0.15 vs. exc+inh = 1.76 ± 0.27 , $p < 0.05$, Fig. 25A-B) compared to both exc and simulated_inh conditions. Notably, VS and CI showed no differences between exc and simulated_inh conditions, confirming that the inhibition accounts for the observed improvement of temporal precision by narrowing the integration window. However, CorrNorm (middle variation: 200 Hz: exc = 0.85 ± 0.13 vs. exc+inh = 0.83 ± 0.02 , $p < 0.001$, Fig. 25C) and EI (Fig. S6A) were consistently reduced.

In summary, these results demonstrate that excitatory input variability-driven changes in encoding strategies are preserved under the physiologically more realistic condition of inhibition. Moreover, the data indicate that inhibitory modulation actively enhances temporal precision and reshapes rate-based envelope tracking, rather than merely reducing AP output.

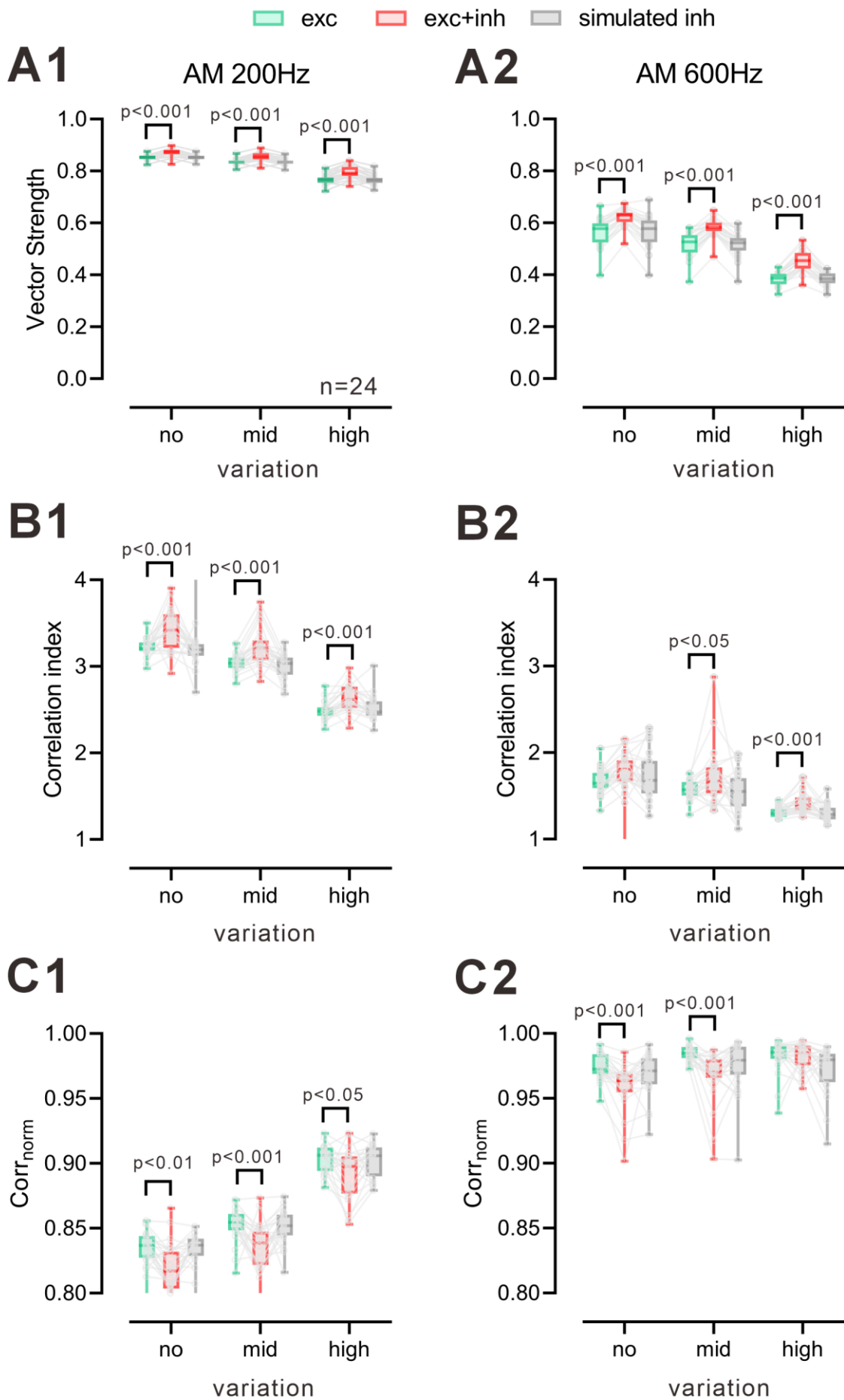


Figure 25. Inhibition specifically enhances temporal precision but decreases rate-coding of AM sounds. (A-

B) Comparison of temporal precision metrics (VS, and CI) among excitatory-only (exc), combined excitatory-inhibitory (exc+inh), and simulated inhibitory control (simulated_inh) conditions for AM frequencies 200 Hz and 600 Hz. Simulated_inh, spikes were randomly removed to match the same number of spikes that were observed under exc+inh. Inhibition (Exc+Inh) increased VS and CI compared to both exc and simulated_inh conditions. Note no significant difference between exc and simulated_inh conditions. (C) Envelope-tracking fidelity (CorrNorm) was reduced under inhibition for both 200 Hz and 600 Hz modulation frequencies. Box plots represent median, interquartile range (IQR), whiskers $1.5 \times \text{IQR}$; data points indicate individual cells (n=24). Statistical analysis via repeated-measures two-way ANOVA with Bonferroni correction.

5. Discussion

Synaptic heterogeneity is a common feature of neural circuits, influencing neuronal processing and information relay. In the auditory brainstem, GBCs receive excitatory inputs via multiple endbulbs of Held, exhibiting prominent variability in size even among inputs converging onto the same postsynaptic neuron (Nicol & Walmsley, 2002; Spirou et al., 2005; Spirou et al., 2023). Despite this well-documented morphological diversity, the functional implications of such input strength variation at the endbulb–GBC synapse have remained largely unexplored. In this study, I tested how heterogeneity in excitatory endbulb inputs shapes the encoding properties of GBCs across acoustic stimulus conditions (e.g., pure tones and SAM tones). In addition, I examined how excitatory input variability interacts with inhibitory input to determine its influence on GBC encoding metrics. Low synaptic input variation enhanced temporal precision, indicated by increased VS, CI, and improved timing of the first spike. Conversely, high input variation increased overall firing rates and enhanced the fidelity of firing rate encoding (CorrNorm) in response to SAM stimuli at the expense of temporal precision. The effects induced by synaptic input variation were preserved under inhibition, which further increased the temporal precision (VS, CI) but reduced rate coding (CorrNorm) of SAM stimuli. Together, these results demonstrate that the encoding strategy employed by GBCs depends on the distribution of convergent excitatory inputs and inhibitory modulation, underscoring the importance of input heterogeneity in auditory processing.

5.1 Input heterogeneity shapes GBC encoding strategy

Across recordings and coincidence-counting modeling (developed in collaboration), variation in the strength of converging endbulb inputs revealed a timing-rate trade-off in GBC coding. When inputs are uniform and subthreshold (no variation), APs require near-coincident EPSPs within the short integration window, yielding low jitter and strong phase locking. As input heterogeneity increases (high variation), a few stronger terminals can reach threshold with reduced temporal synchrony, elevating overall firing and improving envelope following at the cost of temporal precision. This single organizing principle accounts for responses to both pure-tone and SAM stimulations: heterogeneity promotes rate/envelope

codes, whereas uniformity favors temporal codes. Mechanistically, the observed effects can be attributed to the EPSP dynamics arising from the integration of converging endbulb inputs. Under the collaborator's simulations and my experimental results, the spontaneous rate shows a "jump" at a variation of 0.7-0.8 (Fig. 14B3, Fig. S1A2), which indicates that the largest synaptic input switches from subthreshold to suprathreshold. Consistent with this transition, morphological analysis indicates a dividing line around 180 μm^2 for the size of the largest endbulb: GBCs with all inputs below this size operate in a coincidence-detection mode (all inputs subthreshold), whereas those with one or two endbulbs exceeding this size enter a "mixed-mode", in which strong suprathreshold inputs can drive spiking without strict coincidence (Spirou et al., 2023). In the no-variation regime examined here, each individual EPSP was subthreshold and thus failed to evoke an AP. The combination of somatic endbulb inputs with fast EPSC kinetics and a short membrane time constant supports a short effective integration window, so several near-coincident EPSPs are required to evoke an AP with low jitter (Eatock, 2003; Rothman & Manis, 2003; Wang & Manis, 2006; Antunes et al., 2020). When the input variation is high, suprathreshold EPSPs can trigger spikes without coincident input. This reduces the requirement for precise input synchrony, resulting in shorter first-spike latency and increased firing rates, but also reduced temporal precision.

These observations align with earlier studies showing how convergence and the temporal distribution of synaptic input arrival govern spike-timing precision. When many subthreshold inputs arrive nearly simultaneously within a rapidly rising, short window, jitter is strongly reduced (Xu-Friedman & Regehr, 2005a). With only a few inputs, high precision is still possible if individual inputs are near-threshold, have rapid onsets, and if late inputs are effectively curtailed by refractoriness (Xu-Friedman & Regehr, 2005b). In my framework, the low-variation regime corresponds to the many-input (subthreshold) case that favors coincidence detection and low jitter, whereas the high-variation regime introduces strong inputs that elevate rate coding at the expense of phase locking.

This mechanism was further verified in anatomy-constrained experiments, with an input distribution range corresponding to morphological data (Fig. 16-17). Moreover, a simplified coincidence-counting model reproduced the experimental results, indicating that the morphological variability in endbulb sizes is sufficient to cause this timing-rate trade-off on

GBCs. Taken together, variability in converging excitatory inputs emerges as a key factor shaping the coding strategy of GBCs. Low input variability promotes coincidence detection and precise spike timing, whereas high variability enhances firing rates and envelope tracking at the expense of temporal precision. The distinction between timing- and rate-dominant operation also points to differential roles in downstream circuits. The MSO depends on sub-millisecond spike-time precision to compute ITDs (Yin & Chan, 1990; Brand et al., 2002), which is consistent with the greater temporal fidelity produced under low input variability. By contrast, inhibitory projections to the LSO and SPN remain temporally sensitive but are generally considered to rely more on firing-rate and envelope cues for ILD processing and gap detection (Joris & Yin, 1998; Behrend et al., 2002; Tollin, 2003; Kadner & Berrebi, 2008). Thus, the output observed under high input variability appears well matched to the computational demands of LSO and SPN, highlighting how input heterogeneity contributes to functional specialization in the auditory brainstem.

Similar organizational motifs are evident beyond GBCs. Within the auditory brainstem, the avian nucleus magnocellularis shows frequency-dependent convergence: low characteristic frequency neurons receive many smaller inputs, while higher characteristic frequencies are supported by fewer, larger inputs, thereby tuning integration windows and preserving timing where it matters most (Köppl, 1994; Fukui & Ohmori, 2004). Oline et al. (2016) defined a principle of this tonotopic optimization by showing that neurons at low characteristic frequencies achieve higher temporal precision through the convergence of many relatively weak ANF inputs, whereas with increasing frequency, precision is maintained by fewer, stronger inputs. The present results extend this framework from a population-level tonotopic gradient to a within-cell-type mechanism in GBCs, showing that varying the dispersion of endbulb strengths shifts the operating point along a timing-rate continuum. Outside the auditory system, the primate retina offers a clear convergence-coding relationship. Different input convergence onto bipolar neurons determines the balance between resolution and sensitivity. In the fovea, high-threshold cones provide near one-to-one input to midget bipolar cells and support high spatial acuity and precise temporal sampling (Kolb & Marshak, 2003; Masri et al., 2020). With increasing eccentricity, convergence onto midget ganglion cells rises; in the periphery, low-threshold rods converge in large numbers onto rod bipolar cells (via All amacrine pathway), increasing sensitivity but reducing acuity (Field et al., 2009; Tian

et al., 2010; Grimes et al., 2018). Thus, variation in input number and relative synaptic weight sets a resolution-sensitivity trade-off in the retina. In the thalamus, input composition determines coding. The driver-modulator framework establishes that a few strong driver inputs carry the feedforward signal, whereas weaker modulators regulate gain and context (Sherman & Guillery, 1998). In several nuclei, two driver-like sources can converge on the same relay neurons (e.g., corticothalamic layer-5B together with ascending sensory input in the posterior medial thalamic nucleus; retinal together with tectogeniculate input in the dorsal lateral geniculate nucleus shell; retinal together with cortical layer-5 input in the ventral lateral geniculate nucleus). When the relative weight of these convergent inputs changes, the relay shifts what it transmits downstream—toward more faithful, temporally precise feedforward transmission when the ascending/retinal driver dominates, or toward integrated, context-weighted output when the cortical/tectal driver gains influence (Groh et al., 2014; Bickford et al., 2015; Govindaiah et al., 2023). Together, these examples highlight input-strength heterogeneity as an important feature of neuronal coding across systems.

5.2 Role of Inhibition in Regulating GBC Encoding Strategies

Among the central questions of this study is whether inhibition modifies the timing-rate balance established by input heterogeneity or merely operates within it. In vivo recordings in SBCs indicate that inhibition acts as gain control: it reduces EPSP slopes and prolongs the EPSP-to-spike transition (Nerlich et al., 2014; Keine & Rübsamen, 2015). The elevated effective threshold lowers the likelihood of spikes driven by weaker or less synchronous excitation, thereby improving phase locking without shifting the preferred phase. My experimental results showed that under pure-tone stimulation, adding an inhibitory conductance reduced spike counts, while VS at each variation level was unchanged relative to excitation alone (Fig. 21). A plausible explanation is that GBCs receive more numerous endbulb inputs from ANFs than SBCs (Sento & Ryugo, 1989; Spirou et al., 2005). Therefore, under pure-tone stimulation that supports phase-locking, multiple endbulb inputs onto GBCs are likely to generate well-synchronized, phase-locked EPSPs per cycle (Joris et al., 1994; Spirou et al., 2023). Even if inhibition suppresses weaker or poorly synchronized events, the remaining EPSPs still drive APs at a similar phase of the cycle, so temporal precision (VS)

remains unchanged. Another plausible explanation is frequency dependence. Published VS values for gerbil BCs are already high at these low-mid frequencies (≈ 0.8 - 0.9 at 300-400 Hz; ≈ 0.6 - 0.8 at around 1 kHz (Kuenzel et al., 2011)). In the excitation-only condition, VS were comparable to these values (350 Hz: no variation = 0.89 ± 0.01 , medium variation = 0.88 ± 0.02 , high variation = 0.83 ± 0.01 ; 1 kHz: no variation = 0.69 ± 0.04 , medium variation = 0.69 ± 0.04 , high variation = 0.64 ± 0.04). Consistent with the report that inhibitory effects on phase locking are modest at ~ 350 and ~ 1000 Hz (Kuenzel et al., 2011), adding inhibition reduced spike counts but did not measurably increase VS at 350 or 1000 Hz; at 3.5 kHz (above the effective band).

Previous studies have shown that inhibitory currents in SBCs exhibit rapid onset but notably slow decay kinetics, leading to gradual accumulation of tonic inhibition over time (Xie & Manis, 2013b; Nerlich et al., 2014; Kuenzel et al., 2015). As a result, initial spikes are less affected, while subsequent spikes are significantly suppressed as inhibitory conductance progressively summates (Kuenzel et al., 2015). Adding inhibition in my experiments prolonged first-spike latency and reduced jitter. A plausible explanation arises from a methodological difference: In this study, excitation and inhibition were time-aligned (zero relative delay). In vivo, however, sound-evoked inhibition in BC circuits typically begins after excitation and decays more slowly: juxtacellular recordings in gerbil SBCs show a delayed inhibitory onset relative to excitation (Kuenzel et al., 2011), and mouse VCN work describes frequency-specific inhibition delayed by an additional synapse, consistent with feed-forward routes via DCN tuberculoventral or D-stellate cells (Wickesberg & Oertel, 1990). Given this physiological lag, time-aligning excitation and inhibition (zero delay) provides an upper bound on onset effects of inhibition. Inhibition has already accumulated by the time of the first spike, delaying its occurrence. As stimulation continues, inhibition further summates, progressively suppressing later discharges. Thus, the present paradigm may overestimate onset effects compared to the physiological situation. This effect was observed for no/medium-variation conditions, whereas stronger (suprathreshold) inputs presumably overcome such initial inhibition under high-variability conditions, thereby minimizing changes. Anatomical studies show that BCs receive inhibitory input not only at the soma but also on dendrites, indicating potential complexity in inhibitory control mechanisms (Spirou et al., 2005; Campagnola & Manis, 2014; Spirou et al., 2023). In this study, inhibition was

considered somatic, potentially overlooking dendritic inhibitory effects. In general, dendritic inhibition acts as a local increase in conductance that divisively scales EPSPs arising on those dendrites and attenuates their transfer to the soma, with little direct effect on large, well-timed somatic endbulb transients (Chance et al., 2002; Mitchell & Silver, 2003). Within the context of this study, dendritic inhibition would be expected to slightly tighten spike-time precision in low-variation (coincidence-detection) conditions, and to have only minor effects on spike initiation in high-variation conditions where spikes are already driven by single suprathreshold endbulb inputs.

Studies in SBCs reported that inhibition sharpens spike timing and improves the reliability of temporal coding under complex acoustic conditions by making spike output sparser and more reproducible across trials. In addition, inhibition regulates rate-level functions by reducing overall spike output and compressing the growth of firing rate with increasing sound level, often interpreted as a form of gain control (Keine & Rübsamen, 2015; Keine et al., 2016). The GBC data in this study show similar effects on timing during SAM encoding (VS/CI increased) together with a reduction in rate coding (CorrNorm decreased). This pattern is consistent with inhibition reducing the impact of late or poorly aligned excitatory inputs, not by canceling individual EPSPs, but through its slow, cumulative conductance that effectively narrows the temporal window for integration.

Under SAM stimulation, inhibition further improved temporal precision, shifting the balance without altering the operation mode: low-variation remains timing dominant, and high-variation remains rate dominant. The role of inhibition in enhancing temporal precision has also been demonstrated in other systems. In the somatosensory cortex, coordinated excitation and inhibition stabilize membrane potential fluctuations, thereby constraining spike timing (Higley & Contreras, 2006). In the retina, correlations between excitatory and inhibitory inputs reduce response variance and improve trial-to-trial reproducibility of spike timing (Cafaro & Rieke, 2010). Theoretical analyses likewise support this principle, showing that specific E-I correlation structures can reduce synaptic-drive variance without substantially altering its mean, thereby promoting more stable spike timing (Salinas & Sejnowski, 2000).

5.3 Experimental Limitations and Physiological Relevance

This study did not model short-term depression (STD) because converging evidence indicates that, at the timescales and conditions relevant here, STD would not confound the comparisons. At the ANF-BC synapse, the paired-pulse ratio is similar across SGN subtypes recorded within the same BC (Wong et al., 2025), indicating no subtype-specific difference in short-term plasticity. In vivo, SBCs maintain stable input-output functions and preserved temporal precision during sustained stimulation (Kuenzel et al., 2011), and the data do not indicate a prominent progressive, inter-spike interval-dependent depression of excitatory drive (Keine et al., 2016). In vivo research showed that another large synaptic terminal, the calyx of Held, exhibits mild STD due to its lower release probability, supporting more reliable transmission during ongoing activity (Lorteije et al., 2009; Sonntag et al., 2011). On this basis, this study implemented excitation as static, non-depressing conductances to isolate the effect of input-strength heterogeneity. Because identical ANF spike trains were used across all variation conditions and strength was altered only by static scaling, the average STD would be expected to be similar between recordings.

Recordings were obtained in gerbil slices. ANF spike trains were generated with an auditory-periphery model. I used the model's cat configuration because gerbil ANF responses closely resemble those of cats (Versteegh et al., 2011; Huet et al., 2016; Heeringa et al., 2023), making this a reasonable surrogate for gerbil input. Within each cell, periphery parameters were held constant across all conditions, so between-condition differences in this study are attributed to the imposed synaptic input variability.

The number of converging ANFs onto a single GBC differs across mammals (around 20 in cat; 5-12 in mouse (Liberman, 1991; Spirou et al., 2005; Cao & Oertel, 2010; Spirou et al., 2023)). Because gerbil counts are still unavailable, I used 10 ANFs for the main simulations. For a given cell, the input count was kept fixed across its conditions so that differences reflect the distribution of input strengths rather than the number of inputs. In a separate morphology-constrained condition, the input count was set to 8 to match the available mouse endbulb dataset (Spirou et al., 2023). This adjustment ensured that the simulated input distributions reflected empirical anatomical constraints, while all other settings were kept identical.

Across cells, both the parametric (γ) and the morphology-derived input variations produced the same qualitative trade-off between timing and rate. In other words, changing only the allocation of excitatory weight across converging endbulbs, from uniform to more variable, was sufficient to shift GBC output along the continuum from coincidence-based, sub-millisecond timing toward rate/envelope coding. This shows that the pattern of synaptic strength across inputs, rather than input number or the auditory-periphery setting, is the critical determinant of where a cell operates on the timing-rate axis.

Together with the inhibition results, these findings suggest a compact organizing principle: input heterogeneity sets the operating point of GBCs along the timing-rate continuum, and inhibition sharpens temporal precision around that point without switching modes. This framework links measurable anatomical variability to functional diversity in early auditory pathways and yields concrete, testable predictions for downstream circuits.

Supplementary

Table 3. Summary of optimal alignment metrics (correlation coefficient and standard deviation at 0.04 ms shift) across nine synaptic stimulus conditions (three frequencies with three variation levels each) in two GBC recordings.

cell1	Correlation Coefficient	Standard Deviation
350Hz High	0.9942	0.0789
350Hz Medium	0.9997	0.0452
350Hz No	0.9919	0.0383
1000Hz High	0.9929	0.0692
1000Hz Medium	0.9957	0.0444
1000Hz No	0.9835	0.0842
3500Hz High	0.9951	0.0534
3500Hz Medium	0.9936	0.0501
3500Hz No	0.9937	0.0463
cell2	Correlation Coefficient	Standard Deviation
350Hz High	0.9963	0.0419
350Hz Medium	0.9926	0.0837
350Hz No	0.9959	0.0607
1000Hz High	0.9954	0.05 26
1000Hz Medium	0.9919	0.0665
1000Hz No	0.9946	0.0513
3500Hz High	0.9986	0.0738
3500Hz Medium	0.9945	0.0483
3500Hz No	0.9941	0.0458

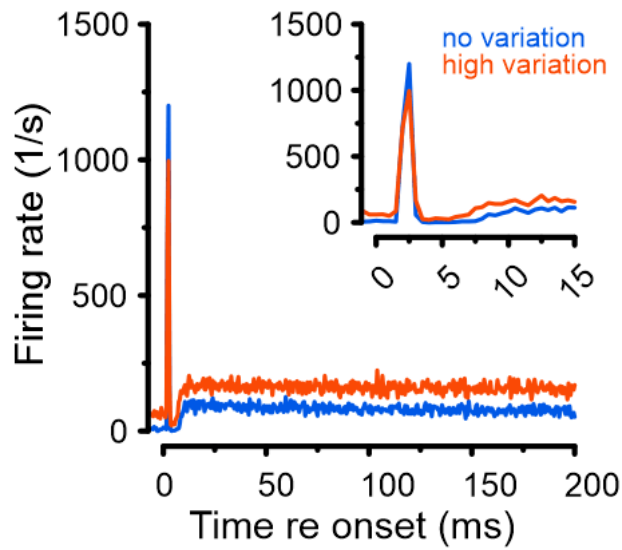
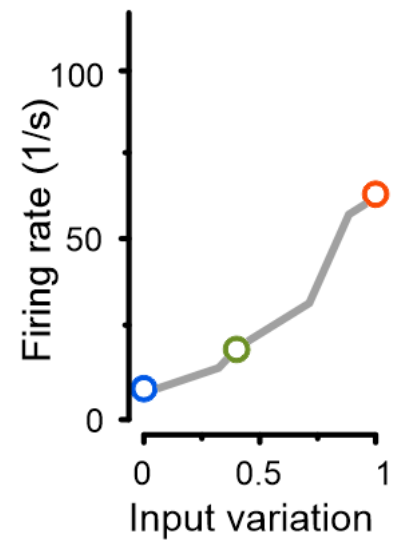
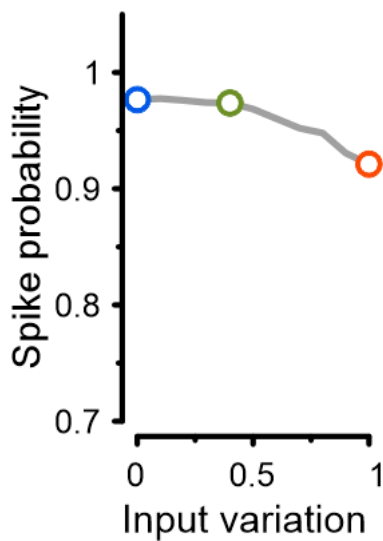
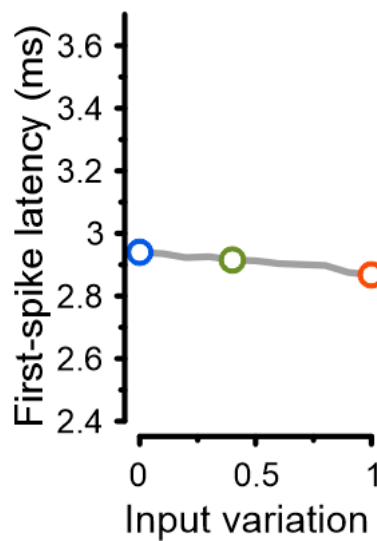
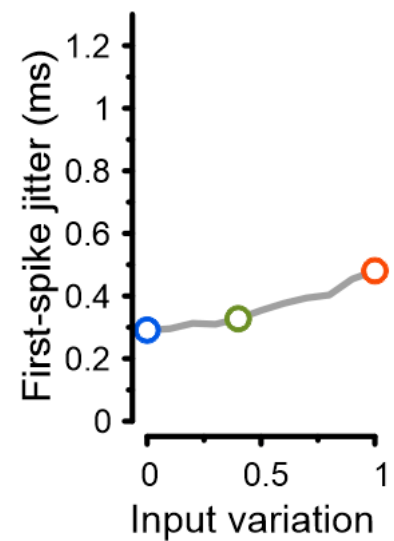
A1**A2** *Spontaneous***A3** *Onset spike***A4** *First-spike latency***A5** *First-spike jitter*

Figure S1. Onset response of GBC model. Output of the GBC model in response to a 3.5 kHz pure-tone under no variation (blue) and high variation (orange) conditions, mirroring the experimental data (A1). Spontaneous firing rate (A2), onset spike probability (A3), first-spike latency (A4), and first-spike jitter (A5) across input variations. Colored dots indicate the input variations used in the *in vitro* experiments. Results of the model match the experimental results. Increased input variation decreased onset spike probability and decreased temporal precision.

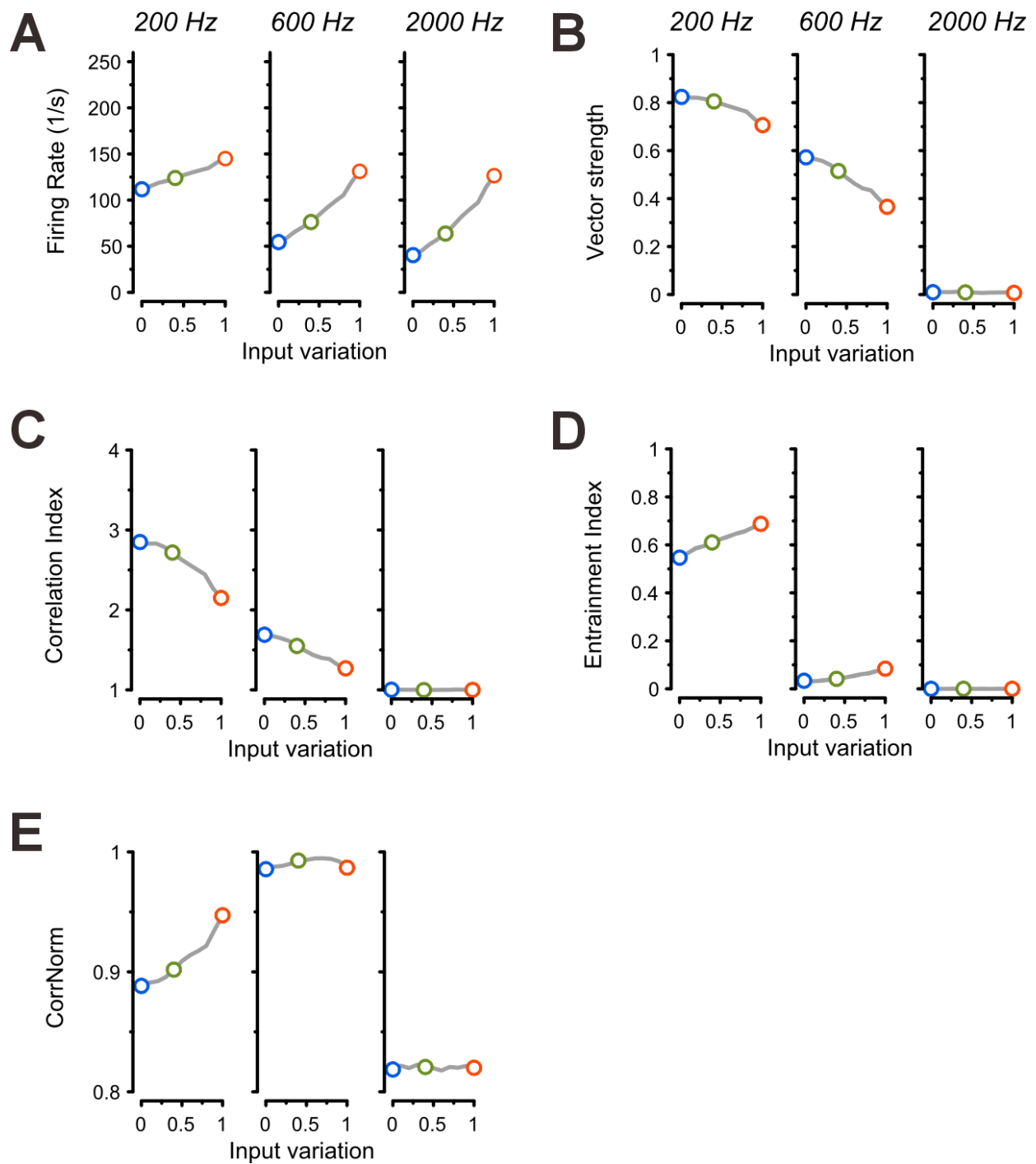


Figure S2. GBC model responses to SAM stimuli. GBC model response to AM sounds with modulation frequencies of 200Hz, 600 Hz, and 2000 Hz across three input variations. (A) The average firing rates generally increased with input variation across all modulation frequencies. (B-C) VS and CI decreased significantly with input variation. (D-E) Both EI and CorrNorm increased with input variation at modulation frequencies 200Hz and 600Hz, whereas they remained unchanged at 2000Hz modulation frequency. Colored dots indicate the input variations used in the *in vitro* experiments. The model results match the experimental results, with higher input variation increasing firing rate and rate coding while decreasing temporal precision.

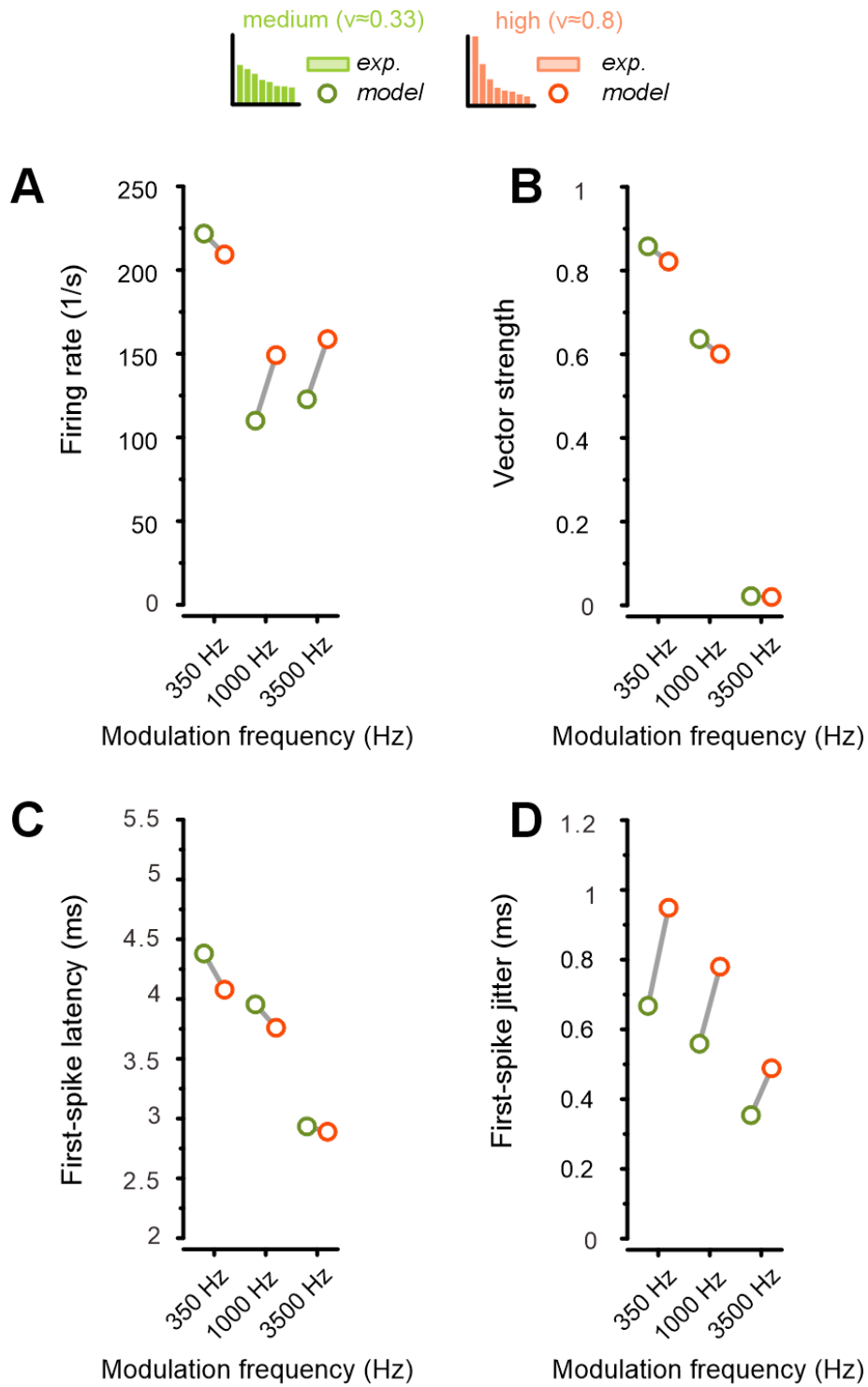


Figure S3. Morphological input variation reproduces the pure-tone timing-rate trade-off in the GBC model.

GBC model response to pure-tone stimulation under two input variation conditions derived from morphological estimates: ‘medium variation’ ($CV \approx 0.33$, green, GBC21) and ‘high variation’ ($CV \approx 0.8$, orange, GBC18L) (Spirou et al., 2023). (A) Input variation increased the firing rate at 1000Hz and 3500Hz. (B) Temporal precision was reduced when GBC received highly variable inputs. (C) First-spike latency is shortened at the expense of an increase in first-spike jitter (D) with high input variations. Colored dots indicate the input variations used in the *in vitro* experiments. Results of the model match the experimental results.

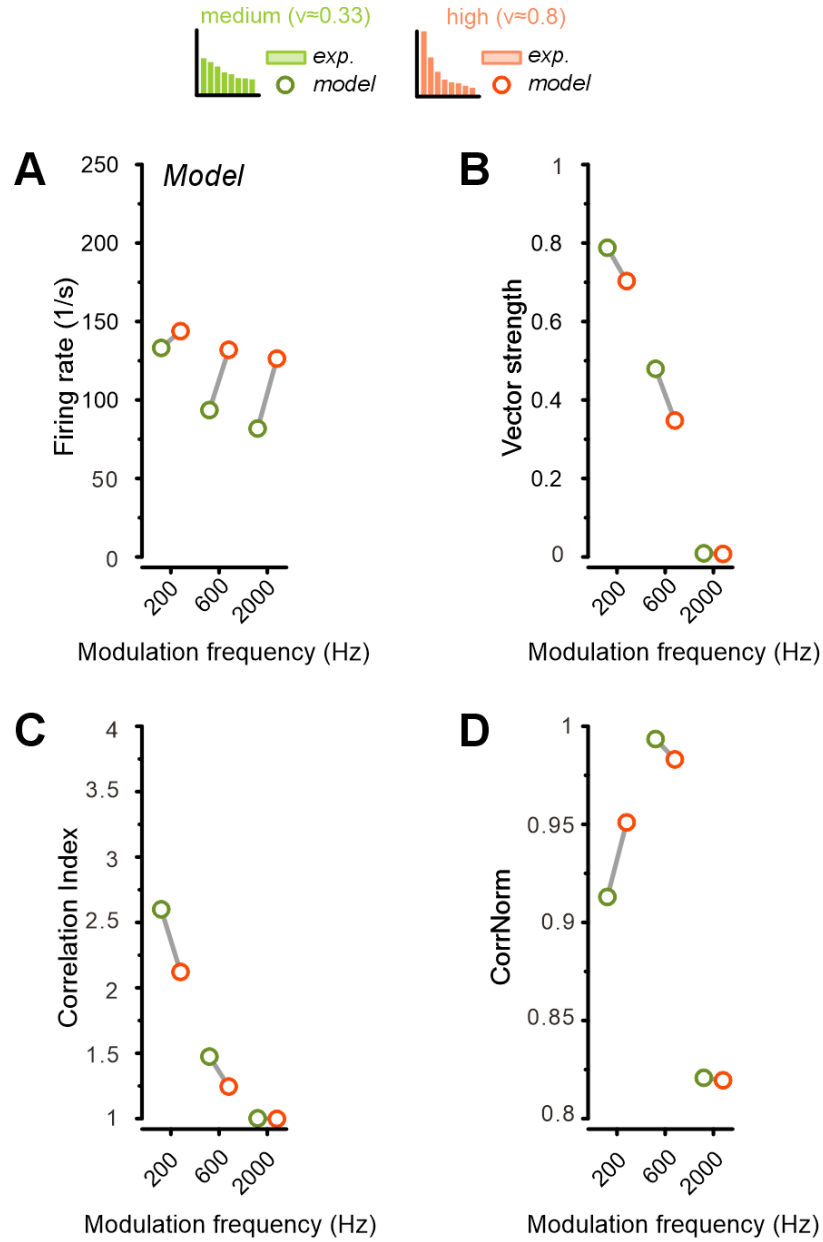


Figure S4. Morphology-derived input variation reproduces the SAM timing-rate trade-off in the GBC model.

GBC model response to SAM stimulation under two input variation conditions derived from morphological estimates: 'medium variation' (CV ≈ 0.33, green, GBC21) and 'high variation' (CV ≈ 0.8, orange, GBC18L) (Spirou et al., 2023). (A) Input variation increased the overall firing rate, (B-C) decreased temporal precision, and (D) improved rate coding. Colored dots indicate the input variations used in the *in vitro* experiments. The model results are consistent with experimental results.

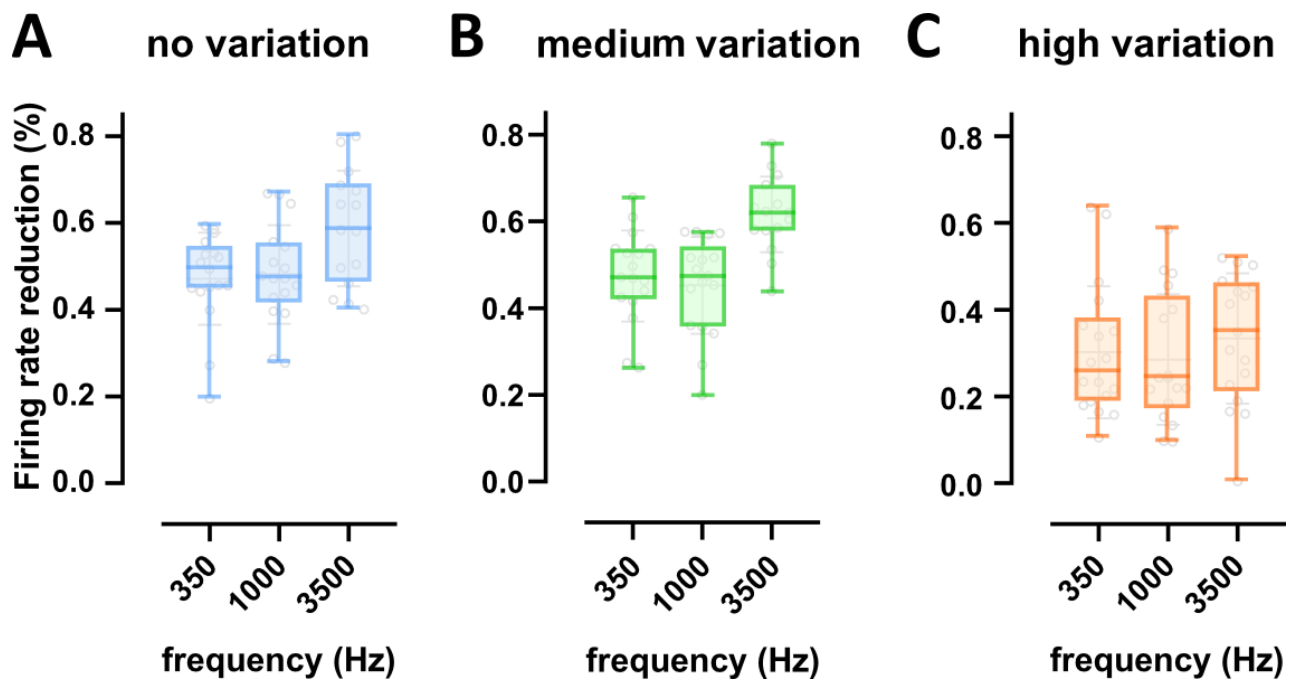


Figure S5. Reduction of APs under pure-tone stimulus with inhibitory conductance. Percent reduction in spike count due to inhibitory conductance. (A-B) In the no- and medium-variation conditions, inhibitory conductance reduced firing rate by at least 50–60% across all three frequencies. (C) In the high-variation conditions, inhibition reduced firing by approximately 30–40%.

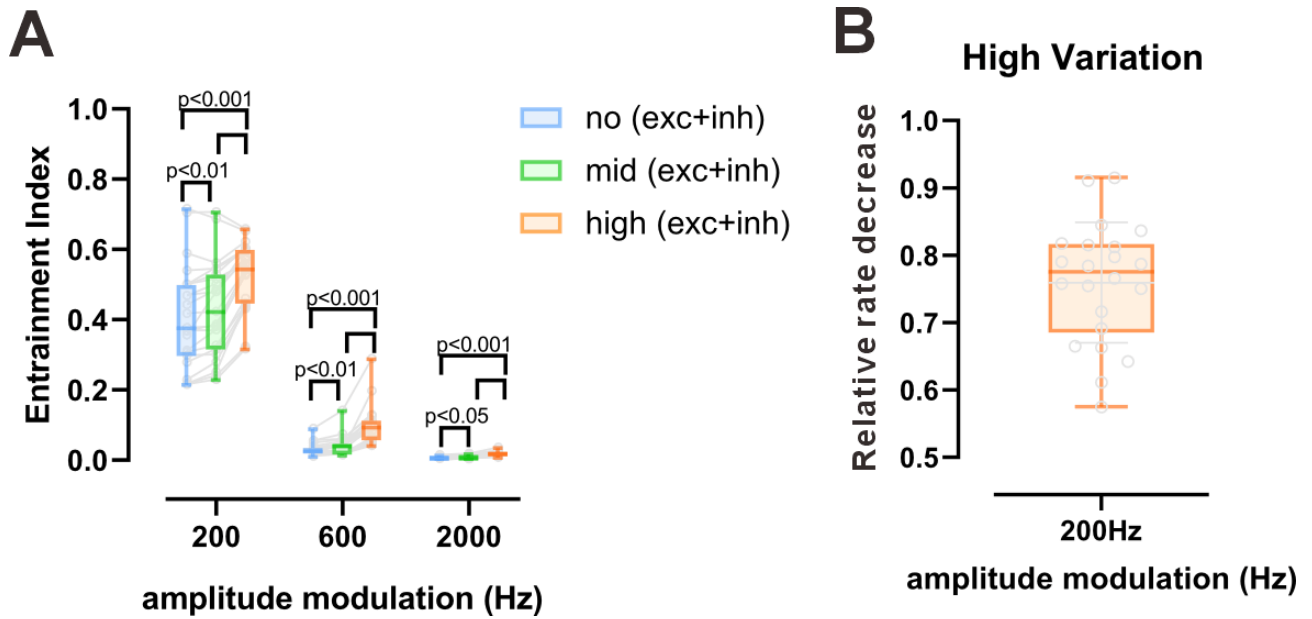


Figure S6. SAM-evoked responses under the excitation and inhibition (exc+inh) condition. (A) Envelope-tracking fidelity measured by EI. Higher input variability increased EI values. (B) Quantification of the inhibitory effect: In the high-variability, excitatory-only condition (Exc only, high variation) at 200 Hz, introducing inhibitory conductance reduced the spike count by approximately 70%. Data are presented as box plots (median, interquartile range [IQR], whiskers $1.5 \times \text{IQR}$); individual cells are represented by circles. Statistical analysis performed via repeated-measures two-way ANOVA with Bonferroni correction.

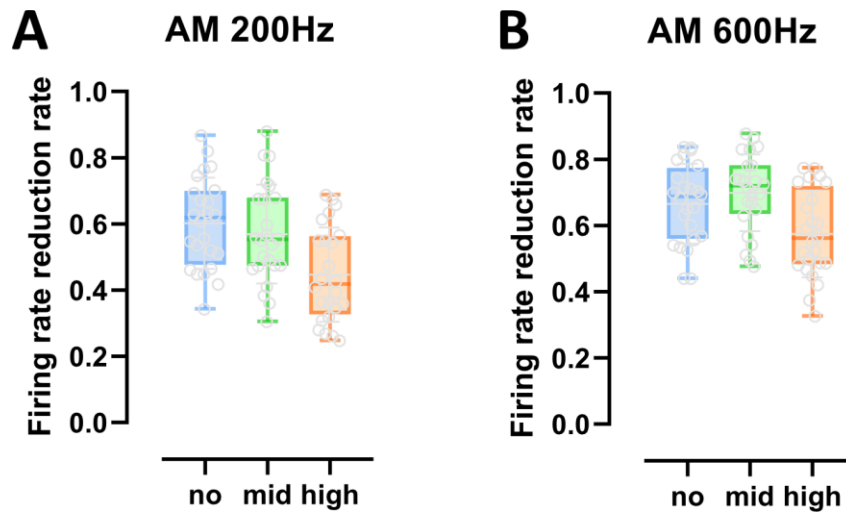


Figure S7. Firing rate reduction induced by inhibition at different AM modulation frequencies. (A) At 200Hz, direct comparison of excitatory-only (exc) and excitatory-inhibitory (exc+inh) conditions showed an approximately 60% reduction in spike count with inhibitory input. (B) At 600Hz, a comparable analysis showed an approximately 70% reduction in spike count with inhibition, supporting the conclusion that inhibitory conductances introduce physiologically relevant and robust suppression of neuronal firing. Box plots show median, interquartile range (IQR), whiskers represent $1.5 \times \text{IQR}$; individual cells represented by circles (n=24).

Publication

Synaptic input variation enhances rate coding at the expense of temporal precision in cochlear nucleus neurons

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Authors' contributions

CW: Investigation; **GA:** Conceptualization, Methodology, Software, Verification, Formal Analysis, Resources, Data Curation, Writing - Review & Editing; **CK:** Conceptualization, Methodology, Software, Verification, Formal Analysis, Resources, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization, Supervision, Funding acquisition; **IM:** Conceptualization, Resources, Writing - Review & Editing, Supervision, Project administration

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Versicherung

Hiermit erkläre ich, dass ich diese Dissertation selbständig verfasst und alle benutzten Hilfsmittel vollständig angegeben habe. Des Weiteren lag und liegt diese Dissertation weder in ihrer Gesamtheit noch in Teilen einer anderen wissenschaftlichen Hochschule zur Begutachtung in einem Promotionsverfahren vor. Innerhalb dieser Dissertation wurden die Leitlinien guter wissenschaftlicher Praxis der Carl von Ossietzky Universität Oldenburg befolgt und ich habe im Zusammenhang mit dem Promotionsvorhaben keine kommerziellen Vermittlungs- oder Beratungsdienste in Anspruch genommen.