Is GABA neurotransmission enhanced in auditory thalamus relative to inferior colliculus?

Rui Cai, Bopanna I. Kalappa, Thomas J. Brozoski, Lynne L. Ling, and Donald M. Caspary

1 Southern Illinois University School of Medicine, Department of Pharmacology, Springfield, Illinois; and 2 Southern Illinois University School of Medicine, Division of Otolaryngology, Springfield, Illinois

Submitted 5 August 2013; accepted in final form 20 October 2013

Cai R, Kalappa BI, Brozoski TJ, Ling LL, Caspary DM. Is GABA neurotransmission enhanced in auditory thalamus relative to inferior colliculus? J Neurophysiol 111: 229–238, 2014. First published October 23, 2013; doi:10.1152/jn.00556.2013.—Gamma-aminobutyric acid (GABA) is considered the major inhibitory neurotransmitter in the central auditory system. Sensory thalamic structures show high levels of non-desensitizing extrasynaptic GABA<sub>A</sub> receptors (GABA<sub>A</sub>Rs) and a reduction in the redundancy of coded information. The present study compared the inhibitory potency of GABA acting at GABA<sub>A</sub>Rs between the inferior colliculus (IC) and the medial geniculate body (MGB) using quantitative in vivo, in vitro, and ex vivo experimental approaches. In vivo single unit studies compared the ability of half maximal inhibitory concentrations of GABA to inhibit sound-evoked temporal responses, and found that GABA was two to three times (P < 0.01) more potent at suppressing MGB single unit responses than IC unit responses. In vitro whole cell patch-clamp slice recordings were used to demonstrate that gaboxadol, a δ-subunit selective GABA<sub>A</sub>R agonist, was significantly more potent at evoking tonic inhibitory currents from MGB neurons than IC neurons (P < 0.01). These electrophysiological findings were supported by an in vitro receptor binding assay which used the picrotoxin analog [3H]TBOB to assess binding in the GABA<sub>A</sub>R chloride channel. MGB GABA<sub>A</sub>Rs had significantly greater total open chloride channel capacity relative to GABA<sub>A</sub>Rs in IC (P < 0.05) as shown by increased total [3H]TBOB binding. Finally, a comparative ex vivo measurement compared endogenous GABA levels and suggested a trend towards higher GABA binding. Furthermore, GABA<sub>A</sub>Rs have a role in controlling gain of stimulus-specific adaptation in IC (Perez-Gonzalez et al. 2012). In general, the role of GABAergic inhibition in IC involves shaping and controlling gain of responses to a variety of simple and complex acoustic stimuli.

Gamma-aminobutyric acid (GABA) is considered the major inhibitory neurotransmitter of the mammalian central nervous system, including sensory systems, where it functions to control gain, improve signal-to-noise, localize environmental cues, and, in general, shape the ascending acoustic message. The inferior colliculus (IC; auditory midbrain) and the medial geniculate body (MGB; auditory thalamus) are key structures of auditory neuraxis. Previous studies demonstrated that acoustic information about stimulus identity is further refined/reduced in single unit recordings from MGB relative to similar IC units recordings (Chechik et al. 2006).

The auditory midbrain (IC) is rich in sources of GABAergic neurotransmission. IC receives ascending GABAergic inputs from the dorsal, intermediate, and ventral nucleus of the lateral lemniscus and the superior paraolivary nucleus of the superior olivary complex (Kulesza et al. 2003; Zhang et al. 1998). In addition, diverse GABAergic neurons form collaterals within IC (Oliver et al. 1994). From a functional perspective, studies in chinchilla cochlear nucleus and IC suggested that glycine and/or GABA could selectively alter near or below best modulation frequency (BMF) responses changing band-pass responses into more low-pass responders. A study by Koch and Grothe (1998) concluded that GABA inhibition sharpened frequency modulation tuning for the majority of neurons in IC of the big brown bat. In addition, GABA<sub>A</sub> receptor (GABA<sub>A</sub>R) blockade alters responses to sinusoidal amplitude modulated stimuli (SAM) in IC of rats and bats by exerting a gain control effect on temporal and rate modulation (Burger and Pollak 1998; Caspary et al. 2002). Dynamic control of discharge rate near best frequency was found as the major role for GABA inhibition in IC of chinchilla, guinea pig, and bats (Le Beau et al. 1996; Palombi and Caspary 1996; Park and Pollak 1993). Furthermore, GABA<sub>A</sub>Rs have a role in controlling gain of stimulus-specific adaptation in IC (Perez-Gonzalez et al. 2012). In general, the role of GABAergic inhibition in IC involves shaping and controlling gain of responses to a variety of simple and complex acoustic stimuli.

Compared to IC, rat MGB has few intrinsic inhibitory interneurons (~1%) (Bartlett and Smith 1999; Winer and Larue 1996). The two major GABAergic inputs to MGB are from IC (Ito and Oliver 2012; Peruzzi et al. 1997; Winer and Larue 1996) and the thalamic reticular nucleus (Rouiller et al. 1985). Recently, Saldaña (2013) described unexpectedly large inhibitory projections from subcollicular sources to nonlemniscal auditory thalamus. Descending auditory corticothalamic projections terminate on MGB neurons in a region-specific manner, with each auditory cortical region projecting to specific MGB subnuclei (Andersen et al. 1980; Bajo et al. 1995; Diamond et al. 1969; Kelly and Wong 1981; Lee et al. 2004; Lee and Winer 2005; Pandya et al. 1994; Pontes et al. 1975; Rouiller and de Ribaupierre 1985; Sousa-Pinto and Reis 1975; Winer et al. 2001; Wong and Kelly 1981). A recent series of detailed studies by Bartlett and Wang (2007, 2011) suggest that MGB neurons display unique, complex responses to modulated and click train stimuli compared with neurons in IC. Based on the disparate nature of inputs and known differences in the nature of GABA<sub>A</sub>Rs between IC and MGB, it is reasonable to expect distinct differences in functional GABAergic neurotransmission between these two auditory structures. Few studies have directly compared the potency of GABAergic inhibition between these two structures in response to acoustic stimuli in vivo. Based on differences between
these structures in the expression and distribution of GABA_ARs subtypes, a direct IC and MGB comparison of GABA efficacy could provide insights into coding characteristics of these structures.

GABA_A Rs are heteromeric pentamers made up of 19 possible subunits (α1–6, β1–3, γ1–3, δ, ε, θ, π, and ρ1–3). A limited number of GABA_A R constructs are prevalent and are regionally distributed in different proportions throughout different brain structures where they display differences in ligand binding affinity, receptor kinetics, Cl⁻ conductance, and subcellular localization (Belelli et al. 2009; Brickley and Mody 2012; Bright and Brickley 2008; Semyanov et al. 2004; Walker and Semyanov 2008). Wild-type (2α1, 2β2, and γ2) GABA_A Rs make up as much as 70% of the distribution of GABA_A R constructs and represents the overwhelming majority of GABA_A R constructs in IC (McKernan and Whiting 1996; Pirker et al. 2000). Immunocytochemical and quantitative receptor binding studies showed that α4- and δ-subunits coexist and are prevalent in significant numbers in sensory thalamic nuclei, including MGB (Belelli et al. 2009; Cope et al. 2005; Richardson et al. 2011). They have not been functionally described in IC. Thalamocortical neurons show unique tonic inhibitory properties mediated by extrasynaptic, α4- and δ-subunit containing, non-desensitizing GABA_A R constructs (Cope et al. 2005; Richardson et al. 2011). The survey study by Pirker et al., of GABA_A R subunit protein expression, describes high levels of α4- and δ-subunits in MGB, and also found low levels of the GABA_A R δ-subunit in IC (Pirker et al. 2000). The nature of the GABA_A R subunit construct determines affinity and efficacy for a given ligand. GABA_A Rs with a γ2-subunit mediating rapidly desensitizing inhibitory postsynaptic currents is characterized by a relatively low affinity (EC₅₀ ~ 6–14 μM) for the endogenous agonist GABA (Farrant and Nusser 2005). In contrast, δ-subunit containing GABA_A Rs (6GABA_A R) show slow desensitization kinetics and high affinity (EC₅₀ ~ 0.3–0.7 μM) for ambient GABA and a higher affinity (EC₅₀ ~ 30–50 nM) for the selective δGABA_A R agonist gaboxadol (GBX) (Farrant and Nusser 2005; Meera et al. 2011).

Based on its critical role in sensory gating and the presence of high-affinity extrasynaptic GABA_A Rs, one might predict an enhanced sensitivity to GABA inhibitory neurotransmission in the sensory thalamus relative to other auditory structures. To test this hypothesis, four distinct sets of experiments were designed as follows: 1) In vivo iontophoretic unit recordings comparing GABA potency in IC and MGB; 2 and 3) In vitro whole cell patch-clamp and receptor binding-assessing agonist evoked Cl⁻ currents and channel activations in IC and MGB slices; and 4) Ex vivo proton magnetic resonance spectra (¹H-MRS) comparing endogenous GABA levels.

**MATERIALS AND METHODS**

All experiments were completed using Fischer Brown Norway (FBN) or Long-Evans (LE) male rats maintained on an ad libitum diet and reversed light-dark cycle. Procedures were done in accordance with protocols (No. 41-10-002 and 41-09-024) approved by the Laboratory Animal Care and Use Committee of Southern Illinois University School of Medicine.

The FBN and LE rats were 4–10 mo of age and considered adult rats, based on average life span (Schroeder et al. 1965; Turner and Caspary 2005).

Iontophoresis. Thirty-seven adult male FBN rats (4–6 mo) were initially anesthetized with injection (1.4 ml/kg im) of a 3:1 mixture of ketamine-HCl (100 mg/ml) and xylazine (20 mg/ml). Anesthesia was maintained with intraperitoneal injections of 100% urethane initially 1.3 ml/kg, and then maintained at one-third of the initial amount ( booster doses). Urethane was chosen as the anesthetic agent because its actions are on multiple neurotransmitter systems rather than simply potentiating the effects of inhibitory systems, thus it has less net effect on GABAergic neurotransmission than barbiturates or other anesthetic agents (Hara and Harris 2002). Rats were placed in a modified stereotaxic frame in an IAC sound-attenuating booth. For MGB, a 2 × 2-mm craniotomy was drilled, exposing the dorsal surface of the cortex (−5.5 mm, bregma; 3.5-mm lateral from midline). The IC was approached dorsally by exposing the calvarium just rostral to the lambda and lateral to the midline (2 mm) at a 15–20° angle (Caspary et al. 2002). A carbon fiber electrode attached to a five-barrel iontophoretic electrode, Carbostar-6 (Kation Scientific, Minneapolis, MN), was coupled to the headstage, then to a preamplifier, and controlled by a PC-based Multichannel Acquisition Processor (MAP) system running MAP software (Plexon, Dallas, TX). Spikes were visualized using Sort Client (Plexon) for real-time spike sorting. A piezoelectric driver (DAVID-Kopf Instruments, Tujunga, CA) advanced the electrode to the dorsal aspect of MGB or IC using a broadband noise (BNN) search signal. Single units (3:1 SNR) were discriminated based on waveform morphology and/or principal component analysis. In a few cases, small clusters were studied. Stimulus presentation, real-time data display, and analysis used Auditory Neurophysiology Experiment Control Software (ANECS; Ken Hancock, Blue Hills Scientific, Boston, MA) coupled to TDT System III hardware. Acoustic signals were amplified (ED1), transduced (EC1), and juxtaposed to the right ear canal using polypropylene tubing. The sound system was calibrated offline using a quarter-inch Bruel & Kjaer model 4938 microphone (Naerum, Denmark) into a simulated rat ear (2–46 kHz ± 2 dB) (Palombi and Caspary 1996). SAM carrier frequency was set at the unit’s characteristic frequency (CF) or BBN; rate modulation transfer functions (rMTFs) were determined for each unit at 30 dB above CF threshold in response to 2-s SAM stimuli (4-ms raise-fall time, 100% depth) with modulated frequency (fm) stepped between 2 Hz and 512/1,024 Hz. Stimuli were 450 ms in duration (presented randomly across the trial among different fms) with spikes collected over a 500-ms period following stimulus onset (10 stimuli/envolve frequency). Multi-barrel iontophoretic electrodes were coupled to a constant current system (BH-2 Neuro Phore System). The balancing barrel was filled with KAc (2 M); other barrels were filled with GABA (500 mM, pH 4.0; Sigma-Aldrich, St. Louis, MO) and GBX (10 mM, Sigma-Aldrich, St. Louis, MO). Retaining currents were set at −15 nA with ejection currents between 0 and 100 nA. A reversible change greater than 15% of control was considered a positive drug effect. Neurons reported here showed full baseline recovery following cessation of drug application. Repeated runs were frequently used to confirm small effects.

Rats were cardiac perfused with phosphate-buffered saline (0.1 M, pH 7.4) followed by paraformaldehyde (4%). Brains were removed, placed in paraformaldehyde (1–2 h), transferred to sucore (20%) overnight, sectioned at 50 μm, and stained with fast thionin for localization of recording sites (Palombi and Caspary 1996).

Patch clamp. Patch-clamp experiments were conducted using procedures and equipment previously described (Kalappa et al. 2010; Richardson et al. 2011). Briefly, adult male FBN rats (4–6 mo) were anesthetized with 2.5–3.0% isoflurane gas and decapitated. Their brains were rapidly removed and transferred to ice-cold sucrose-rich solution (in mM): 250 sucrose, 3 KCl, 1.23 NaH2PO4, 5 MgCl2, 0.5 CaCl2, 26 NaHCO3, 10 glucose (pH 7.4). Horizontal sections, 200–300 μm, containing either the central nucleus of IC or the ventral division of MGB were prepared using a Vibratome 1000 Plus (Leica Microsystems, Wetzlar, Germany). Postsectioning, slices were transferred to a storage chamber, perfused (30 min) at 30°C with artificial
cerebrospinal fluid (ACSF) (in mM): 125 NaCl, 3 KCl, 1.26 NaH2PO4, 2 CaCl2, 1 MgCl2, 26 NaHCO3, 10 glucose, and maintained at room temperature for up to ~8 h. For patch-clamp recordings, slices were transferred to the recording chamber and perfused with ACSF at a rate of 1 ml/min. A MultiClamp 700B amplifier, with a Digidata 1440A A/D converter (Molecular Devices, Sunnyvale, CA) was used to sample data (10 kHz) with a 2.2-kHz low-pass filter. Patch pipettes were pulled using Sutter P-97 horizontal puller (Sutter Instruments, Novato, CA) with a resistance of ~4–6 MΩ when filled with the internal solution. Following the formation of a stable gigahm seal (>2 GΩ), whole cell configuration was established. To measure the membrane capacitance (Cm), membrane resistance (Rm), and access resistance (Ra), a 10-mV depolarizing step voltage command was applied using the membrane test function integrated in pClamp10 software (Molecular Devices). Voltage-clamp recordings were made at room temperature with a ~10-mV holding potential to enhance our ability to detect Cl−-mediated outward currents. To reduce noise induced by K+–mediated currents, a Cs-methanesulfonate-based internal solution was used (in mM): 140 CsMeSO3, 6 NaCl, 2 MgCl2, 2 Mg-ATP, 0.3 Na-GTP, 10 HEPES, 0.3 CsOH (pH 7.4). GBX and gabazine (GBZ) (Sigma-Aldrich, St. Louis, MO) were bath applied. Membrane voltages were not corrected for the liquid junction potentials: V0CsMeSO3 = 9.89 mV (Kalappa et al. 2010). Access and series resistance was not compensated for in these voltage-clamp experiments. Analysis of data was conducted offline using Clampfit 10.2.

[3H]TBOB binding. [3H]-butylbicycloorthobenzoate ([3H]TBOB) binding used increasing concentrations of GABA (0 nM to 5 μM) to modulate picrotoxin sites within the GABAAR chloride channel in MGB and IC. Concentrations based on Milbrandt et al. (1996) were centered near the Kd, which optimized the potential to quantify binding differences in these two structures. In brief, adult male FBN rats (4–6 mo) were decapitated; brains were rapidly removed and frozen. Brain slices (16-μm thick) were cut using a Leica CM1850 cryostat (Leica Microsystems, Buffalo Grove, IL) at −18°C and stored at −20°C. Sections were prewashed in a buffer containing 50 mM Tris-HCl and 1 mM EDTA (pH 7.4) and placed in the incubation buffer (50 mM Tris-HCl, and 120 mM NaCl, pH 7.4) with [3H]TBOB and GABA (concentration from 0 nM to 5 μM) for 90 min at room temperature. Cold picrotoxin (20 μM) was added as a displacer. Autoradiograms were generated by apposing the slides to a phosphor screen, subsequently scanned using a Cyclone phosphor screen (Packard BioScience, PerkinElmer, MA). Images were collected at 600 DPI, and the area of MGB and IC was identified and outlined. Binding intensity was analyzed using OptiQuant image analysis software, which provides tools for grayscale quantification in digital light units (DLU). DLU were converted into fmol/mg protein using a standard curve generated from coexposed 14C-embedded plastic standards (ARC, St. Louis, MO). For detailed methods see Milbrandt et al. 1996.

Spectroscopy. Seven adult (mean age 10 mo) male LE rats (Harlan, Indianapolis, IN) were used in ex vivo spectroscopy studies. Volume-limited 1H-MRS were obtained using a vertical bore Varian Unity/Inova 600 MHz NMR spectrometer with a 14.1 T magnet. Calibration spectra were determined for GABA standards (10 mM) dissolved in sterile normal saline. Volume of interest (VOI) GABA levels were determined using the integrated area of the spectral peak in closest approximation to 2.2 ppm (i.e., the calibration standard). Animals were treated 10 min prior to euthanasia with 10 mg/kg ip 3-mercaptopropionic acid (product M5801; Sigma-Aldrich, St. Louis, MO) to arrest postmortem GABA inflation (van der Heyden and Korf 1978). Immediately before acquisition, animals were given a lethal dose of anesthesia (Euthanol; Virbac, Ft. Worth, TX), decapitated, the mandible removed, and excess muscle tissue dissected away from the skull. The head was placed in a polyethylene holder along with a 1-mm-diameter glass capillary filled with CuSO4 (3 mM). The CuSO4 image phantom unambiguously indexed laterality. For each animal, an initial MRI brain scan was used to locate the VOI for 1H-MRS. Contiguous transverse (i.e., coronal) slices, 0.5 mm thick (26-μm planar resolution), were obtained, extending 13 mm caudally from bregma (26 slices total). VOI for 1H-MRS were determined in ventral MGB (vMGB), dorsal MGB (dMGB), and IC. Spectra, as TIFF images, were imported into Image J (ver. 1.44p, http://imagej.nih.gov/ij). Peaks in closest approximation to the calibration peak for GABA were outlined, and the area under each curve (AUC) was determined. AUCs were expressed in spectrum baseline units (i.e., curve lower bound) to correct for image gain. For detailed methods see Brozoski et al. 2012.

RESULTS

GABAAR endogenous and selective agonists alter SAM responses in MGB. SAM stimuli were used to elicit auditory responses in extracellular recordings from MGB units in anesthetized rats. Figure 1 displays examples of the iontophoretic paradigm used to assess potency of GABAARs. A representative unit showing a bandpass response pattern (BP) (30% of MGB response types) displayed a 46% [(653–353)/(653 × 100%)] reduction of SAM-evoked activity with low-dose GABA application (Fig. 1A). A significant decrease in spike rate was evident during drug application near BMF in both the dot raster and the rMTF (Fig. 1, Ab and B). Application of GABA onto 23 MGB BP units (average GABA dose 8.17 ± 1.57 nA) resulted in a 46.24 ± 2.97% decrease of SAM responses on rMTFs at 30 dB above threshold (Fig. 1C). GABA dose was calculated as the smallest dose that produced a greater than 15% change in response. Another MGB SAM response type termed “Mixed” type (Fig. 2C, 35% of the MGB response types) showed a similar reduction in discharge rate of 48.15 ± 3.71% for 31 Mixed units at an average GABA dose of 10.97 ± 2.02 nA (data not shown here). Together, MGB neurons showed exquisite sensitivity to iontophoretic application of GABA.

The selective δGABAAR agonist, GBX, was applied to MGB neurons to test whether more selective activation of extrasynaptic GABAARs would differ from responses to GABA, a less selective endogenous agonist. Responses to both GABA and GBX applications, with recovery to control discharge rates, were obtained from 15 MGB units responding to SAM stimuli. The suppression of SAM responses by GBX was similar but somewhat smaller and less selective than anticipated. The majority of units (11/15) showed greater reduction in total spikes with application of GABA than with application of GBX (Fig. 1, D–F). Average total spikes were 32.59 ± 4.86% under GABA application and 67.15 ± 4.62% under GBX application (Fig. 1F). However, four units showed similar levels of inhibition to the two agonists (Fig. 1, G–I). Due to limitations of the iontophoretic technique, relative sensitivity of GABAARs to different GABA and GBX dose levels is considered difficult to compare (Krogsgaard-Larsen et al. 2004; Mortensen et al. 2004). Based on the present data, both GABA and GBX could inhibit MGB auditory response to a certain extent, but GABA appeared more efficacious than GBX.

GABAAR sensitivity: IC and MGB units, an in vivo comparison. Experience from prior IC iontophoretic studies (Caspy et al. 2002; Palombi and Caspary 1996), initial MGB iontophoretic studies, and the studies described above suggested that MGB neurons are more sensitive to GABA application than IC neurons.
To quantify these suspected GABAAR sensitivity differences, we examined the impact of increasing GABA dose (dose response) using the same electrodes and GABA concentrations to minimize possible experimental design or methods differences in a comparative IC vs. MGB single unit study. Figure 2 compares responses from representative IC and MGB units to GABA application in response to SAM stimuli.

Increasing GABA doses were applied to each unit resulting in increased suppression/inhibition of firing for both units (Fig. 2, A and C). Note that the MGB unit in Fig. 2C responds at very low doses (from leaking, “0” nA) of GABA with increasing suppression of driven activity. A clear decrease in responses at both lower and higher fm could be seen at the 4-nA dose (Fig. 2C). Only onset responses remained relatively unaffected at higher GABA doses. GABA also inhibited SAM responses of IC units but required higher doses of GABA (Fig. 2A). The patterns of suppression of rMTFs in IC units were similarly altered by GABA application with the greatest suppression observed at or near BMF for both structures (Fig. 2, B and D). IC and MGB dose sensitivity to GABA application was quantified by plotting dose against total spikes for each run (normalized) (Fig. 2E). Regression lines showed a dose-dependent response to GABA application. The 50% inhibition dosage (ID$_{50}$) was calculated based on the normalized spike rate. Higher iontophoretic GABA doses were needed to achieve a 50% reduction of discharge rate for IC units (Fig. 2E). The mean ID$_{50}$ was significantly lower (14.01 ± 3.00 nA) for MGB units compared with IC units (49.56 ± 6.75 nA) (**P < 0.01, independent t-test) (Fig. 2F). We also compared ID$_{50}$ for GABA sensitivity between the two major MGB divisions for nine well-localized dMGB and fifteen vMGB units (Fig. 2G). No significant ID$_{50}$ differences in GABAAR sensitivity were found (P > 0.05, independent t-test).
MGB, whole cell voltage-clamp recordings from IC (n = 5; Cm, 75.5 ± 3.19 pF; Rm, 19.85 ± 1.27 MΩ) and MGB (n = 5; Cm, 99.75 ± 8.83 pF; Rm, 349.25 ± 36.06 MΩ) neurons from adult slices were conducted with CsMeSO4 internal solution (see MATERIALS AND METHODS). Once a stable whole cell configuration was established, neurons were held at −10 mV to maximize visualization of tonic Cl− currents upon activation of extrasynaptic -GABAARs. Bath application of increasing doses (0.1–10 μM) of GBX, a -GABAARs subunit-selective agonist, evoked tonic currents from both IC and MGB neurons in a dose-dependent manner. The amplitude of GBX-evoked tonic currents were revealed by addition of 10 μM GBZ, a GABAAR antagonist (Fig. 3B). Comparison of dose response curves of GBX-evoked tonic currents (i.e., response net amplitude per unit of membrane capacitance) from IC (EC50IC = 4.91 μM) and MGB (EC50MGB = 1.18 μM) neurons demonstrated that GBX was still significantly more potent in activating tonic currents in MGB neurons than in IC neurons (normalized curves not shown). A second limitation of this approach is that the voltage-clamp experiments were conducted under the assumption that space clamp in both MGB and IC neurons were similar and adequate. Although the extent of space clamp may differ between these neurons, it would be safe to assume that IC neurons exhibit better space clamp due to lower membrane capacitance than MGB neurons. Hence, if any discrepancies in peak amplitude estimation were to occur due to inadequate space clamp, the tonic currents of MGB neurons may be somewhat underestimated compared with IC neurons which are likely to exhibit better space clamp. Overall, these observations suggested that the differences in adequacy of space clamp and membrane capacitance in IC and MGB neurons may have subtle influence on the absolute values.

One limitation of this approach is the difference in passive membrane properties of IC and MGB neurons. Although IC and MGB neurons exhibited comparable membrane resistance, IC neurons demonstrated lower membrane capacitance than MGB neurons. However, comparison of EC50 values from normalized dose response curves of GBX-evoked tonic currents (i.e., response net amplitude per unit of membrane capacitance) from IC (EC50IC = 4.91 μM) and MGB (EC50MGB = 1.18 μM) neurons demonstrated that GBX was still significantly more potent [F (1, 64) = 76.68, P < 0.0001; F-test] in activating tonic currents in MGB neurons than in IC neurons (normalized curves not shown).
but may not significantly affect the overall conclusion of this voltage-clamp study.

Modulation of binding at the GABA<sub>AR</sub> picrotoxin site: IC and MGB, in vitro comparison. An estimate of total GABA-evoked chloride flux was assessed using a ligand that binds in the open GABA<sub>AR</sub> chloride channel at the picrotoxin binding site. The ability of [3H]TBOB, a picrotoxin competitive analog (Lawrence et al. 1985), to bind at, and be modulated by increasing concentrations of GABA (0 nM-5 μM), was used to compare the maximum number of available GABA<sub>AR</sub> in both IC and MGB neurons. Amplitudes of GBX-evoked tonic currents were revealed by addition of 10 μM gabazine (GBZ). Figure 3C compared the [3H]TBOB modulation with increasing concentrations of GABA (0 nM to 5 μM) was performed on IC and MGB slices. 0 nM GABA was set as the control condition and represented the resting/control openings of GABA<sub>AR</sub>Cl<sup>-</sup> channels. At low concentrations, both structures showed increased binding indicative of increased GABA<sub>AR</sub>, Cl<sup>-</sup> channels openings. Peak percent increase in binding occurred at 100 nM for MGB (*P < 0.05, 2-way ANOVA) and 50 nM for IC, with a significantly larger area (shadow) under the MGB curve (black) suggesting a greater MGB neuronal total chloride flux capacity relative to IC neurons. Both MGB and IC showed desensitization (1,000 nM and 5,000 nM) reflecting a greater percentage of closed GABA<sub>AR</sub>Cl<sup>-</sup> channels than in the control condition.

**Fig. 3. In vitro: GBX induced tonic inhibition and [3H]-butylbicycloorthobenzoate ([3H]TBOB) binding in brain slices. Whole cell patch-clamp recordings from 5 IC and 5 MGB neurons from adult slices were used to compare the relative sensitivity of extrasynaptic GABA<sub>AR</sub> in IC and MGB neurons. A: tonic currents were plotted against increasing dosages of GBX. GBX was significantly more potent in activating tonic currents in MGB units than in IC units [EC<textsubscript>50MGB</textsubscript> = 2.25 μM; EC<textsubscript>50IC</textsubscript> = 4.45 μM; F (1, 54) = 30.17, P < 0.0001; F-test]. B: bath application of increasing doses (0.1–10 μM) of the δ-GABA<sub>AR</sub> subunit-selective agonist, GBX, evoked tonic currents in both IC and MGB neurons. Amplitudes of GBX-evoked tonic currents were revealed by addition of 10 μM gabazine (GBZ). C: modulation of [3H]TBOB channel (picrotoxin) binding with increasing concentrations of GABA (0 nM to 5 μM) was performed on IC and MGB slices. 0 nM GABA was set as the control condition and represented the resting/control openings of GABA<sub>AR</sub>Cl<sup>-</sup> channels. At low concentrations, both structures showed increased binding indicative of increased GABA<sub>AR</sub>, Cl<sup>-</sup> channels openings. Peak percent increase in binding occurred at 100 nM for MGB (*P < 0.05, 2-way ANOVA) and 50 nM for IC, with a significantly larger area (shadow) under the MGB curve (black) suggesting a greater MGB neuronal total chloride flux capacity relative to IC neurons. Both MGB and IC showed desensitization (1,000 nM and 5,000 nM) reflecting a greater percentage of closed GABA<sub>AR</sub>Cl<sup>-</sup> channels than in the control condition.**
(52x241) results suggest elevated GABA level in the auditory thalamus and MGB; wild-type GABAARs (2 synaptic and extrasynaptic GABAARs by GABA and GBX is in MGB may be higher than in IC and that activation of tonically evoked adult MGB neurons compared with similarly examined IC neurons; 3) significantly increased $[^3H]TBOB$ binding following GABA application, in MGB compared with IC; and 4) a clear trend towards increased GABA tissue concentrations in MGB compared with IC using $[^1H]$MRS imaging. Collectively, data from these four studies suggest that GABA levels in MGB may be higher than in IC and that activation of synaptic and extrasynaptic GABAARs by GABA and GBX is enhanced in MGB compared with IC.

GABAAR composition and efficacy in IC and MGB. In IC and MGB, wild-type GABAARs ($2\alpha_1$, $2\beta_2$, and $2\gamma_2$) are thought to mediate fast GABAergic inhibition and are activated by release of the inhibitory neurotransmitter GABA (Mckernan and Whiting 1996; Milbrandt et al. 1997; Pirker et al. 2000). In addition, other heteromic GABAAR constructs are highly expressed in sensory thalamic structures. $\alpha_2$- and $\delta$-subunit containing GABAAR show high affinity for GABA and the subunit selective agonist GBX (Brown et al. 2002; Mortensen et al. 2010). They are localized to extrasynaptic postsynaptic sites and show tonic non-desensitizing, hyperpolarizing chloride currents (Cope et al. 2005; Richardson et al. 2011). The discussion below suggests that our findings of increased GABA sensitivity in MGB neurons compared with IC neurons may reflect the existence of higher levels of extrasynaptic GABAAR in MGB. By contrast, these GABAAR constructs are not prominent in IC, since $\alpha_4$ and $\delta$ subunit protein levels were low, and $\alpha_4$ and $\delta$ message levels were below the level of detection (Pirker et al. 2000; Wisden et al. 1992). We probed this using the methodology of Richardson et al. (2011) adapted from Cope et al. (2005) and found, for the first time, that tonic currents could be evoked by GBX application in IC neurons, in a manner similar to what has been seen in thalamus. However, as predicted, the ability of the subunit-selective agonist GBX to evoke tonic currents was reduced by 50% in IC neurons compared with MGB neurons.

A recent series of studies on marmoset suggest that MGB neuronal responses to modulated and click stimuli display more complex responses than do neurons in IC (Barlett and Wang 2007, 2011). In addition, sensory thalamic neurons are able to switch their discharge pattern from the so-called “tonic” mode to “burst” mode, depending on thalamocortical rhythmicity regulating sleep and attention and depending on the strength and nature of the stimulus (Steriade et al. 1993). These transformations are likely critical to the way sensory pathways process information (see reviews: Sherman 2001; Sherman and Guillery 1996). We hypothesized that these thalamic attributes were due to unique GABA circuits and receptors within MGB. While examining GABA’s role in coding modulated signals in a separate study, it became evident that response suppression by GABA application onto MGB units responding to SAM stimuli was far more potent than observed previously in IC (Burger and Pollak 1998; Caspary et al. 2002; Koch and Grothe 1998). We initiated a parallel dose response comparison using the exact same methodology between structures (electrodes, anesthesia, and animal) to compare GABA efficacy. GABA was two to three times ($P < 0.01$) more potent at suppressing SAM-evoked MGB unit responses than SAM-evoked IC unit responses, further supporting the enhanced GABA sensitivity of sensory thalamic neurons.

GABA concentration in IC and MGB. GABA is the major inhibitory neurotransmitter in the central auditory system. The extrasynaptic GABAARs in sensory thalamus suggest that their presence and regulation of this GABAAR construct might reflect ambient GABA concentrations. In the present study, a trend toward higher GABA levels in MGB relative to IC was detected using ex vivo $[^1H]$MRS. These findings are consistent with our previously published findings showing significantly higher MGB GABA levels relative to IC, using an earlier MRS data set (Brozoski et al. 2012). $[^1H]$MRS ex vivo GABA levels include all GABA compartments, including astrocytic (Papp et al. 2004; Sperlagh et al. 2002) and other non-vesicular stores.
(Demarque et al. 2002). Reports of GABA levels/concentrations by others used different methods to quantify GABA in IC and MGB with varying results. A recent HPLC study in hamster found relatively lower GABA levels in MGB subdivisions (7.6 \sim 8.2 \text{ mmol/kg dry wt}) than IC subdivisions (8.3 \sim 12.4 \text{ mmol/kg dry wt}) (Godfrey et al. 2012). In HPLC studies in human tissue, it was found that there were lower GABA levels in MGB (3.31) than in IC (5.20) (Banay-Schwartz et al. 1989), while these same authors working with rats found similar GABA levels between these two structures (MGB: 85 vs. IC: 83) (Banay-Schwartz et al. 1993). The above-cited studies showing either similar GABA levels/concentration between the two structures or somewhat higher IC GABA levels were carefully conducted but differ significantly in methodology from the present study. The differences between these studies are likely due to use of indirect measures, using different sample/tissue treatments, and species differences. The present \textsuperscript{1}H-MRS studies used direct measures of GABA concentration against a known standard and found a trend toward higher MGB GABA concentrations relative to IC GABA concentrations, in agreement with our previously published study (Brozoski et al. 2012). The present findings support the notion that elevated GABA levels in MGB could underpin the unique distribution of GABA\(_{\text{A}}R\) high-affinity constructs found in thalamus which include extrasynaptic \(\alpha_5\beta_2\) GABA\(_{\text{A}}R\) constructs that are thought to be regulated by endogenous local GABA concentrations (Belletti et al. 2009; Cope et al. 2005; Richardson et al. 2011).

Acoustic information processing through IC and MGB. The presence of extrasynaptic GABA\(_{\text{A}}R\), elevated GABA concentrations, and unique response modes to inhibition make the thalamus a distinctive auditory structure. The present study used parallel methods in IC and MGB to reveal more potent GABAergic inhibition in MGB relative to IC. Previous studies showed that acoustic representation in the IC differs from that in MGB and primary auditory cortex (A1), which suggests a change in coding strategy, from a mixed rate and temporal code to a more refined rate code in MGB (Joris et al. 2004; Liang et al. 2002). Studies in cat also demonstrated coding changes as acoustic information ascended from IC through MGB to A1. Information redundancy presented in IC was thought to be reduced in MGB (Checchi et al. 2006; Las et al. 2005). In support of unit studies in animals, fMRI studies in humans found trends showing a population-based neural representation of the beginning and end of distinct perceptual events that is weak or absent in IC but emerges at the level of MGB (Harms and Melcher 2002). A recent review reported a more efficient temporal coding strategy at sensory thalamocortical levels relative to midbrain coding strategies (“multiplexed temporal processing scales”) (Panzeri et al. 2010), which operates multiple neural codes simultaneously at different temporal scales. Together with previous studies, the hypothesis that stimulus coding diverges in MGB compared with IC is plausible and is supported by neurophysiological data. Thus, we assume that the existence of synaptic/extrasynaptic receptors, with properties of fast/slow decay time and low/high agonist sensitivity, may enable MGB to be a multiplexed router.

Summary. The present study shows enhanced GABA sensitivity in MGB compared with IC, and this enhancement is likely mediated by both high-affinity extrasynaptic and synaptic components. This increased GABA sensitivity may serve as a basis of the vital filtering role of the auditory thalamus in processing ascending acoustic information.

ACKNOWLEDGMENTS
The authors thank Dr. Brandon C. Cox for reading and editing an earlier version of this manuscript and Dr. Ben D. Richardson and Daniel D. Duque for help with the experiments and valuable discussions.

GRANTS
These studies were supported by National Institute on Deafness and Other Communication Disorders Grants DC-00151 and DC-008532 (to D. M. Caspary), DC-009669 (to C. Bauer and T. J. Brozoski), and Office of Naval Research Grant N000141210214 (to D. M. Caspary).

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
R.C. and D.M.C. conception and design of research; R.C., B.I.K., T.J.B., and L.L.L. performed experiments; R.C., B.I.K., T.J.B., and L.L.L. analyzed data; R.C., T.J.B., and L.L.L. interpreted results of experiments; R.C., B.I.K., T.J.B., and L.L.L. prepared figures; R.C. and B.I.K. drafted manuscript; R.C., B.I.K., T.J.B., L.L.L., and D.M.C. approved final version of manuscript; D.M.C. edited and revised manuscript.

REFERENCES
Brozoski T, Odintsov B, Bauer C. Gamma-aminobutyric acid and glutamic acid levels in the auditory pathway of rats with chronic tinnitus: a direct determination using high resolution point-resolved proton magnetic resonance spectroscopy (H-MRS). Front Syst Neurosci 6: 9, 2012.

J Neurophysiol • doi:10.1152/jn.00556.2013 • www.jn.org


