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GABAERGIC INHIBITION SHAPES SAM RESPONSES IN RAT AUDITORY THALAMUS

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Abstract—Auditory thalamus (medial geniculate body [MGB]) receives ascending inhibitory GABAergic inputs from inferior colliculus (IC) and descending GABAergic projections from the thalamic reticular nucleus (TRN) with both inputs postulated to play a role in shaping temporal responses. Previous studies suggested that enhanced processing of temporally rich stimuli occurs at the level of MGB, with our recent study demonstrating enhanced GABA sensitivity in MGB compared to IC. The present study used sinusoidal amplitude-modulated (SAM) stimuli to generate modulation transfer functions (MTFs), to examine the role of GABAergic inhibition in shaping the response properties of MGB single units in anesthetized rats. Rate MTFs (rMTFs) were parsed into ''bandpass (BP)'', ''mixed (Mixed)'', ''highpass (HP)'' or ''atypical'' response types, with most units showing the Mixed response type. $GABA_A$ receptor blockade with iontophoretic application of the $GABA_A$ receptor ($GABA_AR$) antagonist gabazine (GBZ) selectively altered the response properties of most MGB neurons examined. Mixed and HP units showed significant $GABA_AR$-mediated SAM-evoked rate response changes at higher modulation frequencies (*f*ms), which were also altered by N-methyl-D-aspartic acid (NMDA) receptor blockade (2R)-amino-5-phosphonopentanoate (AP5). BP units, and the lower arm of Mixed units responded to $GABA_AR$ blockade with increased responses to SAM stimuli at or near the rate best modulation frequency (rBMF). The ability of GABA circuits to shape responses at higher modulation frequencies is an emergent property of MGB units, not observed at lower levels of the auditory pathway and may reflect activation of MGB NMDA receptors (Rabang and Bartlett, 2011; Rabang et al., 2012). Together, $GABA_ARs$ exert selective rate control over selected *f*ms, generally without changing the units' response type. These results showed that coding of modulated stimuli at the level of auditory thalamus is at least, in part, strongly controlled by GABA neurotransmission, in delicate balance with glutamatergic neurotransmission.

Key words: auditory thalamus, SAM response, inhibition–excitation balance.

INTRODUCTION

The temporally complex features of acoustic stimuli are used by most species as specific communication calls, including speech for humans. Sinusoidal amplitude-modulated (SAM) stimuli are frequently used as a reliable proxy to examine how different substations of the auditory system process temporally changing stimuli as one ascends the auditory neuraxis. Neurons at higher levels of the auditory system respond to SAM stimuli with large and complex variations in spike rate. The response properties of auditory neurons to amplitude-modulated stimuli were well described for the auditory cortex (AC) and inferior colliculus (IC) (Krishna and Semple, 2000; Wallace et al., 2000, 2002; Lu and Wang, 2004). The medial geniculate body (MGB), the auditory thalamic ''relay station'', receives excitatory and inhibitory ascending information from the IC and direct and indirect descending excitatory and inhibitory input from AC (Winer et al., 1996; Bartlett et al., 2000; Malmierca, 2003). Studies have examined the temporal processing features of MGB units, comparing them to other auditory structures, including studies in anesthetized cat (Rouiller et al., 1979), guinea pig (Wallace et al., 2007), and recent series of in-depth studies in unanesthetized marmoset (Bartlett and Wang, 2007, 2011). Collectively, these studies suggested that MGB neurons display unique and more complex responses to modulated and click train stimuli than do neurons in the IC (Bartlett and Wang, 2007, 2011). This conclusion is based, in part, on finding a large number of MGB neurons exhibiting both synchronized and non-synchronized discharge patterns (Bartlett and Wang, 2007, 2011). These MGB findings contrast, quantitatively, to response properties described at lower levels of the auditory neuraxis using similar stimuli (Krishna and Semple, 2000; Joris et al., 2004). A number of studies at lower levels of the auditory neuraxis suggested that glycinergic and/or GABAergic inhibition play a role in shaping responses to

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Abbreviations: AC, primary auditory cortex; AP5, (2R)-amino-5-phosphonovaleric acid; BBN, broadband noise; BMF, best modulation frequency; BP, bandpass; CF, characteristic frequency; d/vMGB, dorsal/ventral medial geniculate body; *fc*, carrier frequency; *fm*, modulation frequency; GABA, γ-aminobutyric acid; $GABA_AR$, $GABA_A$ receptor; GBZ, gabazine; HP, highpass; IC, inferior colliculus; KAc, potassium acetate; MAP, multichannel acquisition processor; MGB, medial geniculate body; MTF, modulation transfer function; NMDA, N-methyl-D-aspartic acid; r/tBMF, rate/temporal best modulation frequency; r/tMTF, rate/temporal modulation transfer function; rWMF, rate worst modulation frequency; TRN, thalamic reticular nucleus; SAM, sinusoidal amplitude modulation; SNR, signal-to-noise ratio; VS, vector strength.

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modulated stimuli (Burger and Pollak, 1998; Koch and Grothe, 1998; Caspary et al., 2002). Studies in chinchilla cochlear nucleus and IC suggested that glycine or GABA selectively alters the response-rate at or below best modulation frequency (BMF), frequently changing band-pass (BP) responses to more low-pass responses (Burger and Pollak, 1998; Caspary et al., 2002). Other rat IC studies found that AMPA or N-methyl-D-aspartic acid (NMDA) receptor blockade resulted in decreased discharge rates while $GABA_A$-receptor blockade produced an increase in firing rate (Kelly and Zhang, 2002). Koch and Grothe (1998) found that GABAergic inhibition sharpened tuning of frequency-modulated signals for a majority of IC neurons in the big brown bat. Three separate IC iontophoretic studies found that $GABA_A$-receptor blockade increased near-characteristic frequency (CF), tone-evoked discharge rates, suggesting that inhibitory inputs onto IC neurons had co-tuned frequency response areas, with some inhibitory neurons likely tuned more broadly than the IC neurons onto which they are projected (Yang et al., 1992; Le Beau et al., 1996; Palombi and Caspary, 1996). These experiments suggested that glutamatergic and GABAergic inputs selectively regulate IC response properties to SAM stimuli.

Based on the identification of high-affinity $GABA_A$Rs mediating tonic inhibitory current and enhanced GABA sensitivity in the auditory thalamus (Richardson et al., 2011; Cai et al., 2013), and the findings and models by Bartlett, Wang and colleagues (Bartlett and Wang, 2007, 2011; Rabang and Bartlett, 2011; Rabang et al., 2012), the present study sought to characterize the role of GABAergic inhibition in shaping responses to SAM stimuli for commonly observed SAM-response-types seen for MGB neurons.

EXPERIMENTAL PROCEDURES

All experiments were carried out in accordance to protocols approved by the Laboratory Animal Care and Use Committee of the Southern Illinois University School of Medicine.

Surgery procedure

Thirty-seven adult male FBN (Fisher Brown Norway) rats (4–6 mos) were initially anesthetized with I.M. injection (1.4 ml/kg) of a 3:1 mixture of ketamine–HCl (100 mg/ml) and xylazine (20 mg/ml). Anesthesia was maintained by ip injections of urethane (initially 1.3 ml/kg, then one-third initial amount for maintenance doses; 750 mg/kg, Sigma, St. Louis, MO, USA). 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 may have less net effect on GABAergic neurotransmission than other anesthetic agents (Hara and Harris, 2002). Rats were placed in a modified stereotaxic frame in an IAC sound-attenuating booth (Industrial Acoustic Co., Inc., New York, NY, USA) with body temperature maintained at 37 ± 0.5 °C by a thermostatically controlled heating blanket. Similar to Caspary et al. (2005), prior to surgery, auditory brainstem responses (ABRs) to click and 4, 8, 12, 16, 24 and 32-kHz tones (3-ms duration, 1-ms ramp) were obtained. None of the animals used in the present study showed any signs of hearing loss. To access the left MGB, a 2 × 2-mm craniotomy was drilled, exposing the dorsal surface of cortex (−5.5 mm from bregma; 3.5 mm lateral from midline). MGB unit recording depth was between 4800 to 6800 μm from the surface (Paxinos and Watson, 2007; Cai et al., 2013).

Acoustic stimuli and electrophysiological recording

Similar to Cai et al. (2013), a carbon fiber electrode attached to a five-barrel iontophoretic electrode, Carbostar-6 (Kation Scientific, Minneapolis, MN, USA), was coupled to a headstage preamplifier, multichannel acquisition processor (MAP) system and PC running MAP software and Sort Client (Plexon Inc., Dallas, TX, USA) for real-time spike sorting. A piezoelectric advancer (David Kopf Ins., Tujunga, CA, USA) advanced the electrode into the left MGB using 70–80-dB broadband noise (BBN) pips as search signal. Single units (3:1 signal-to-noise ratio (SNR)) were discriminated based on waveform morphology and principle component analysis. Stimulus presentation real-time data display and analysis used ANECS software (Blue Hills Scientific, Dr. K. Hancock, Boston, MA, USA) coupled to TDT System III hardware. Acoustic signals were amplified (TDT-ED1), transduced (TDT-EC1) and presented to the right ear canal using polypropylene tubing. The sound system was calibrated off-line using a 0.25-inch Bruel & Kjaer model 4938 microphone (Naerum, Denmark) into a simulated rat ear (2–48 kHz ± 2 dB) (Palombi and Caspary, 1996). SAM carrier frequency (*fc*) was set at the unit's CF or BBN; rate modulation transfer functions (rMTFs) and temporal modulation transfer functions (tMTFs) were determined for each unit at 30 dB above the CF threshold in response to 2/s, 450-ms long SAM stimuli (4 ms raise-fall time, 100% depth) with *f*ms stepped between 2 and 512 Hz. Spikes were collected by the carbon fiber over a 500-ms period, following stimulus onset for 10 stimulus repetitions at each envelope frequency.

Iontophoresis and histology

A multi-barrel electrode was coupled to a constant current system (BH-2 NeuroPhore System). The balancing barrel was filled with potassium acetate (KAc, 2 M), with remaining barrels filled with γ-aminobutyric acid (GABA, 500 mM, pH = 4.0, Sigma–Aldrich, St. Louis, MO, USA), gabazine (SR-95531) (GBZ, 10 mM, Sigma–Aldrich, St. Louis, MO) and (2R)-amino-5-phosphonovaleric acid (AP5) (100 mM, pH = 7.4). Retaining currents were set at −15 nA as previous studies (Cai et al., 2013; Duque et al., 2013). The KAc filled balancing barrel neutralizes all currents by passing current equal and opposite currents being used. Additional control runs using the same current used to eject the test drug, but unbalance through the balancing barrel, were routinely utilized to rule out possible current effects. Once an MGB unit was located, drug delivering was performed with ejection currents kept between 0 to