

# Stimulus-Specific Adaptation Occurs in the Auditory Thalamus

Lucy A. Anderson,<sup>1</sup> G. Björn Christianson,<sup>1</sup> and Jennifer F. Linden<sup>1,2</sup>

<sup>1</sup>Ear Institute, University College London, London WC1X 8EE, United Kingdom, and <sup>2</sup>Department of Neuroscience, Physiology and Pharmacology, University College London, London WC1E 6BT, United Kingdom

Neurons in the primary auditory cortex respond less strongly to a commonly occurring “standard” tone than to the same tone when it is rare or “deviant.” This phenomenon, called “stimulus-specific adaptation” (SSA), has been proposed as a possible single-neuron correlate of the mismatch negativity, a cortical evoked potential associated with stimulus novelty. Previous studies in cat did not observe SSA in single neurons in the auditory thalamus. However, these reports did not differentiate between the auditory thalamic subdivisions and did not examine the effects of changing the stimulus presentation rate. To explore the possibility of thalamic SSA more completely, we recorded extracellularly from 30 single units and 22 multiunit clusters in the ventral, medial, and dorsal subdivisions of the mouse medial geniculate body (MGB), while presenting the anesthetized animals with sequences of standard and deviant tones at interstimulus intervals of 400, 500 and 800 ms. We found SSA in the auditory thalamus at all three stimulus presentation rates, primarily in the medial subdivision but to a lesser degree also in the ventral MGB. Thalamic SSA was evident from the earliest onset of tone-evoked activity, although the latencies of responses to standard and deviant tones were not significantly different. Together with related findings of SSA in neurons of the “belt” regions of the inferior colliculus, these results demonstrate that SSA is present at subcortical levels, primarily in but not restricted to the nonlemniscal auditory pathway.

## Introduction

Stimulus-specific adaptation (SSA), or frequency-selective habituation to commonly occurring tones, was recently described in the cat primary auditory cortex and proposed as a possible neuronal correlate of the mismatch negativity (Ulanovsky et al., 2003, 2004). The authors found no evidence for SSA in the main nucleus of the auditory thalamus [the medial geniculate body (MGB)] and suggested that SSA might, therefore, be a cortical phenomenon. However, those thalamic studies used only stimuli presented at an interstimulus interval of 800 ms (~1.25 stimuli/s), and the locations of the recordings among the auditory thalamic subdivisions were not specified. We wondered if SSA might be observed in the MGB only for stimuli presented at faster rates or only in particular thalamic subdivisions. To address these possibilities, we recorded single-unit and multiunit activity in the medial, ventral, and dorsal subdivisions of the mouse MGB, using a stimulus identical to that of Ulanovsky et al. (2004) but with interstimulus intervals of 400, 500, and 800 ms. Preliminary versions of this study have appeared previously in abstract form (Linden and Anderson, 2008; Anderson et al., 2009a).

## Materials and Methods

**Animals.** Ten adult male CBA/Ca mice, 6–24 weeks of age, were used as subjects in this experimental study. Mice were anesthetized with ketamine and medetomidine, prepared for recording and monitored using procedures similar to those described by Linden et al. (2003). A craniotomy ~3 mm in diameter, centered 2 mm lateral to midline and 3 mm caudal to bregma, was performed on the left-hand side, enabling vertical access to the MGB. Extracellular recordings were made across all MGB subdivisions using custom-made tungsten-in-glass electrodes (tip sizes, 10–20  $\mu\text{m}$ ; impedance typically, 1–2  $\text{M}\Omega$ ) (Bullock et al., 1988). All experiments were performed in accordance with the United Kingdom Animal (Scientific Procedures) Act of 1986.

**Auditory stimuli.** Free-field auditory stimuli were directed at the animal's right (contralateral) ear, and a sound-attenuating plug was placed in the left ear. Before the start of each experiment, acoustic stimuli were calibrated with a microphone positioned as close as possible to the opening of the animal's auditory canal. The sound system frequency response was flat to within  $\pm 1.5$  dB from 2 to 90 kHz. Responses to tone pips of variable frequency and intensity were used to estimate the frequency-response area and characteristic frequency (CF) for each MGB neuron. If the neuron showed a clear response to tonal stimuli (as indicated by the frequency response area), stimulus-specific adaptation was assessed using two frequencies (f1 and f2) presented in a “switching oddball” paradigm exactly as used for analysis of SSA in cat auditory cortex (Ulanovsky et al., 2004). Briefly, the switching oddball paradigm consisted of blocks of 800 trials, 20 repetitions of a “frozen” sequence of 40 trials. For the first 20 trials, the frequencies f1 and f2 occurred with probabilities 0.8 and 0.2, respectively; in the second 20 trials, the probabilities were reversed. Individual tones were 200 ms long, with a cosine-squared rise/fall time of 5 ms, and were presented at 20 dB SPL above threshold at CF. The interval between successive tones in the stimulus [interstimulus interval (ISI)] was either 400, 500, or 800 ms. As in Ulanovsky et al. (2004), the frequencies f1 and f2 were separated by no more than 0.5 octaves, with the central

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Correspondence should be addressed to Dr. Jennifer F. Linden, Ear Institute, University College London, 332 Gray's Inn Road, London WC1X 8EE, UK. E-mail: j.linden@ucl.ac.uk.

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frequency ( $f_2 \times f_1$ )<sup>1/2</sup> close to the neuron's characteristic frequency. This condition was equivalent to the condition  $(f_2 - f_1)/(f_2 \times f_1)^{1/2} = 0.37$ , described by Ulanovsky et al. (2003, 2004).

**Histology.** Localization of recording sites to each of the three major subdivisions within the MGB was accomplished by reconstruction of electrolytic lesions and recording tracks from brain sections stained for cytochrome oxidase. Histological procedures were exactly as described by Anderson et al. (2009b).

**Data analysis.** A neuronal SSA index ("neuronal SI") was defined as in Ulanovsky et al. (2003, 2004): neuronal SI =  $[\text{dev}(f_1) + \text{dev}(f_2) - \text{std}(f_1) - \text{std}(f_2)]/[\text{dev}(f_1) + \text{dev}(f_2) + \text{std}(f_1) + \text{std}(f_2)]$ , where  $\text{dev}(f_1)$  is the average neuronal response to  $f_1$  when  $f_1$  is deviant,  $\text{std}(f_1)$  is the average response to  $f_1$  when  $f_1$  is standard, and likewise for  $\text{dev}(f_2)$  and  $\text{std}(f_2)$ . For each neuron, the significance of this neuronal SI value was assessed by comparing the actual value to the distribution expected under the null hypothesis of no difference between responses to standard and deviant stimuli (approximated using a Monte Carlo simulation with 10,000 randomizations of the assignments of tone responses to standard or deviant stimuli). A given neuronal SI value was considered significant if its probability in this randomization test was  $<0.01$ . Using a similar Monte Carlo approach, we also tested for significant differences in first-spike latency (defined as the mode of the first spike times after tone onset) between the standard and deviant conditions.

## Results

We recorded from 30 single units and 22 multiunits in the MGB of 10 mice. Similar trends were observed among single units and multiunits separately, so results are reported here for pooled single-unit and multiunit data. All neurons responded to pure tones and showed similar responses to both frequencies chosen for the switching oddball stimuli. Responses were generally transient, consisting of a short burst of spikes after tone onset. Neuronal firing rate was, therefore, analyzed in the 0–60 ms interval after tone onset, which encompassed the entire response evident in population peristimulus time histograms (PSTHs) (Fig. 1). Larger analysis windows produced qualitatively similar results.

Population PSTHs averaged over all recordings in the MGB demonstrated that when a tone was presented as a standard (Fig. 1A, blue), it evoked a weaker response than when it was presented as the deviant stimulus (Fig. 1A, red). This stimulus-specific adaptation was evident for switching oddball stimuli with interstimulus intervals of 400, 500, and even 800 ms. Analysis of neuronal SIs revealed significant SSA at all three stimulus presentation rates (Fig. 1B). The median neuronal SI was significantly positive (indicating greater response to deviant than standard tones) at all interstimulus intervals tested (sign-rank test,  $p < 0.01$  in all cases). Moreover, individual neuronal SIs were significant in 11–16% of the population, depending on presentation rate ( $p < 0.01$  by a randomization test; see Materials and Methods). Thus, at interstimulus intervals of 400, 500, and even 800 ms, there was significant SSA in the mouse MGB both at the population level and in a subpopulation of individual neurons.

To better illustrate the time course and magnitude of the SSA, Figure 1C presents, on an expanded timescale, population PSTHs calculated only for the individually significant neurons. Neurons in this subpopulation responded strongly to the  $f_1$  and  $f_2$  stimuli (compare  $y$ -scale in Fig. 1C to that in Fig. 1A) and had a large difference signal (for example, at 500 ms ISI, the difference signal was approximately half the magnitude of the population response to the standard tone). As is also evident in Figure 1A for the entire population, the latency of the difference signal in Figure 1C was similar to the latency of the standard and deviant population responses themselves at all ISIs, indicating that SSA emerged very early in the population response. At the single neuron level, there was no difference between the first-spike latencies

to either the standard or deviant conditions, regardless of the interstimulus interval ( $p > 0.05$  in all cases using the randomization test; see Materials and Methods). However, over the period 5–15 ms after tone onset, which encompassed the earliest tone responses, the mean difference between responses to deviant and standard tones was significantly positive for 500 and 800 ms ISIs ( $t$  test,  $p < 0.01$ ) and positive although not significantly so for 400 ms ISI.

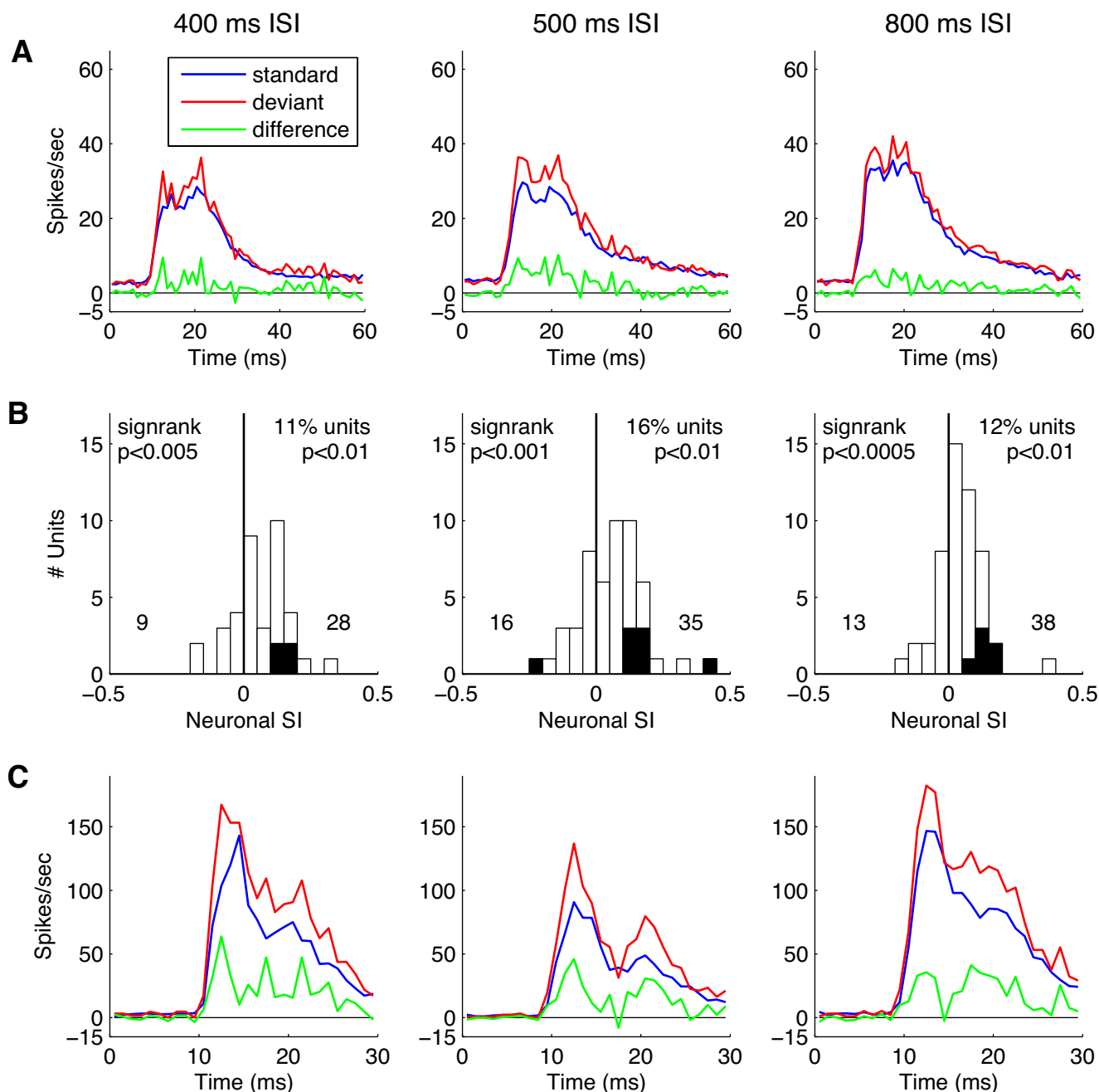
To determine whether the prevalence of SSA differed between MGB subdivisions, we localized recording sites to the medial ( $N = 20$ ), ventral ( $N = 19$ ), or dorsal ( $N = 13$ ) subdivisions by reconstructing electrode tracks in brain sections stained for cytochrome oxidase (supplemental Fig. 1, available at [www.jneurosci.org](http://www.jneurosci.org) as supplemental material). The range of characteristic frequencies of recorded neurons was similar for the three MGB subdivisions. However, population histograms revealed that stimulus-specific adaptation was most pronounced in the medial MGB population, at all ISIs tested (supplemental Fig. 2, available at [www.jneurosci.org](http://www.jneurosci.org) as supplemental material).

This finding was confirmed by analysis of neuronal SI histograms (Fig. 2). Median neuronal SI was significantly positive in the medial MGB (sign-rank test,  $p < 0.01$  at all three ISIs), and ~20% of medial MGB neurons had neuronal SIs that were individually significant at the  $p < 0.01$  level (Fig. 2A). In the ventral MGB, ~10% of the neurons had significant neuronal SIs ( $p < 0.01$ ), but the median neuronal SI for the population was not significantly different from zero (sign-rank test, not significant for any ISIs). A smaller sample of neurons from the dorsal MGB showed no significant SSA at either the population level or in individual neurons (Fig. 2C). Thus, stimulus-specific adaptation occurred primarily in the medial MGB subdivision and was similar for switching oddball stimuli with ISIs of 400, 500, and 800 ms (for further analysis of the effects of ISI, see supplemental Fig. 3, available at [www.jneurosci.org](http://www.jneurosci.org) as supplemental material).

## Discussion

Stimulus-specific adaptation occurred in the mouse MGB at all stimulation rates tested, even the relatively slow 800 ms ISI used in previous studies that had reported no SSA in the cat MGB (Ulanovsky et al., 2003, 2004). However, in the mouse MGB, the SSA was evident primarily in the medial subdivision; the effect was much less pronounced in the ventral subdivision and was not detected in a smaller sample of neurons from the dorsal MGB. Although we observed SSA in the larger ventral MGB, it was not significant at the population level; thus, it may be that thalamic SSA was not observed in the cat MGB, since the blind sampling strategy may not have included a sufficiently large population from the medial MGB. Alternatively, it may be that SSA is an exclusively cortical phenomenon in the cat but not in rodents.

The medial subdivision of the MGB is part of the nonlemniscal auditory pathway and receives the majority of its projections from the belt regions of the inferior colliculus (Winer, 1992). In addition to auditory projections, the medial MGB has strong connections with the amygdala (LeDoux et al., 1990) and somatosensory pathways among other areas (Rouiller et al., 1989; Winer, 1991, 1992). The medial MGB is also known to contain neurons with short-latency responses to acoustic stimuli (Anderson et al., 2006). Based on these observations, it has been suggested that the medial MGB may mediate alerting responses to acoustic stimuli. Stimulus-specific adaptation, which essentially amounts to habituation of auditory responses to frequently occurring sounds, may play an important role in these alerting re-

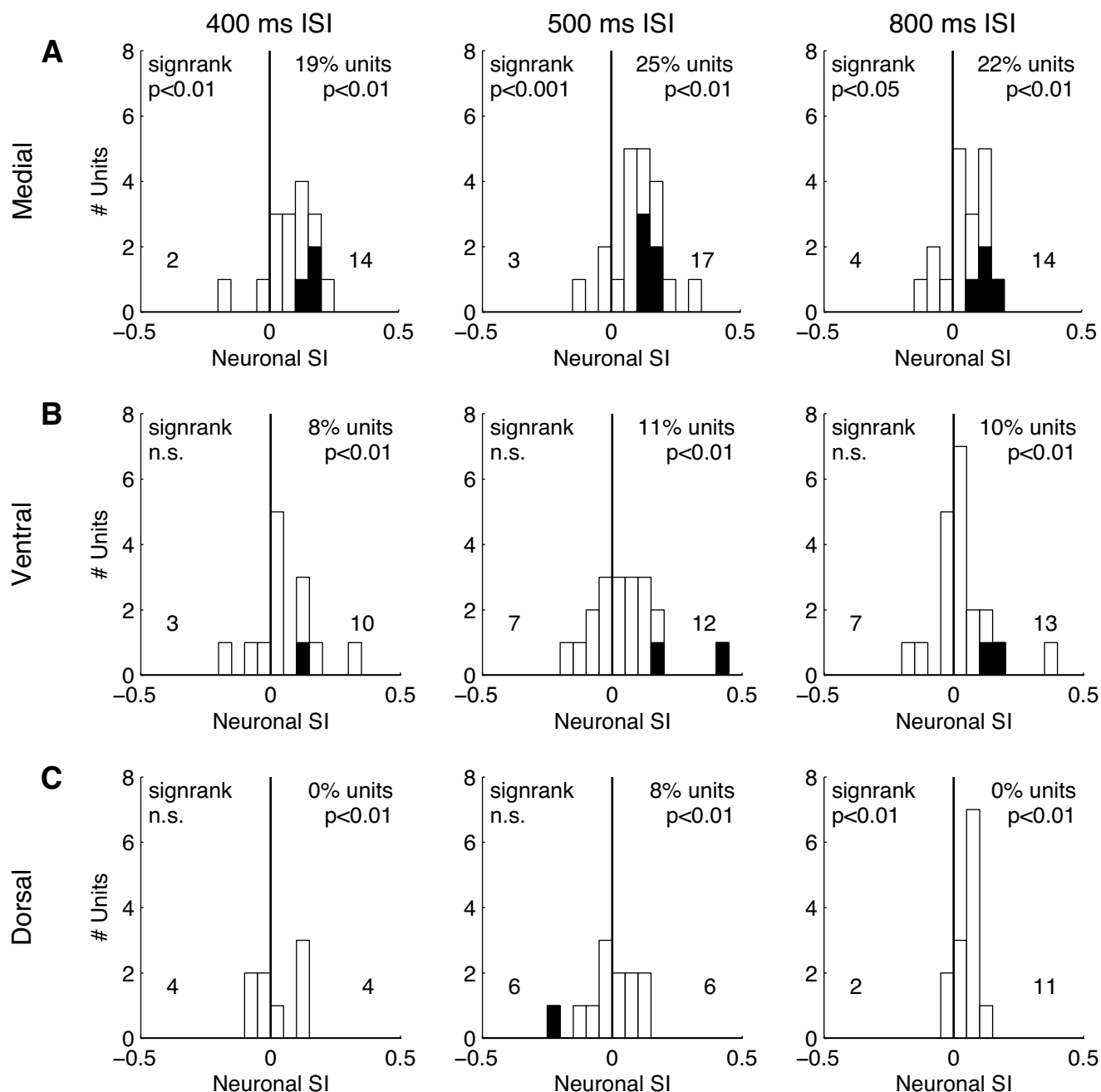


**Figure 1.** Stimulus-specific adaptation in the mouse MGB. **A**, Population PSTHs averaged over all MGB recordings, showing responses to switching oddball stimuli with interstimulus intervals of 400 (left), 500 (middle), or 800 ms (right). Each plot compares the average neuronal response to tones presented as standards (blue) with the average response to the same tones presented as deviants (red); the average difference in response is also shown (green). **B**, Histograms of neuronal stimulus-specific adaptation indices (neuronal SIs) for tone responses recorded using switching-oddball stimuli with interstimulus intervals of 400 (left), 500 (middle), or 800 ms (right). Recordings with individually significant neuronal SIs ( $p < 0.01$ ) are indicated with black bars. Numbers and text within each plot indicate the following: (bottom left and right) the number of recordings with neuronal SIs above and below zero; (top left) the result of a sign-rank test of the hypothesis that the median neuronal SI was zero; and (top right) the percentage of recordings for which the neuronal SI was individually significant by a randomization test ( $p < 0.01$ ). **C**, Population PSTHs showing responses averaged over only those MGB recordings for which the neuronal SIs were significant ( $p < 0.01$ ) and with an expanded timescale around response onset. Conventions as in **A** but note different x-scale and y-scale.

sponses, by suppressing responsiveness to common sounds and thereby highlighting low-probability, more unexpected sounds.

Like SSA in the cat auditory cortex (Ulanovsky et al., 2003, 2004), SSA in the mouse MGB was evident through the entire duration of the neuronal response. Mouse MGB responses were usually very transient (as are MGB responses in other species) (for review, see De Ribaupierre, 1997), so it was not possible to determine whether SSA in mouse MGB affected sustained activ-

ity  $>60$  ms after tone onset, as demonstrated in the auditory cortex (Ulanovsky et al., 2003, 2004). However, the SSA of the transient portions of the responses in the mouse MGB resembled those in the cat auditory cortex [compare Fig. 1A with Ulanovsky et al. (2003), their Fig. 2A, B]. SSA in mouse MGB differed from the SSA reported in cat auditory cortex in that individual neuronal SI values for mouse MGB neurons were  $<0.5$ , whereas in the cat auditory cortex, a small subpopulation of neurons were re-



**Figure 2.** Stimulus-specific adaptation occurs primarily in the medial subdivision of the mouse MGB. Plotting conventions as in Figure 1*B*. Histograms show neuronal SIs for recordings in the medial (**A**), ventral (**B**), and dorsal (**C**) MGB, for each of the three different interstimulus intervals.

ported to have neuronal SI values exceeding 0.6 (Ulanovsky et al., 2003, 2004). Recent conference abstracts have reported SSA in the MGB of two other rodent species; the neuronal SI values reported here are comparable with those observed in the gerbil (Bauerle et al., 2009) but lower than those reported in the rat (Antunes et al., 2009). These differences in neuronal SI values for MGB neurons in mouse, gerbil, and rat may reflect species differences or could have arisen from variations in experimental protocols, including differences in the type of anesthesia or the neuronal search strategies used.

Stimulus-specific adaptation has a relatively short latency (<15 ms) across the auditory thalamic population in the mouse. In fact, the divergence between the mean response to tones presented as standards and the mean response to the same tones

presented as deviants was evident from the earliest onset of tone-evoked activity (Fig. 1*A, C*). This finding suggests that thalamic SSA is unlikely to arise through stimulus-driven modulation of thalamic responses by cortical feedback, which would be expected to introduce a delay of at least a few milliseconds between the onset of tone responses and the onset of SSA. Fast-onset thalamic SSA could arise through longer-term cortical modulation of thalamic excitability or the excitability of other subcortical auditory areas; however, we observed no difference in first-spike latencies between standard and deviant conditions ( $p > 0.05$ ). Alternatively, SSA might be a general property of many neurons in the auditory system, particularly within the nonlemniscal pathway. SSA is also known to occur in neurons of the nonlemniscal belt regions of the inferior colliculus (Pérez-González et al.,

2005; Reches and Gutfreund, 2008; Malmierca et al., 2009). Together with the data on thalamic SSA presented here, these findings suggest that whatever its origin, SSA is an important feature of subcortical nonlemniscal auditory processing.

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