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Slow Recovery From Excitation of Thalamic Reticular Nucleus Neurons

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Yu XJ, Xu XX, Chen X, He S, He J. Slow recovery from excitation of thalamic reticular nucleus neurons. *J Neurophysiol* 101: 980–987, 2009. First published December 10, 2008; doi:10.1152/jn.91130.2008. Responses to repeated auditory stimuli were examined in 103 neurons in the auditory region of the thalamic reticular nucleus (TRN) and in 20 medial geniculate (MGB) neurons of anesthetized rats. A further six TRN neurons were recorded from awake rats. The TRN neurons showed strong responses to the first trial and weak responses to the subsequent trials of repeated auditory stimuli and electrical stimulation of the MGB and auditory cortex when the interstimulus interval (ISI) was short (<3 s). They responded to the second trial when the interstimulus interval was lengthened to ≥3 s. These responses contrasted to those of MGB neurons, which responded to repeated auditory stimuli of different ISIs. The TRN neurons showed a significant increase in the onset auditory response from 9.5 to 76.5 Hz when the ISI was increased from 200 ms to 10 s ($P < 0.001$, ANOVA). The duration of the auditory-evoked oscillation was longer when the ISI was lengthened. The slow recovery of the TRN neurons after oscillation of burst firings to fast repetitive stimulus was a reflection of a different role than that of the thalamocortical relay neurons. Supposedly the TRN is involved in the process of attention such as attention shift; the slow recovery of TRN neurons probably limits the frequent change of the attention in a fast rhythm.

INTRODUCTION

The dorsal thalamus relays sensory and motor information to the cerebral cortex and receives strong modulation from its projection cortex. Both thalamocortical and corticothalamic projections send collaterals to the thalamic reticular nucleus (TRN) in the ventral thalamus. The GABAergic neurons of the TRN project only to the dorsal thalamus (Houser et al. 1980; Jones 1975; Steriade et al. 1997; Yen et al. 1985) and exhibit burst firing and rhythmic oscillations reflecting the interaction of thalamus and cortex (Bal and McCormick 1993; Deschênes et al. 1985; McCormick and Prince 1986; Steriade and Deschênes 1984). Its location, unique connections, and firing patterns have led to the TRN being proposed both as an important player in the internal attention searchlight circuit (Crick 1984) and as a bridge linking the specific and nonspecific pathways, leading to a large-scale coordination of neuronal processes (Llinás and Paré 1997).

In the auditory region of the TRN, neurons show various response patterns to the acoustic stimulus: burst response, phasic ON response and OFF response (Shosaku and Sumitomo 1983; Simm et al. 1990). Acoustical stimulation can evoke 5- to 13-Hz oscillations in TRN neurons, while TRN activation induces focal Gamma waves of ~35–55 Hz in the cortex (Cotillon and Edeline 2000; Cotillon-Williams and Edeline 2003; Shosaku and Sumitomo 1983; Xu et al. 2008). The TRN neurons are tuned to a broad range of frequency and are not tonotopically organized (Simm et al. 1990).

Using in vivo extracellular recordings from both anesthetized and conscious animals, we examined the temporal features of TRN neurons with acoustic stimulus and with electrical stimulation of the auditory cortex and the medial geniculate body. We also compared the response properties of TRN neurons with those of thalamic relay neurons.

METHODS

Animal preparation

Wistar rats of either sex, weighing 280–360 g with clean external ears served as subjects. Anesthesia was initially induced with urethane (1.5g/kg, 20% solution ip, Sinopharm Chemical Reagent) and maintained by supplemental doses of the same anesthetic (0.5 $g \cdot kg^{-1} \cdot h^{-1}$) during the surgical preparation and recording. Atropine sulfate (0.05 mg/kg sc) was given 15 min before anesthesia to inhibit tracheal secretion. A local anesthetic (xylocaine 2%) was liberally infiltrated in the wound to reduce discomfort. Animals were surgically prepared as described previously (Guo et al. 2007; He 2001; Xiong et al. 2004). Briefly, the subject was mounted in a stereotaxic device following the induction of anesthesia. A midline incision was made in the scalp, and craniotomies were performed to enable us to vertically access the medial geniculate body (MGB) and the auditory sector of TRN and to perpendicularly access the auditory cortex in the left hemisphere. The dura mater was removed at the positions above the MGB, TRN, and auditory cortex. The procedures were approved by the Animal Subjects Ethics Sub-Committee of the Institute of Biophysics.

Acoustic stimulus

Acoustic stimuli were generated digitally by an Auditory Work Station (Tucker-Davis Technologies, TDT, Alachua, FL), which was controlled by a PC computer. Acoustic stimuli were delivered to the subject via a coupled electrostatic speaker (EC1, TDT) mounted in a probe. The sound pressure level (SPL) of the speaker was calibrated with a condenser microphone (Center Technology). The subject was placed in a double-walled sound-proof room. Repeated noise bursts with varied inter-stimulus-interval (ISI) from 200 ms to 30 s were used to examine the TRN and MGB neurons.

Recording

Tungsten microelectrodes with impedances of 2–7 MΩ (Frederick Haer, Bowdoin, ME) were advanced by a stepping-motor microdrive, which was controlled outside the sound-proof room. The microelectrode and the acoustic stimulus signals were amplified and stored in

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the PC computer using TDT software (OpenEX, TDT) and Axoscope software (Axon Instruments, Sunnyvale, CA). The time of spike occurrence relative to stimulus delivery was calculated with Matlab software (Mathworks, Natick, MA).

The TRN and MGB were accessed vertically from the top of the brain in the stereotactically positioned subject. The penetrations were made according to a rat brain atlas (Paxinos and Watson 2005). The vertical coordinate of the electrode was determined at a point slightly above the cortical surface at the first penetration. A single electrode was used for each experiment so that the depth coordinates could be kept consistent for different penetrations during the experiment for the recording in the TRN. This technique enabled us to reconstruct a physiological map of a whole frontal TRN plane containing many penetrations by applying only a single lesion at the last penetration. Multi-channel recording was performed to simultaneously record the neuronal activities of a TRN neuron and a MGB neuron.

Electrical stimulation of the MGB and auditory cortex

Stimulating electrodes were implanted in the MGB and auditory cortex in 34 subjects. Responses of the TRN neurons to repeated electrical stimulations of varied ISI with a range of 200 ms to 10 s were recorded. Single electric shock (1-ms biphasic pulse; 100 μA) was used to activate the MGB or the auditory cortex.

Recording from conscious animals

Surgical preparation was similar to the anesthetized subjects. However, we implanted an electrode array (4 electrodes in 0.3-mm spacing, 1 M&OHgr;Ω, glass-coated, homemade) in the TRN that targeted the auditory region. The location of the implanted electrode array was examined with its auditory responses. The electrode array was connected to a socket which could be plugged into a homemade telemetric circuit (pictured as in Supplemental Fig. S7)[1] to amplify and transmit the neuronal signals to a computer. The socket was fixed on the skull with dental cement and stainless screws. The subject was then released from the stereotaxic device after a single administration of analgesia and antibiotics.

The subjects were recovered for 6 days before the telemetric circuit was plugged into the socket for recording. A two-channel telemetric circuit (Supplemental Fig. S7) picked up the neuronal signals from the two implanted electrodes and sent them via two FM transmitters (~80 MHz) to the receiver (pictured as in Supplemental Fig. S7). The neuronal signals were then forwarded and stored in the PC computer with the assistance of TDT software (OpenEX) and Axoscope software as mentioned earlier. Acoustic stimuli were delivered to the subject via electrostatic speaker (EC1, TDT) 2 m above the subject.

Anatomical confirmation

After the recording session, the subjects were deeply anesthetized with sodium pentobarbital and perfused transcardially with 0.9% saline followed by 0.4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.3). The brains were removed and stored overnight in 0.1 M phosphate buffer containing 30% sucrose. The thalami were cut transversally into 50-μm-thick sections with a freezing microtome. All sections were stained with the Nissl method. The Nissl sections were overlapped with the physiology map, through the electrode penetration tracks and the lesion for guidance. Nine subjects were used for the purpose of anatomical confirmation.

RESULTS

Results presented in the present report were mainly obtained from anesthetized subjects (103 neurons from 42 anesthetized subjects and 6 neurons from 3 awake subjects). The recordings from the awake subjects were used to confirm our major findings.

Auditory responses of TRN and MGB neurons

With repeated auditory stimuli in a fast repetition, TRN neurons responded strongly to the first trial and weakly to the subsequent trials before showing a good response some seconds later. The neurons showed an increased firing rate in the auditory response when the ISI was lengthened gradually from 200 ms to 30 s. The first spike latency was shortened as the ISI was increased. Figure 1 shows neuronal responses to repeated stimuli of different ISIs from three TRN neurons in the auditory sector. The responses to the first and subsequent trials of varied ISI are shown in Fig. 1. *Aa–Ca.* All neurons were located in the TRN as shown in the microphotographs of Nissl staining for their recording sites (*bottom row,* Fig. 1, *A–C, a*). The neuronal responses to repeated auditory stimuli of varied ISI were illustrated in the raster displays (Fig. 1, *A–C, b*).

The neuron in Fig. 1*A* responded to the first presentation of the noise-burst stimulus with oscillatory firings whatever the ISI was (Fig. 1*Aa*). The oscillatory firings lasted for >600 ms. Although it was difficult to differentiate whether the long-lasting oscillatory firings was purely evoked by the first presentation of the stimulus or by the first four presentations of the stimulus, the neuron showed adaptation to the following trials of the stimulus when the ISI was 200 ms for >5 s before showing some spikes again (*1st trace* in Fig. 1*Aa*). The multiunit firings of the *second trace* in Fig. 1*Aa* suggest that the refractory period of the neuron decreased when the ISI was lengthened to 1 s. The neuron responded to the second presentation of the stimulus when the ISI was lengthened further to 3 s although the oscillations observed in the second response were not as long as the first. The neuron in Fig. 1*B* responded only to the first few trials of the repeated stimuli when the ISI was 200 ms (*1st trace* in *a*) but responded to every trial when the ISI was lengthened to 1 and 3 s (*middle* and *bottom traces* in *a*). The neuron in Fig. 1*C* showed response to the first trial of the repeated stimuli of all ISIs (Fig. 1*Ac*). The neuron showed very limited responses to the subsequent 10 trials when the ISI was 200 ms (*1st trace* in Fig. 1*Ac*).

All examples in Fig. 1 show that TRN neurons responded to the first trial of repeated stimuli when the ISI was short, e.g., 200 ms. They showed increased responses to the subsequent trials of the stimuli when the ISI was lengthened to 1 and 3 s. Typical TRN neurons responded in the second trial with a similar firing as in the first trial when we lengthened the ISI to 3 s as shown in Fig. 1, *A–C* (lowest traces in *a*). The raster displays in Fig. 1 show responses to repeated stimuli of different ISIs from 200 ms to 7 and 30 s. Neuronal responses increased when the ISI was increased for all neurons. It was also obvious that the latency of the onset responses to the repeated stimuli was shortened when the ISI was lengthened (most obvious in Fig. 1*Bc*).

Among the neurons in Fig. 1, those in *A* and *B* were recorded from anesthetized subjects, whereas the neuron in Fig. 1*C* was recorded from a conscious subject. The recordings from the conscious subjects were of low signal-to-noise ratio. The adaptation of the neuron in Fig. 1*C* was best observed from the onset responses to the repeated stimuli of different ISIs (Fig. 1*Cc*). (See

[1] The online version of this article contains Supplemental material.

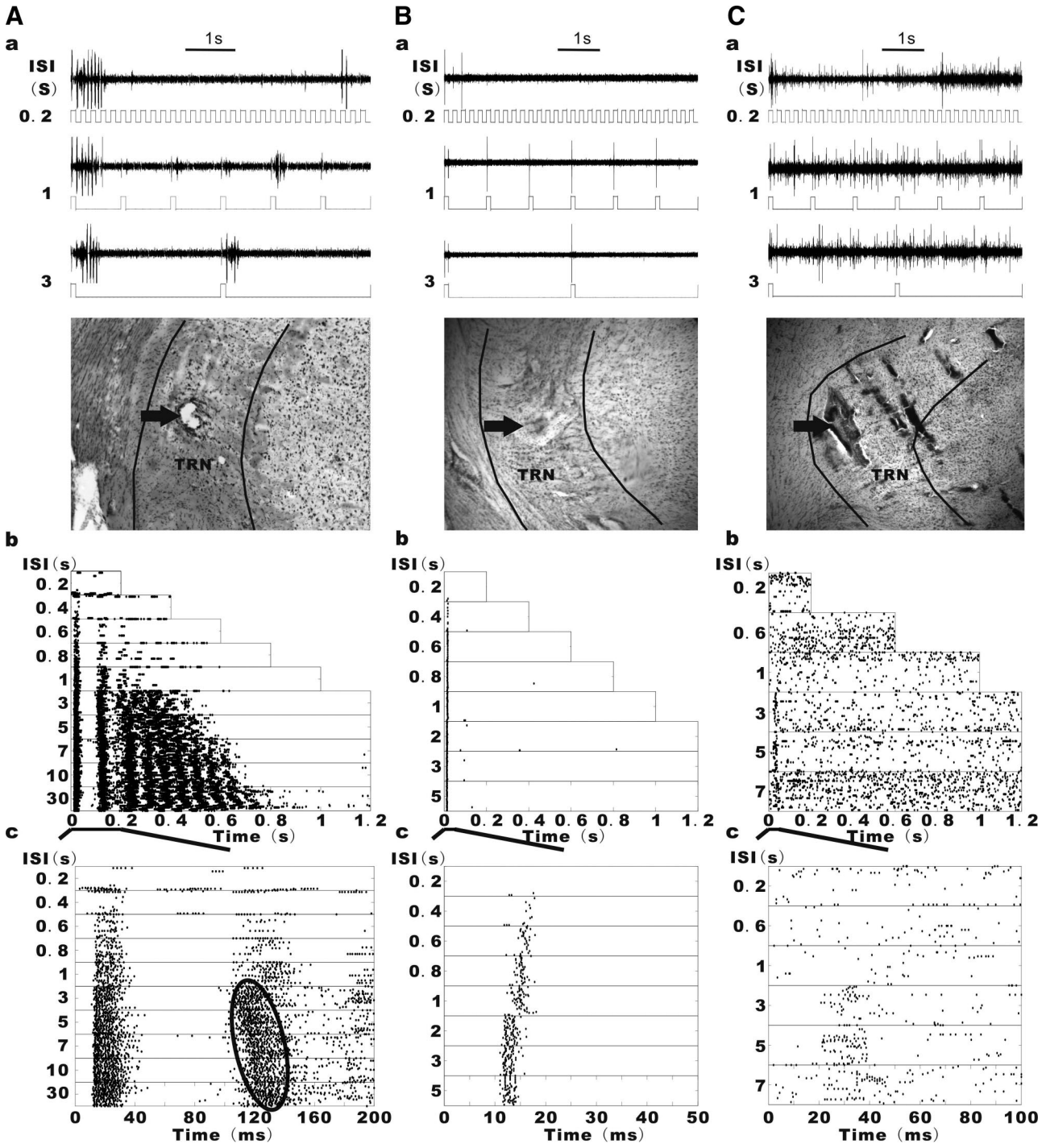

FIG. 1. Auditory responses of 3 thalamic reticular nucleus (TRN) neurons. *A—C, a, top*: extracellular recordings show neuronal responses to repeated noise bursts of 3 different interstimulus intervals (ISIs). Noise bursts of 100-ms duration and 5-ms rise/fall time were indicated as rectangle pulses below the extracellular recording traces. ISI is shown on the *left*. *Bottom*: photomicrographs of Nissl-stained sections show the electrode track and lesions of the recorded sites in the auditory sector of the TRN. →, locations of recording neurons in the TRN. Border lines of the TRN were drawn based on cytoarchitectual differences. *B*: raster displays show neuronal responses to noise bursts of varied ISIs (from 200 ms to 30 s). The noise burst was presented for 30 repetitions for each ISI. The order of trials for each repeated stimulus was presented from the bottom to the top. These conventions apply to the following figures. The first 50- to 200-ms responses were depicted and shown in *c;* the spikes in the circle were the 2nd cluster of spikes.

supporting information, Supplemental Fig. S8 for another full example of recording from conscious subject, Supplemental Fig. S7 for the methodology and telemetric system, and Supplemental Fig. S9 for another example of recording from an anesthetized subject with single-spike responses.)

MGB neurons also responded strongly to the repeated auditory stimuli of longer ISI, but the differences of responses were not comparable with TRN neurons. Figure 2 shows responses of an MGB neuron to repeated stimuli of different ISIs. The neuron responded to every repeated stimulus when the ISI was

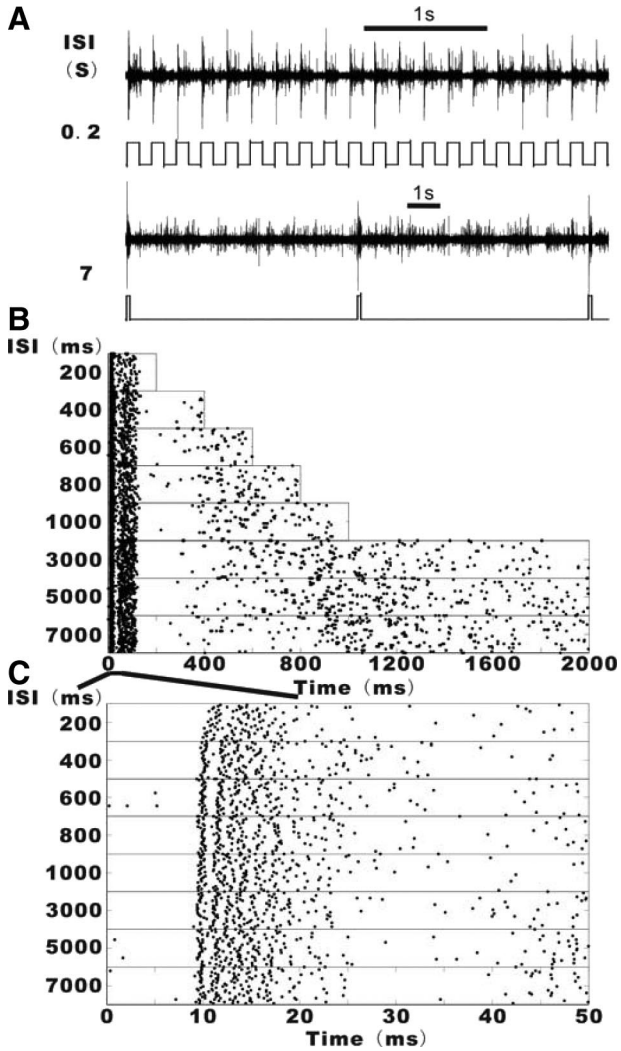

FIG. 2. Auditory responses of a medial geniculate body (MGB) neuron. *A*: extracellular recordings show neuronal responses to repeated noise bursts of 2 ISIs: 200 ms and 7 s. Noise bursts of 100-ms duration were indicated as rectangle pulses below the extracellular recording traces. *B*: raster displays show neuronal responses to noise bursts of varied ISIs (from 200 ms to 7 s, each with 30 repetitions). The onset responses of 50 ms were depicted and shown in *C*.

even 200 ms (Fig. 2*A*, *top trace*). The neuron showed no obvious difference in its onset response (defined as the 1st spike cluster with latency of <100 ms) to the noise-burst stimuli with different ISIs (Fig. 2, *A*, *bottom trace*, and *B*). The first spike latency, however, was shortened as the ISI was increased, which was similar to that for the TRN neurons.

Simultaneous recording of TRN and MGB neurons

Simultaneous recordings allowed us to confirm the aforementioned differences between TRN and MGB responses to repeated acoustic stimuli of different ISIs. Figure 3 shows a simultaneous recording of their responses to repeated stimuli of different ISIs from a pair of TRN and MGB neurons. Similar to the neuron in Fig. 1*A*, the TRN neuron showed oscillatory firing response to the first trial of the repeated stimuli and weak or no responses to the following trials when the ISI was 200 ms. However, the MGB neuron responded similarly to every trial of the repeated stimuli when the ISI was 200 ms (Fig. 3*Aa*). Both neurons responded to every trial of the repeated noise bursts when the ISI was lengthened to 7 s (Fig. 3*Ab*).

The firing rate of the onset response for both neurons was calculated and shown as a function of ISI in Fig. 3*B*. The ON response of the MGB neuron remained constant when the ISI increased from 200 ms to 30 s, whereas the TRN neuron showed a fourfold increase as the ISI increased from 200 ms to 30 s. The TRN neuron showed a steep increase when the ISI increased from 200 ms to 3 s and saturated when the ISI was >3 s.

Auditory responses as a function of the repeated trials

TRN neurons responded to the later trials differently than to the first trial when the ISI was short (Fig. 1). We calculated the responses to the stimulus in different trials over 95 TRN neurons at different ISIs (Fig. 4). Neurons showed similar onset auditory responses to the first trial of the repeated stimuli at different ISIs. They showed a slight decrease in their responses to subsequent trials of repeated stimuli when the ISI was 1 s. It took several trials to reach the stable onset response when the ISI was <1 s. The shorter the ISI, the greater the response decrease to the subsequent stimuli.

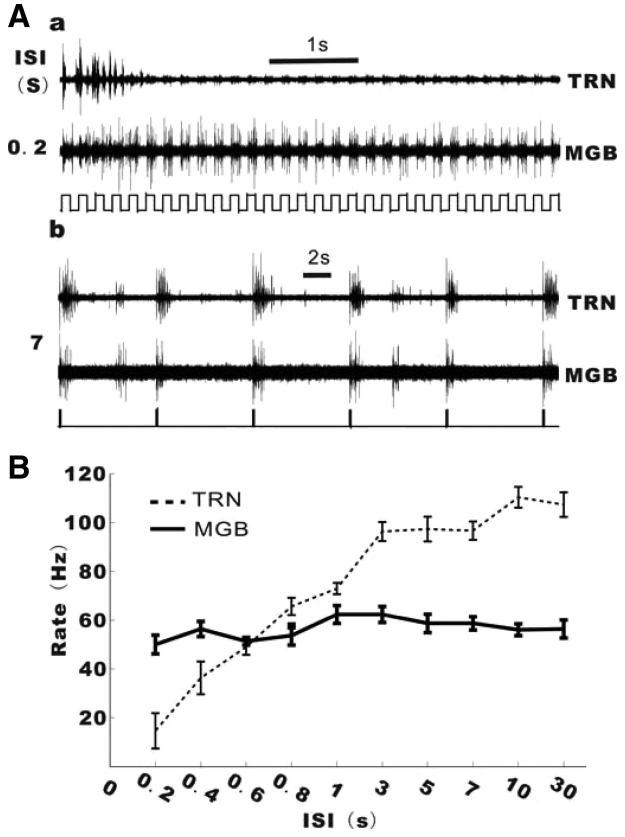

FIG. 3. Auditory responses of a pair of neurons from the TRN and MGB. *A*: simultaneous recordings of a pair of TRN and MGB neurons show neuronal responses to repeated noise-bursts of 2 ISIs: 200 ms in *a* and 7 s in *b*. Noise bursts of 100-ms duration were shown below the extracellular recording traces. *B*: firing rate of onset response is shown as a function of ISI for both neurons. Each data point was calculated over 30 presentations of the noise burst for specific ISI. The firing rate was calculated over the auditory response during stimulus presentation (100 ms).

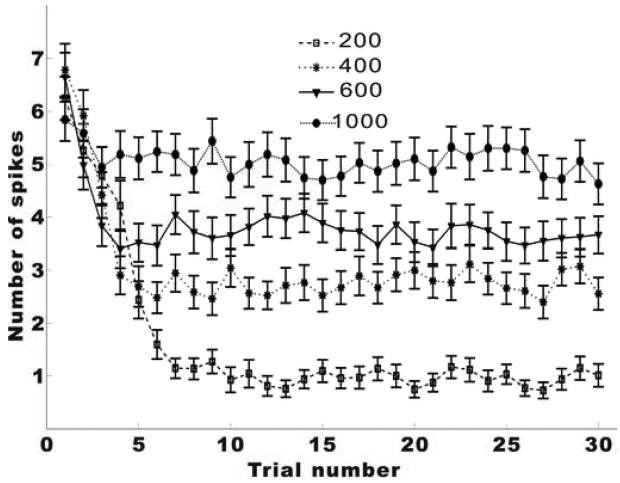

FIG. 4. TRN neurons response as a function of serial number of repeated stimulus. The mean number of spikes for different trials of the repeated stimuli was calculated during the 1st 100-ms peristimulus time over 95 TRN neurons. Although 9 ISIs were examined, only 4 different ISIs, 200, 400, and 600 ms and 1 s, were presented here for clarity.

Comparison of the auditory responses between the TRN and MGB neurons

We calculated the mean response rate and latency to auditory stimuli at different ISIs for TRN neurons and MGB neurons. The response rate was normalized to their response of 7 s. TRN neurons showed a statistically significant increase in the onset auditory response from 9.5 to 69.9 Hz when the ISI was increased from 200 ms to 7 s ($P < 0.001$, ANOVA, results before normalization in Fig. 5). Normalized data showed significant differences between the responses of ISIs $\leq$ 1 s and that of ISI = 7 s (all $P < 0.001$) for TRN neurons (Fig. 5). Neurons in the MGB also showed an increase in the onset auditory responses from 19.8 to 31.9 Hz when the ISI was increased from 200 ms to 7 s ($P = 0.0563$). No significant differences were detected in other data points for the MGB neurons. Significant differences were found between data points when curves after normalization based on the common stable point (ISI = 7 s) were compared (Fig. 5*A*; ISIs: 0.2, 0.4, 0.6, 0.8, and 1 s, all $P < 0.001$).

The first spike latency decreased for both TRN and MGB neurons as the ISI increased. The latency of the TRN neurons decreased from 31.6 to 14.9 ms when the ISI increased from 400 ms to 7 s ($P < 0.001$), whereas that of the MGB neurons decreased from 22.3 to 16.9 ms. The difference of the change was not significant ($P = 0.058$). The MGB neurons showed an only significant decrease from 26.1 to 16.9 ms in the latency comparing the ISIs of 200 ms and 7 s ($P < 0.05$), whereas the TRN neurons showed still a significant decrease between the data points of ISIs of 3 and 7 s ($P < 0.05$).

Responses of TRN neurons to electrical stimulation of MGB and auditory cortex

To examine whether the adaptation of the TRN neurons was caused intrinsically or through the neuronal network that includes the ascending pathway and corticothalamic loop, we investigated their onset responses to electrical stimulation of their immediate inputs: the MGB and auditory cortex. There were very few responses to repeated electrical stimuli of 200-ms ISI in the MGB but increased responses to repeated electrical stimuli of longer ISIs (Fig. 6*A*). TRN neurons showed almost no response to repeated electrical stimuli of ISI $\leq$ 1 s in the auditory cortex but responded substantially to those of ISI $\geq$ 3 s (Fig. 6*B*). After the first 10 ms of the raster displays were depicted, the gradually shortened first spike latency could be observed in Fig. 6, *middle.* The response rate as a function of ISI for both MGB stimulation and cortical stimulation was similar to that for auditory stimuli as shown in Fig. 6 (*right*). There was no obvious difference between the cortical and MGB stimulations.

DISCUSSION

We examined the responses of TRN neurons and MGB neurons to repeated auditory stimuli and/or electrical stimulation of the MGB and auditory cortex in both anesthetized and awake animals. TRN neurons showed strong responses to the first trial and weak responses to the subsequent trials of the repeated auditory stimuli and electrical stimulation of the MGB and auditory cortex when the ISI was short (<3 s). They responded to the second trial similarly to the first trial when the ISI was lengthened to ≥3 s. These responses contrast to those of MGB neurons, which responded similarly to the repeated auditory stimuli of different ISIs (≥200 ms in the present study). Although the majority of the results were obtained from anesthetized subjects, the major finding that the TRN neurons

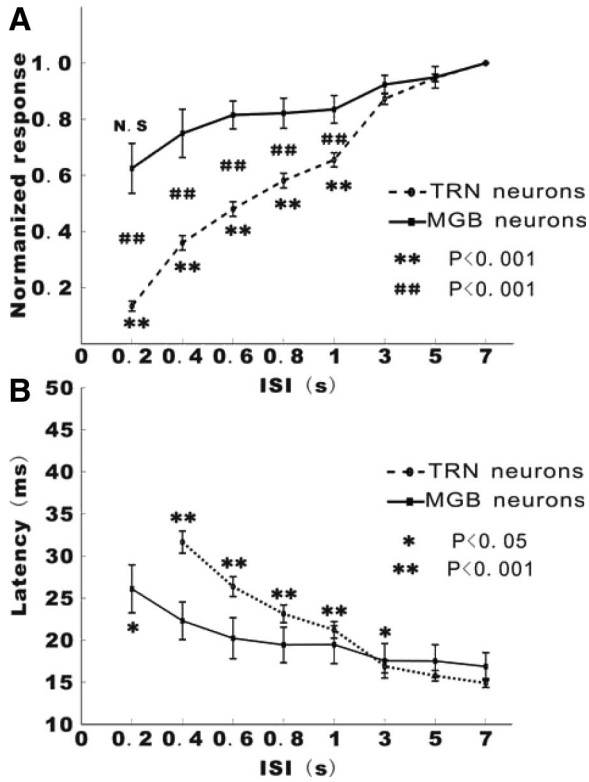

FIG. 5. Normalized response (*A*) and latency (*B*) as functions of ISI for TRN and MGB neurons. Mean firing rate was normalized based on the responses to auditory stimuli with 7-s ISI for both TRN and MGB neuron groups. Comparisons between different ISIs and 7-s ISI are shown (*, $P < 0.05$; ** $P < 0.001$). Comparison between TRN and MGB neurons are shown (##, $P < 0.001$). The calculation was based on trials from 11 to 30. The 1st 10 trials were excluded from the calculation as they would not reflect the adaption.

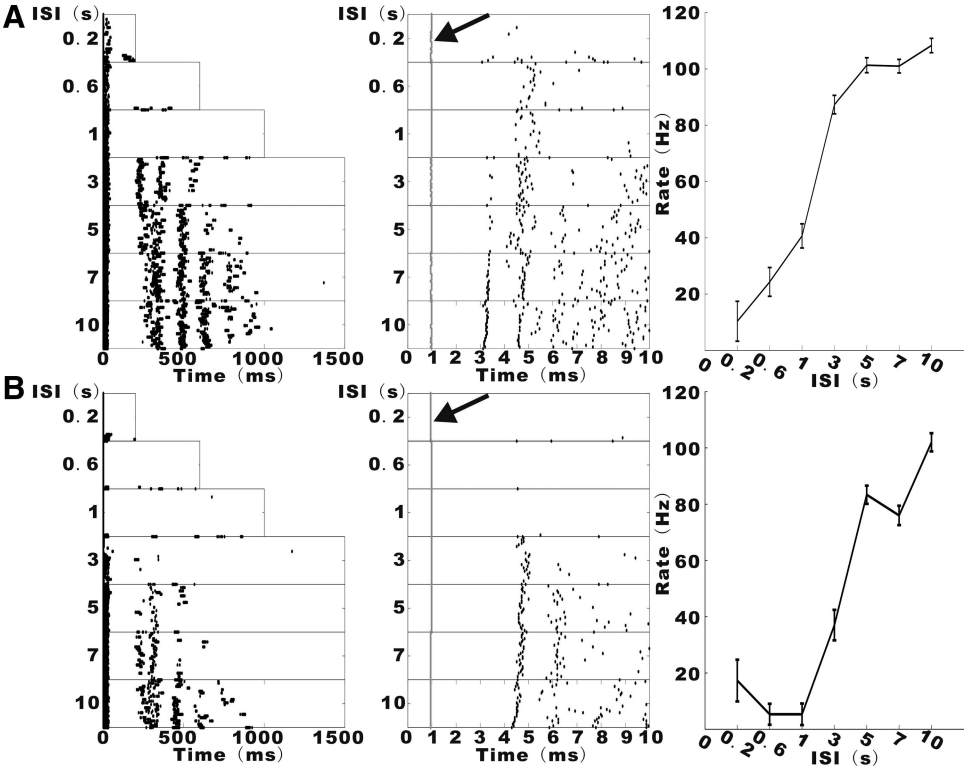

FIG. 6. Responses of a TRN neuron to repeated electrical stimuli in the MGB (*A*) and the auditory cortex (*B*). Raster displays in the *left panel* show neuronal responses to electrical stimuli of varied ISIs (from 200 ms to 10 s). Similar to the presentation of sound stimulus, the repeated electrical stimuli was presented for 30 trials for each ISI. The order of trials was from the bottom to the top in the raster displays. The 1st 10-ms responses of the raster displays in the *left panel* were depicted and shown in the *middle panel*. *Right*: the firing rate of the 1st 100-ms as a function of ISI. Here the calculation of firing rate started at 3 ms after the electrical stimulus was delivered to the MGB and the auditory cortex. Arrows indicate the artifacts in dim lines caused by electrical stimulation.

showed rapid adaptation was confirmed in recordings from awake rats. The neuronal firing patterns between the anesthetized and conscious subjects was different, this topic needs further investigation.

The major sources of input to the TRN are the collaterals of thalamocortical and corticothalamic fibers, all of which pass through the TRN (Jones 1975). The thalamocortical and corticothalamic collaterals display an anatomical topography in the distribution of their terminals in the TRN (Yen et al. 1985). Although there are multisensory neurons in the TRN, a sector of the TRN is dedicated to each major thalamic nucleus (Crabtree 1992, 1996, 1998, 1999; Crabtree et al. 1998; Jones 1975; Montero et al. 1977; Shosaku et al. 1984, 1989; Simm et al. 1990; Steriade et al. 1984). Recently, Crabtree and Isaac (2002) showed the TRN′s involvement in the inter-modal interactions of the somatosensory and motor nuclei in the thalamus.

All TRN neurons are GABAergic (Houser et al. 1980; Oertel et al. 1983) and project back to the dorsal thalamus, applying strong inhibition to the thalamocortical neurons (Crabtree 1996; Jones 1975; Rouiller et al. 1985; Steriade et al. 1984; Zhang et al. 2008). TRN axons projecting to the principal sensory nuclei exhibit restricted arbors, whereas those projecting to the second-order or multisensory nuclei have more widespread arbors in those nuclei (Cox et al. 1996; Jones 1975; Liu et al. 1995; Uhlrich et al. 1991).

Physiologically the TRN has been considered as the pacemaker for various thalamic oscillations (Contreras and Steriade 1996; Golshani and Jones 1999; Grenier et al. 1998; Steriade and Deschênes 1984; Steriade et al. 1993a,b). Recordings from the auditory regions of the TRN in the present study confirm the oscillatory responses. The interspike intervals among the successive spikes in the bursts of the spindle rhythm (Fig. 1*A*, Supplemental Fig. 10A, and Supplemental Fig. S11), suggest that the bursts were low-threshold calcium spike (LTS) bursts, which exhibited *accelerando*–decelerando spike patterns (Contreras et al. 1996; He and Hu 2002; Jahnsen and Llinás 1984a,b; Llinás and Steriade 2006; Xu et al. 2008).

The auditory region of the TRN was activated by the electrical stimulation of the auditory cortex in the present study. The peak amplitude of corticothalamic excitatory postsynaptic conductance is ~2.5 times higher in the TRN neurons compared with that of thalamocortical neurons (Golshani et al. 2001; Liu et al. 2001), indicating that the corticofugal input to the TRN has a strong control over their excitability (Steriade 2001). The TRN neurons extend dendrites within the thin reticular sheet, which enables them to receive projections from a wide cortical region (Jones 1985).

The TRN is involved in both "specific" and "nonspecific" thalamocortical pathways (Jones 1975; Llinás and Paré 1997). Both thalamocortical pathways/loops can reverberate, producing thalamocortical re-entry activity because the neurons involved are all capable of oscillatory activity (Llinás and Paré 1997; Steriade 2006). It was proposed that the specific and nonspecific pathways could integrate with each other through the TRN (Llinás and Paré 1991, 1997). An intermediary role for the TRN is plausible, as some neurons exhibit multimodal responses and projections across nuclei (Crabtree and Isaac 2002; Shosaku and Sumitomo 1983; Simm et al. 1990). The binding of the specific and nonspecific pathways through the TRN was proposed to be involved in the process of attention and cognition (Llinás and Paré 1997).

One major finding in the present study was that the TRN neurons depended on the ISI in responses to repeated stimuli. This ISI dependence applied to both anesthetized and awake subjects and to neurons in different firing modes. In contrast, the MGB neurons responded to repeated stimuli. In other words, the MGB neurons recovered much faster than the TRN neurons, implying that the MGB neurons could relay the

auditory information to the cortex in both the frequency and time domain. In the present study, no further localization of subnuclei of the recorded neurons was made, and the possibility that other MGB neurons adapt to repeated stimuli could not be ruled out. In the present study, the MGB neurons showed minor adaptation when the ISI was 200 ms, with a decreased response and lengthened latency (Fig. 5).

Compared with MGB neurons, TRN neurons showed strong adaptation to ISIs in the hundreds of milliseconds. The short-latency responses (<5 ms) of TRN neurons to electrical stimulation in the MGB and auditory cortex were likely monosynaptic and would reflect only their direct responses to the inputs of MGB or auditory cortex (Fig. 6). Therefore the adaptation of the short-latency responses indicated that the adaptation was likely caused intrinsically by the TRN neurons themselves not accumulatively through the ascending or recurrent thalamocortical circuit.

It is also of interest to note that the latency of the second spike cluster of the stimulus-evoked oscillation was correlated with sustained oscillations (Supplemental Fig. S10*B*). If the oscillation consisted of LTS bursts, the degree to which the oscillation was sustained should depend on the appearance of bursting cycles, i.e., the longer the oscillation was sustained, the more the oscillatory cycles. The latency of the first spike cluster was shortened as the ISI was increased, whereas that of the second was lengthened. The difference between the latencies of the second and first spike clusters would reflect the refractory period of the LTS and also the depth of the hyperpolarization of the TRN neuron after the first LTS burst (Contreras and Steriade 1996; Jahnsen and Llinás 1984a). The depth of the hyperpolarization of the TRN neurons should be reflected in the sustained oscillation.

The slow recovery of the TRN neurons to fast repetitive stimuli reflects a different role than that of the thalamocortical relay neurons. If the TRN was to play a role in processes such as attention shift (Crick 1984; Llinás and Paré 1997), their slow recovery might limit the rate of change of attention. Alternatively, TRN neurons may respond to novel stimuli as do some IC neurons (Perez-Gonzalez et al. 2005). Novel stimulus detection would play a role in the search light hypothesis (Crick 1984). The process of attention and cognition for binding of the specific and nonspecific pathways may also be related to novelty detection (Llinás and Paré 1997; Perez-Gonzalez et al. 2005; Ulanovsky et al. 2003). Our result adds to the recent proposal that novelty detection happens only in the cortical level (Ulanovsky et al. 2003).

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