Tonotopic Control of Auditory Thalamus Frequency Tuning by Reticular Thalamic Neurons

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INTRODUCTION

The thalamus is often considered as a "gate" for the transfer of information to neocortex. Understanding the key factors controlling this gate has been the subject of intense research, but several points remain unresolved. The reticular nucleus of the thalamus (TRN) has a key anatomical position and chemical composition to modulate thalamocortical activity. It receives inputs from both thalamus and cortex and sends outputs back to the thalamus (Jones 1975). Exclusively composed of GABAergic neurons (Arcelli et al. 1997; Benson et al. 1991; Houser et al. 1980), it is a major source of inhibition for all thalamic nuclei (Jones 1985). In some species and for some sensory modalities, it is the quasi-exclusive source of inhibition in the thalamus, whereas in others it shares this role with local inhibitory interneurons (reviewed in Jones 1985; Sherman and Guillery 2001). The lateral geniculate nucleus possesses around 20% of inhibitory interneurons in all species (Barbaresi et al. 1986; Benson et al. 1992). The somatosensory thalamus and the auditory thalamus possess 20–25% of inhibitory interneurons in primate and cat (Benson et al. 1991; Fitzpatrick et al. 1984; Montero 1989; Penny et al. 1983; Spreafico et al. 1983) but <1% in rat (Winer and Larue 1988, 1996). In the auditory thalamus (medial geniculate nucleus, MG) of rat and cat, an additional source of GABAergic inhibition is directly provided by the sensory inputs coming from the inferior colliculus (Peruzzi et al. 1997; Saint Marie et al. 1997; Winer et al. 1996).

The TRN contains several distinct sectors, each related to a particular functional group of thalamocortical pathways (review in Guillery et al. 1998). There is no simple general rule governing cortical and thalamic connections in TRN because clear differences exist depending on sensory modality and species. Nonetheless, the connections among the cortex, the thalamus, and the modality-related TRN sector are, in all cases, spatially organized: each sensory sector is divided into subsectors that are determined by the connections with the first-order (i.e., primary or lemniscal) and higher-order (i.e., nonprimary or nonlemniscal) cortical areas and thalamic nuclei. The arrangement of the cortico-reticular projections has relatively well characterized: the visual (Crabtree and Killackey 1989; Lozsádi et al. 1996), somatosensory (Crabtree 1992a,b), and auditory (Conley et al. 1991; Kimura et al. 2005) sectors receive mapped inputs from the related primary cortical areas.1 The projections from nonprimary cortical areas are topographic (Kimura et al. 2005) or nontopographic (Conley et al. 1991), and they partially overlap with those coming from primary areas in the auditory (Kimura et al. 2005) and somatosensory (Crabtree 1992b) modalities but not in the visual modality (Coleman and Mitrofanis 1996; Conley and Diamond 1990). The organization of the reciprocal connections between dorsal thalamic nuclei and TRN has been less precisely described. In each modality, the projections to and from first- and higher-order thalamic nuclei are segregated (Coleman and Mitrofanis 1996; Conley et al. 1991; Crabtree 1996, 1998). However, reticular cells projecting to different thalamic nuclei can occupy overlapping territories in the TRN (Crabtree 1996, 1998);
some individual reticular cells even project to more than one thalamic nucleus (Crabtree 1996, 1998; Yen et al. 1985; but see Coleman and Mitrofanis 1996; Pinault et al. 1995a,b). To what extent the co-existence, and sometimes overlap, of topographic and nontopographic projections from cortex and thalamus onto TRN cells is compatible with physiological functional maps is still largely undefined.

Another poorly documented question concerns the influence that TRN cells exert on the functional properties of thalamic relay cells. Very few studies have directly examined to what extent the TRN shapes the receptive fields (RF) of thalamic cells. In the somatosensory thalamus, a single study showed a direct effect of the TRN on thalamic receptive fields (Lee et al. 1994a). A few hours after excitotoxic lesions of the somatosensory TRN, the RF size of ventral posterior (VP) neurons was increased, but the cells remained sensitive to the direction of motion and kept their center-surround organization. In contrast, in another study minor effects on evoked responses and on RF properties were obtained by glutamate injections in the somatosensory TRN (Warren and Jones 1994). Other studies using iontophoretic applications in thalami have provided evidence for a GABAergic control on thalamic RFs. On the one hand, in the rat VP, application of the GABAB agonist, baclofen, increased the RF size (Lee et al. 1994b), an effect not observed with the GABA_A agonist, bicuculline (Hicks et al. 1986; Lee et al. 1994b). On the other hand, studies performed in the visual (Sillito and Kemp 1983) or in the auditory (Suga et al. 1997) thalamus reported that bicuculline injections produced RF enlargements. However, in these two later experiments, the contribution of TRN neurons to the observed effects cannot be distinguished from those coming from local interneurons (in the visual thalamus) or from inferior colliculus neurons (in the auditory thalamus).

That changes in TRN activity indeed influence the responsiveness of auditory thalamus neurons was initially substantiated by results showing that electrical stimulation of auditory TRN neurons suppressed spontaneous and evoked discharges of MG neurons (Shosaku and Sumimoto 1983). Inactivation of the auditory sector of the TRN has opposite effects. In an experiment on evoked oscillations in the thalamocortical system (Cotillon and Edeline 2000), as well as in a subsequent methodological study (Edeline et al. 2002), we observed that spontaneous and evoked multiunit activity in MG was increased after cannula injections of the GABA_A agonist, muscimol, in the auditory TRN. Similarly, multiunit activity in MG was increased in the few minutes following GABA injection (unpublished data; see Fig. S1). Such techniques are, however, inappropriate to study how TRN neurons influence the functional properties of MG cells: the very long-lasting effects of muscimol prevent observing a recovery in electrophysiological experiments and, more generally, cannula injections generate mechanical instability incompatible with single-unit recordings. Thus in the present experiment, iontophoretic applications were used to modulate the activity of clusters of TRN cells. Single units were recorded in the MG while the activity of auditory TRN cells was locally controlled by ejections of GABA or of glutamate. The frequency tuning curve and the rate-level function of each MG cell was evaluated before, during, and after drug ejection in TRN. Parts of the results were presented in an abstract format (Cotillon-Williams et al. 2004).

Methods

Subjects and surgery

Forty-five urethan-anesthetized Sprague Dawley rats (300–500 g) were used as subjects (1.5 g/kg, supplemented when necessary by 0.5 g/kg to maintain an areflexive state). A tracheotomy was performed, and the body temperature was maintained around 37°C by a heating pad. After the scalp was opened, a local anesthetic (Xylocaine, 2%) was liberally infiltrated in the wound. For the recordings in the auditory TRN, the coordinates (in mm) were 3.3 posterior to Bregma and 3.5 lateral and 4.5–5.5 below dura. For the recordings in MG, the coordinates (in mm) were 5.8 posterior to Bregma and 3.5 lateral and 6–6 below dura. As the size of the stereotaxic micromanipulators did not allow a simultaneous vertical approach of the two investigated structures, the electrode penetrations to reach the MG were made with an angle of 35–40° relative to vertical.

Three silver balls were inserted between bone and dura: one was used as reference during the recording sessions; the other two, placed over the frontal and parietal cortex, served to monitor the cortical electroencephalogram (EEG). The cisterna magna was drained to minimize brain pulsations and to stabilize neuronal recordings. All surgical procedures conformed to national (JO 887-848) and European (86/609/EEC) legislation on animal experimentation, which is similar to that described in the Guidelines for the Use of Animals in Neuroscience Research of the Society of Neuroscience.

Electrodes and drug ejection

Single units were recorded in the MG by glass pipettes (~10 MΩ at 1,000 Hz) filled with pontamine sky blue (2.5% in 3 M NaCl). Multiunit recordings were collected in the auditory TRN via nichrome electrodes (1–5 MΩ) glued to a multibarrel iontophoretic electrode from which they protruded by 20–50 μm. Five-barrel pipettes were used for drug ejection: one barrel was filled with 1 M NaCl for automatic current balance; the others were filled with GABA (0.5 M, pH = 3.8) or glutamate (1 M, pH = 6–7). Drugs were ejected by a iontophoretic system (Bionic), which included automatic current compensation. The retaining currents were 5–15 nA, depending on the impedance of the iontophoretic electrodes (25–80 MΩ). To control large populations of auditory TRN cells, high ejection currents (between 100 and 200 nA) were used, and, in most of the cases, each drug was ejected simultaneously from two barrels. The impedance of the iontophoretic electrodes was tested before starting the protocol on each MG cell to verify that the drugs could be correctly ejected.

Recording procedures

All electrodes were slowly advanced in the target structure under electrophysiological control. The auditory TRN was localized based on the presence of short-latency responses (<30 ms) and of typical long-duration bursts (30–200 ms) as previously described (Cotillon and Edeline 2000; Simm et al. 1990; Villa 1990). The signal was amplified (gain: 5,000), filtered (band-pass 0.6–10 kHz), and sent to an audio monitor and to a voltage window discriminator. The waveform of each single unit was digitized (50-KHz sampling rate), continuously displayed, and stored on the computer hard drive. The data collection was stopped each time the waveform became unstable. The TTL pulses corresponding to each action potential (AP) were sent to the acquisition board of a Pentium II computer; their time of occurrence was known with a 50-μs resolution. The cortical EEG was permanently monitored on a polygraph (Grass model 79D, band-pass

But as described at the cortical level, iontophoretic applications of bicuculline can have nonspecific effects (Kurt et al. 2006).

The online version of this article contains supplemental data.
Acoustic stimuli

The sound-generating system was previously described (Edeline et al. 2000; Manunta and Edeline 1997, 1999). Briefly, pure tone frequencies were generated by a remotely controlled wave analyzer (Hewlett-Packard, Model No. HP 8903B) and attenuated by passive programmable attenuators (Wavetek, P557, maximal attenuation 127 dB). The wave analyzer and the attenuators were controlled by a microcomputer via an IEEE bus. The stimuli were delivered through a calibrated speaker (Beyer DT-48), placed close to the ear canal. Calibration of the system was done using a standard reference tone (1 kHz at 94 dB re 20μPa) generated by a Bruel and Kjaer (Model No. 4230) sound level calibrator. This sound-delivery system can deliver tones of 80 dB ≤20 kHz and of 70 dB ≤35 kHz.

The frequency tuning curve (TC) and the rate-level (R-L) function were determined for each MG cell. The frequency TC was determined by presenting pure tone frequency (rise-fall time: 5 ms; duration: 100 ms; inter-tone interval: 1 s) from 80 dB to threshold. After evaluating the characteristic frequency (CF), the frequency TC was determined at 20 dB above threshold by 10 repetitions of a set of 11 isointensity pure tone frequencies centered on the CF. At this intensity, the cell’s best frequency (BF) was defined as the frequency providing the strongest excitatory responses. The CF was selected to test the neuron R-L function. The R-L function was determined by 10 replications of a set of 11 intensities using either 5- or 10-dB steps depending on the dynamic range of the cell. Testing time for each frequency TC, and for each R-L function, was 110 s.

Experimental protocol

Simultaneous recordings were obtained in auditory TRN and in MG. On a given animal, the recording site was kept the same in the auditory TRN, and single-unit recordings were collected in the MG (1–6 cells/animal). The tuning curve and the CF of the recording site in TRN were determined at the beginning of each experiment, but in most of the cases, they were also tested simultaneously with the cell recorded in MG. At the beginning of most experiments (42/45 animals), iontophoretic ejections of GABA and glutamate were used to verify that the ejection pipette was correctly operating. In most of the cases (39/42), both GABA and glutamate produced large (>50%) and reproducible changes in spontaneous activity. In 3/42 experiments, only GABA was effective in controlling TRN activity.

As for most of the MG cells, the frequency TC and the R-L function were tested with both GABA and glutamate ejections: 100 TCs and 96 R-L functions were tested with the two drugs. After two (or 3) control TCs (or control R-L functions), GABA or glutamate was ejected in the auditory TRN for 120 s and the TC (or the R-L function) was determined during drug application. Drug ejection started 5 s after determination of the frequency TC (or the R-L function). Two tests of the TC (or of the R-L function) were performed one and four minutes after the end of ejection (tests “post 1” and “post 4”, respectively).

Data analysis

ANALYSIS OF THE MG SINGLE-UNIT RECORDINGS. For each cell, spontaneous and evoked activity (averaged across the 11 frequencies or intensities) collected during and after drug ejection were compared (using 2-tailed paired t-test) with the values obtained before drug ejection. The significance level of P < 0.05 was used to determine if the drug ejection induced an excitation, an inhibition or no change in firing rate for the recorded cell. Trials during which changes in the EEG spectrum were detected were systematically discarded from analysis.

As in previous studies (Edeline et al. 2000, 2001; Manunta and Edeline 1997, 1999), complementary indices were used to quantify the response selectivity and the breadth of tuning. First, the response selectivity was quantified by an index (Iselect) that quantifies the relative weight of the BF responses in the responses evoked within the RF: 

\[ \text{Iselect} = \frac{\text{response at BF} - \text{mean evoked response}}{\text{response at BF}} \times 100. \]

An index approaching 100 means that responses were present only at the BF, whereas an index of 0 means that the cell gave similar responses across the 11 frequencies used to test the TC. Second, the breadth of the frequency tuning was quantified by two indices. The first was the Q20dBselect defined as \( [\text{CF} / (\text{f}2 - \text{f}1)] \) where f2 and f1 indicate the high and low limits of the TC at 20 dB above threshold; with this index, the higher the value the sharper the frequency TC. The second was the square root transformation \( \sqrt{f2 - f1} \). This measure is independent of the unit’s CF (Calford et al. 1983; Whitfield 1968; Whitfield and Purser 1972); with this index, the lower the value the sharper the frequency TC. These three indices are complementary: the index Iselect takes into account the strength of evoked responses, whereas the breadth of tuning calculated with the Q20dBselect and the square root transformation are independent of the strength of evoked responses.

The acoustic threshold was determined from the rasters and histograms obtained at each phase of the protocol. Based on the raster display, threshold was defined as the intensity level that produced ≥5 responses of 10 presentations of a tone at a given intensity; each response ranged from one to six APs. Threshold determination was made by two investigators blind of the significant effect on the neuron firing rate. The best intensity (I max) was defined as the intensity which triggered the largest excitatory evoked responses. The slope of the R-L-function was defined as follows: slope = \[ \frac{\text{response at } I_{\text{max}} - \text{response at threshold (APs/I)}}{\text{I}_{\text{max}} - \text{threshold (dB)}} \].

The R-L functions were classified as monotonic or as nonmonotonic according to the criteria of Phillips and Irvine (1981): when spike counts evoked at the higher stimulus level were ≤50% of those evoked at the lower stimulus level, the cell was classified as nonmonotonic.

ANALYSIS OF THE TRN ACTIVITY. In all but three cases, TRN multiunit activity was collected during determination of the TC and of the R-L function of each MG cell. The spontaneous (500 ms before each tone presentation) and evoked activities were quantified, and the CF was defined as for MG cells. For each phase of the protocol, spontaneous and evoked activities collected before, during, and after drug injection were compared with the values obtained before drug application. The significance level of P < 0.05 (paired t-test, 2-tailed) was used to decide whether GABA and glutamate induced an excitation, an inhibition, or no change in spontaneous and/or in evoked activity in the TRN.

EVALUATION OF THE BURSTINESS OF MG AND TRN NEURONS. Both thalamic relay cells and reticular cells can display two modes of discharge, a tonic mode, usually observed during wakefulness, and a burst mode, usually observed during slow-wave sleep and anesthetized states. To assess the burstiness of thalamic and reticular cells before, during, and after pharmacological modulation of TRN activity, we used, as an empirical gauge, the “burstiness index” used in previous studies (Edeline et al. 2000; Guido et al. 1992; Lu et al. 1992) by computing the percentage of intervals ≤4 ms in the interspike interval distribution. This index is only an indirect measure of the number of bursts in spike trains, we also isolated bursts from single APs by looking for groups of two or more APs with an interspike interval <5 ms preceded by a silent period of ≥100 ms, a

4 Except if it is measured at 20 dB above threshold, the Q20dB denotes the lowest threshold at which the neuron responds to at least 50% of the stimulus presentations.

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criteria that was previously applied to extracellular recordings in anesthetized or awake animals (Guido and Weyand 1995; Guido et al. 1992; Massaux et al. 2004; Ramcharan et al. 2000; Swadlow and Gusev 2001, 2002).

Histology

At the end of each experiment, pontamine sky blue was ejected in the MG via the recording electrode (15 min, 5–20 μA). This ejection was made at the depth of the last recording site (for 44/45 animals, MG recordings came from the same electrode track). The animals received a lethal dose of pentobarbital (200 mg/kg), and the brain was rapidly removed from the cาวarium. After 48 hr in buffered formalin (10% in phosphate buffer, pH = 7.4), the brains were placed in 30% sucrose-formalin until they sank. They were sectioned on a freezing microtome (50-μm-thick serial coronal section). The sections were stained with cresyl violet for Nissl preparation. They were subsequently examined under several microscopic magnifications for determination of recording placements in the MG and of ejection sites in the auditory TRN. To determine the location of each cell, we used the electrode tracks for guidance, the point of entrance in the thalamus, the blue spot of pontamine sky-blue at the bottom of the track, and the depth coordinates read from the microdrive during the experiment. The assignment of each recorded cell to the different MG divisions was based on previously published anatomical descriptions of the rat MG (Clerici and Coleman 1990a,b; Doron and Ledoux 1999; Ledoux et al. 1987; Smith et al. 2006; Winer et al. 1999). Determination of the location of the recording sites was done blind of the electrophysiological results.

RESULTS

Examination of the histological material indicated that all placements in TRN were in the caudal part of the nucleus (from 2.8 to 3.8 posterior to Bregma) where robust tone-evoked responses can be recorded (Cotillon and Edeline 2000; Shosaku and Sumitomo 1983). All the MG recordings exhibited robust excitatory responses.

Initially, 134 cells (1–6 cell/animal) were recorded for 45–90 min each and submitted to different tests. However, the results presented in the following text are from only 66 cells. First, we discarded from analysis 18 cells showing unstable evoked responses during control TCs (and/or during control R-L functions). Second, we discarded 20 cells for which noticeable EEG changes occurred during the recording session because the evoked responses and the functional properties of thalamic cells display drastic changes when the EEG switches from synchronized to desynchronized state or the reverse (Edeline et al. 2000; Funke and Eysel 1992; Livingstone and Hubel 1981; see also Fig. 3B). Third, 30 cells tested in the absence of significant drug effects at the TRN ejection site were also eliminated. Although the experiment started only when drug ejections induced >50% change of spontaneous activity in the auditory TRN (see METHODS), GABA and glutamate were no longer effective during the tests of these 30 MG cells. Besides the obvious possibility that the ejection pipettes were partly clogged, the lack of effect could result from postsynaptic receptor desensitization as it has been already mentioned in the cat somatosensory TRN after repeated pressure injections of 2–4 min (Warren and Jones 1994). Thus the following presentation of the results focuses on the 66 TRN-MG recording pairs tested while drug application produced significant and reliable changes in evoked activity at the TRN ejection site.

Potent control of neuronal activity at the TRN ejection site did not guarantee a modulation of frequency tuning and threshold in MG

EFFECTS OF DRUG EJECTION IN TRN. The effects produced by GABA ejections were homogenous, but those produced by glutamate were not. During the tests of the frequency TC and of the R-L functions, GABA decreased TRN activity in 94% of the cases. As shown in Fig. 1A, the decrease induced by GABA could be either a total suppression of spontaneous activity (Fig. 1A1) or a partial suppression with peaks of activity corresponding to discharges occurring during spontaneous spindles (Fig. 1A2 and 3). In contrast, glutamate induced as many decreases as increases (50.6 and 49.4%, respectively). As shown in Fig. 1B, 1–4, excitatory effects could display different time courses. Inhibitions were not immediate and persisted after the end of the ejection (see Fig. 1B5), indicating that the effect was not generated by the ejection current itself. Figure 1C shows the time course of the effects induced by the two drugs in the auditory TRN during the tests of frequency TC and of R-L functions. On average, the effects were no longer detectable during the tests performed 1 min post-ejection (remember that the tests lasted 2 min).

EFFECTS ON THE RECORDED MG CELLS. On average, no significant effects were observed on the population of 66 MG cells (Fig. 2). For example, the indices used to quantify the selectivity and the breadth of tuning (Iselect, f2/f1, and Q20dB) did not exhibit significant changes (lowest P value, P = 0.19). This lack of effect is illustrated in Fig. 3A: although glutamate produced large increases of spontaneous and evoked responses in the TRN, the frequency TC of the recorded MG cell remained remarkably stable. There was also no change in the burstiness index during drug ejection (25 vs. 27%, P > 0.29).5

Although no significant effect emerged from the whole population, some MG cells did exhibit activity changes in the expected direction, i.e., their responses were enhanced when TRN activity was decreased by GABA ejection, and they were reduced when TRN activity was increased by glutamate ejection.

MG cells showing significant changes in spontaneous and evoked activity during iontophoretic applications in the TRN exhibited frequency tuning and threshold modifications

Individual statistical analyses (see METHODS) revealed that during GABA and glutamate ejections, a fraction of the recorded MG cells (n = 20) exhibited significant modifications of their evoked activity. Figure 4, A–D, presents tests of the frequency TC for a ventral MG (MGv) cell showing increased evoked responses during GABA ejection. This increase was accompanied by an expansion of the frequency TC: Iselect decreased from 69 to 55 and the index f2/f1 increased from 0.34 to 0.45, both indicating that the frequency TC was broader when GABA was ejected in the auditory TRN. This

5 However, concomitant changes could occur in auditory TRN and MG: in a few cases (not considered in our analyses) for which important EEG changes occurred during the protocol, large increases of spontaneous and evoked activity were expressed in parallel in auditory TRN and MG (see Fig. 3B). In all these cases, the burstiness index was decreased in both auditory TRN and MG.
FIG. 1. Diversity of effects observed at the ejection sites in the auditory thalamic reticular nucleus (TRN). A: examples of total (A1) or partial (A2 and A3) suppression of spontaneous activity during GABA application. The peaks of discharges which persisted during GABA application were from spontaneous spindles. The ejection (1 min) is indicated by the thick bar below the histograms. B: examples of changes in spontaneous activity observed in TRN during glutamate application. The 1st 4 examples show different temporal time courses for glutamate-induced excitations. The 5th example shows a decrease in spontaneous activity produced by glutamate application. C: when evoked activity was averaged across effective ejection sites, it appeared that glutamate application induced as many increases as decreases during both the tests of tuning curves and the tests of rate-level functions. In contrast, GABA almost exclusively led to decreased activity. Values are means ± SE.
The effect started to dissipate during the test performed 1 min after the ejection, and 4 min later, the frequency TC matched the control one (Fig. 4E). Glutamate ejection produced opposite effects (Fig. 4F). Figure 5, A–D, displays the R-L function for another MGv cell showing decreased evoked responses during glutamate ejection in the TRN. During ejection (Fig. 5C, 1 and 2), the threshold increased from 50 to 65 dB; there was a progressive recovery during the tests performed 1 and 4 min after ejection (Fig. 5D, 1 and 2). GABA ejection in the TRN decreased the threshold to 40 dB (Fig. 5E). The TC of this cell was also modified: the frequency tuning was sharper during glutamate ejection (Fig. 5F), and it was broader during GABA ejection (Fig. 5G).

Figure 6 shows the evolution of the indices computed on this population of 20 cells. During GABA ejection (Fig. 6A), the threshold was decreased \( t(19) = 3.3; P = 0.01 \), and there was a significant and reversible broadening of the frequency TC: \( I_{\text{select}} \) was decreased \( t(19) = 3.1; P = 0.01 \), the \( Q_{20\text{dB}} \) was decreased \( t(19) = 2.2; P = 0.04 \), and \( \sqrt{f_2} - \sqrt{f_1} \) was increased \( t(19) = 2.8; P = 0.04 \). Opposite effects were obtained during glutamate ejection (all \( P \) values <0.05; see Fig. 6B).

Thus local modifications of auditory TRN activity can impact on the breadth of tuning and on the threshold of MG cells. Therefore the question is twofold: why were so few cells influenced by the pharmacological manipulation of auditory TRN activity and what are the specific characteristics of these cells?

A short octave distance between the CFs in TRN and in MG is necessary for controlling the breadth of tuning and the R-L function of ventral MG cells

As previously reported (Simm et al. 1990; Villa 1990), at suprathreshold intensities, auditory TRN neurons exhibited frequency TCs extending over several octaves (Fig. 7A) and much larger than those of MG neurons (mean values for the
Given the very large TCs in TRN, the frequency TCs of MG and TRN recordings overlapped in 97% of the cases: the overlap was 40% in 36% of the cases and 45% in 64% of the cases (Fig. 7B). No particular difference was detected between these two cell populations during both GABA and glutamate ejections (lowest P value, $P = 0.12$). One can suspect that cells with large TCs exhibited overlapping TCs at supra-threshold intensities whereas, at threshold, their CFs were quite distant. Therefore the octave distance between the CFs should be a better gauge of the potential interactions between TRN and MG.

Whereas on the whole population there was no relationship between the CF values at the recording and ejection sites ($r = 0.15; P = 0.21$), such a relationship existed for the 20 cells exhibiting significant changes ($r = 0.73; P < 0.002$; Fig. 7C). For each of these cells, the octave distance between the two CFs was $<0.25$ octave, whereas it spread $\pm5$ octaves on the whole population. These results suggest that TRN activity modulates the breadth of tuning and the threshold of MG cells when the TRN and MG cells are in close vicinity along the tonotopic axis.

To evaluate if this result could have been obtained by chance, analyses were performed on two other samples of 20

- **Fig. 3.** Potent control of TRN activity did not necessarily lead to changes in frequency tuning of MG cells. A: individual example illustrating the stability of a frequency tuning curve despite large effects on spontaneous and evoked activities in the auditory TRN. The recording site in the auditory RE displayed a characteristic frequency (CF) at 2 kHz, whereas the MG cell simultaneously recorded had a CF at 9 kHz. Glutamate ejection in the auditory RE produced a large increase in spontaneous and evoked activity, without any modification of the tuning curve of the MG cell. B: individual example showing that concomitant changes can occur in auditory TRN and in MG when important electroencephalographic (EEG) changes occurred (these cases were not considered in our analyses, and the animal received immediately a supplement of anesthetics when such effects were observed).
cells (all selected among the 66 cells tested during potent effects in TRN): first, 20 randomly selected cells; second, the 20 cells exhibiting the largest octave distance between the CFs at the recording and ejection sites. For these two sets of cells, there was no significant change for the threshold and for the indices quantifying the selectivity and the tuning breadth, during both glutamate and GABA ejections (all $P$ values $>0.10$). Note also that of the 66 cells, none of the 36 cells for which the CF distance was $>0.25$ octave displayed significant changes. Thus a small octave distance between the CFs seems to be a critical factor to obtain significant effects. Nonetheless, it was not sufficient since for 10 cases with small octave distances between the CFs, modulating the activity of TRN neurons did not change MG cell activity (see Table 1 and Fig. 7C).

The subset of 20 cells significantly affected by drug ejections in TRN did not exhibit modifications of their burst proportions, using either the burstiness index (for example, 25% before vs. 27% during glutamate ejection; $P = 0.35$) or criteria classically used to isolate the bursts from spike trains (see METHODS). For example, the initial burst proportion was $12 \pm 5\%$, and it remained unchanged both during GABA-induced expansions in frequency tuning ($11 \pm 8\%; P = 0.27$) and during glutamate-induced increases in acoustic threshold ($13 \pm 7\%; P = 0.41$).

Due to the angle used to approach the MG (see METHODS), 85% of the collected recordings (115/134) were from the lemniscal MGv. Of the 66 cells kept for analysis, only 8 were from the nonlemniscal divisions: 3 were from the dorsal

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**FIG. 4.** Enlargement of the tuning curve for a ventral MG cell during GABA ejection (250 nA) in the auditory TRN and shrinkage during glutamate ejection (260 nA). During the control tuning curve (A), this cell (CF at 8 kHz at 40 dB) responded from 3 to 8 kHz. GABA was ejected in the auditory TRN at a site exhibiting a CF of 9 kHz. During GABA ejection (B and D), the responses at all the frequencies were facilitated, and new responses emerged at 9 and 10 kHz. One minute after the end of GABA application, some responses were still facilitated (E), but 4 min later the tuning curve was virtually similar to the control (C and E). During glutamate ejection (F), the responses were attenuated for almost all the frequencies. When the rate-level function of this cell was tested during GABA ejection, the threshold shifted from 40 to 30 dB (data not shown).
division (MGd) and 5 from the medial (MGm) division. As shown in Table 1, 19/20 cells exhibiting significant effects were located in MGv. As there were a total of 28 MGv cells for which the CF of the recorded cell was close to the CF of the TRN site, modulating the TRN activity successfully modified the properties of MGv cells in 68% of the cases.

**DISCUSSION**

Controlling auditory TRN activity by iontophoretic ejections of GABA or of glutamate overall failed to modify the activity of MG cells. However, a population of 20 cells, all but 1 located in the ventral division, exhibited changes in their evoked activity, breadth...
of tuning and threshold. The octave distance between the CF of the MG cell and the CF of the TRN ejection site was crucial: it is only when this distance was <0.25 octave that the functional properties of the MG cell were modified as a result of TRN activity changes.

**Methodological considerations**

**RELIABILITY OF THE EFFECTS.** Contrary to previous lesion studies that relied on comparisons between cell populations (Lee et al. 1994a), in the present experiment, each recording was its own control, and the responses of MG neurons were compared before, during, and after pharmacological manipulations in the auditory TRN. At a first glance, the obtained results were weak because only a fraction of MG cells was significantly affected by drug application in TRN. However, for these 20 cells, consistent effects occurred on evoked activity, threshold, and breadth of tuning; all these effects were reversible; the results from the different indices quantifying the selectivity (the $I_{select}$ index) and the tuning breadth (the $Q_{20dB}$ and the square-root transformation) were coherent; and GABA and glutamate ejections led to opposite effects. Furthermore, none of these changes were detected for two other sets of 20 cells, one randomly selected, the other corresponding to the largest CF distances. It is thus unlikely that the observed effects resulted from random fluctuations of the frequency tuning, which was generally fairly reproducible.

The factor that best accounts for the presence or the absence of effects was the CF distance between the TRN neurons at the ejection site and the MG recorded cell. When there was a match between the two CFs (CF distance $<0.25$ octave), the probability of observing an effect was high (68%: 19/28 MGv...
**FIG. 7.** Relationships between the tuning curves obtained in the TRN and in the MG. A: the tuning curves were much broader in auditory TRN than in MG. B: there was a bimodal distribution for the amount of overlap between the tuning curves obtained in the 2 structures. C: for the 20 MG cells exhibiting significant effects (black symbols), there was a significant relation \((r = 0.73; p = 0.0016)\) between the CF of MG cells and the CF of TRN neurons. No such relation \((r = 0.15; p = 0.21)\) was found for the whole population of 66 cells recorded during potent control of TRN activity. In this scattergram, the circles correspond to recordings obtained in the ventral MG, the squares to recordings recorded during potent control of TRN activity. In this scattergram, the circles correspond to recordings obtained in the ventral MG, the squares to recordings recorded during potent control of TRN activity. In this scattergram, the circles correspond to recordings obtained in the ventral MG, the squares to recordings recorded during potent control of TRN activity.

Finally, it is unlikely that the results were biased by urethan anesthesia. First, unlike other anesthetics, urethan does not predominantly target the GABAergic system; it modestly affects multiple neurotransmitter systems at anesthetic concentrations (Hara and Harris 2002). Second, urethan acts on intrinsic membrane properties and produces minimal disruption of GABA-mediated synaptic transmission (at least in the neocortex) (Sceniak and Maciver 2006).

**EFFECTS OBSERVED AT THE TRN EJECTION SITE.** GABA and glutamate were not similarly effective in changing the activity of TRN cells. Whereas GABA induced almost exclusively inhibitions, glutamate induced as many excitations as inhibitions at the ejection sites.

The few cases (6%) of excitatory effects observed with GABA applications might rely on differential consequences of GABA applications at the somatic and at the dendritic level, as it was reported in the cortex (Gulledge and Stuart 2003; Scharfman and Sarvey 1987).

Two mechanisms can mediate glutamate-induced decrease of firing rate in the TRN. First, by acting on group II metabotropic receptors, glutamate can increase K+ conductance and produce hyperpolarizations (Cox and Sherman 1999). Second, intra-reticular mutual inhibitions can also promote decreases in spontaneous and evoked activity. For example, glutamate application on perigeniculate nucleus (PGN) cells created an inhibitory postsynaptic potential (IPSP) in cells recorded at a distance of 50–250 \(\mu\)m (Sanchez-Vives et al. 1997), potentially by reciprocal inhibitory connections within PGN. In recent studies, activation of TRN cells by photostimulation triggered GABA\(_{\text{A}}\) inhibitory postsynaptic currents (IPSCs) for a large proportion of the investigated TRN cells (≈60%) (Deleuze and Huguenard 2006; Lam et al. 2006). Axonal collaterals are absent in the TRN of adult rats, but reciprocal inhibitions can be mediated by dendrodendritic connections (Pinault et al. 1997). In our experiments, as the recording electrode was located 20–50 \(\mu\)m from the tip of the iontophoretic electrode, mutual inhibitions have potentially contributed to the inhibitory effects of glutamate. Depending on the distance between the iontophoretic pipette and the recording electrode and on the spread of glutamate diffusion, excitations or inhibitions were expressed at the TRN recording site.

**TABLE 1.** Summary of the effects in the different anatomical divisions of the medial geniculate nucleus.

<table>
<thead>
<tr>
<th></th>
<th>Significant Effects in TRN</th>
<th>Significant Effects in MG</th>
<th>Significant Effects When the CFs Matched in TRN and MG</th>
</tr>
</thead>
<tbody>
<tr>
<td>MG ventral</td>
<td>58</td>
<td>19/58 (33%)</td>
<td>19/28 (68%)</td>
</tr>
<tr>
<td>MG dorsal</td>
<td>3</td>
<td>0/3 (0%)</td>
<td>0/0 (0%)</td>
</tr>
<tr>
<td>MG medial</td>
<td>5</td>
<td>1/5 (20%)</td>
<td>1/2 (50%)</td>
</tr>
<tr>
<td>Total</td>
<td>66</td>
<td>20/66 (30%)</td>
<td>20/30 (66%)</td>
</tr>
</tbody>
</table>

From an initial population of 134 medial geniculate (MG) cells, 38 cells were first discarded because of unstable frequency tuning curves (TCs) or of obvious EEG changes. Then 30 cells were discarded because they were tested while drugs produced unreliable effects at the thalamic reticular neuron (TRN) ejection site. Results are thus from 66 TRN-MG recording pairs tested while drug application produced significant changes in TRN evoked activity. Values in parentheses as percentages.
Tonotopic organization of the TRN-MG connections

There is general agreement to consider that the connections between TRN and thalamocortical neurons obey to topographic rules. Indeed, there is evidence for topographic projections from primary cortical areas onto the modality-related sector of TRN (Crabtree 1992a,b; Crabtree and Killakey 1989; Kimura et al. 2005). There is also anatomical evidence for a coarse organization of the thalamo-reticular and reticulo-thalamic projections because within a given sensory system, the different thalamic nuclei project to and receive from different TRN subsectors (Coleman and Mitrofanis 1996; Crabtree 1996, 1998; Pinault et al. 1995a,b). In a recent in vitro electrophysiological study, laser photostimulation of reticular neurons allowed a precise determination of the TRN area (the “footprint”) providing inhibitory inputs onto a given thalamic cell (Lam and Sherman 2005). Even if the size of the reticular footprint varied with the laser power, on average it was quite small (310 × 117 μm), and there was a spatial relation between the position of the footprint and the position of the thalamic cell, suggesting a topographic projection from the TRN to the VP nucleus. However, the retinotopic, somatotopic or tonotopic nature of the reticulo-thalamic connections remains to be experimentally demonstrated because no in vivo study has ever performed a functional characterization of the projecting and receiving sites.

In the auditory modality, the available data come from three anatomical studies (Conley et al. 1991; Crabtree 1998; Rouiller et al. 1985). They showed that the auditory TRN has organized connections with all MG divisions but with a species-dependent organization. In bushbaby, the auditory TRN is formed by a core, which receives from and projects to MGv, surrounded by a U-shaped belt, which receives from and projects to MGd and MGm (Conley et al. 1991). In cat, different subsectors of the auditory TRN project to different MG divisions, but some of these subsectors project to two MG divisions (for example, there is a subsector projecting both to the lateral part of MGv and to the MGd) (Crabtree 1998). Thus the fine grain organization of the TRN projections is yet undefined; in particular, unknown is whether in MGv the reticulo-thalamic projections respect the tonotopic gradient formed by the inferior colliculus inputs.

The present study brings indirect evidence suggesting that the reticulo-thalamic connectivity follows tonotopic rules. Despite the lack of anatomical demonstration, the fact that a MG cell exhibited functional changes during drug ejection in TRN argues for a connection between this cell and TRN neurons at the ejection site. Thus by showing that drug-induced changes in TRN activity affected MGv cells if, and only if, there was a small octave distance between the CF of the MGv cell and the CF of the TRN ejection site, our results support the view that tonotopic connections underlie the functional interactions between auditory TRN neurons and MGv neurons. This conclusion applies to the ventral, tonotopically organized, division; for the other divisions, given the small number of recorded cells (3 in MGd and 5 in MGm with only 2 CF matches), it is not possible to evaluate whether the CF distance is a critical factor.

The probability of observing an effect in MGv was high when there was a match between the CFs (19/28 cells, 68%). However, this condition was not sufficient. In nine cases, despite a good CF match, modifying TRN activity did not change the responses of the MGv cell. Several reasons can account for this. First, as the auditory TRN projects to all MG divisions (Crabtree 1998), a lack of effect can simply be due to the fact that the TRN neurons at the ejection site did not project to MGv but to MGm or to MGd. Second, even if these TRN neurons projected to MGv within the frequency band corresponding to the CF of the recorded cell, their terminal zones can reach other cells than the recorded one. Indeed, data from the somatosensory thalamus show that TRN axonal projections display a well-focused terminal field (Pinault and Deschênes 1998). Last, the auditory thalamus of rodent has <1% of local interneurons (Winer and Larue 1988, 1996), but the inhibitory inputs coming from the inferior colliculus (Peruzzi et al. 1997; Saint Marie et al. 1997; Winer et al. 1996) can potentially shape the functional properties of MG neurons. More precisely, it is possible that for some MGv cells, the balance between the excitatory and inhibitory inputs coming from the inferior colliculus exerts a major control on the breadth of tuning and threshold. In these cases, controlling the activity of TRN neurons by iontophoretic applications should not affect the tuning and threshold of MGv neurons.

Impact of TRN activity on the receptive fields of MG cells

For a particular thalamic relay cell, TRN neurons can potentially have two distinct functional roles. First, by feedback inhibition, they can control the cell’s excitability. Second, by lateral inhibition, they can control the cell’s receptive field properties (for reviews, see Pinault 2004; Sherman and Guillery 1996).

Apart from the initial observations by Shosaku and Sumimoto (1983) showing that TRN stimulation suppressed MG activity, only two experiments have assessed the consequences of activation/inhibition of TRN neurons on sensory thalamic neurons. First, using glutamate ejections in the TRN, Warren and Jones (1994) found no dominant effects on the responses of VP neurons to cutaneous stimuli (decrease 29%, increase 24%). The largest effect observed was a reduction of the ON and OFF postinhibition excitation, suggesting a control on the excitability cycle of the cells. The topographic relationships between the ejection and recording sites were not investigated. Second, after kainic acid lesions of the somatosensory sector of the TRN, Lee and colleagues (1994a) reported a two- to fivefold expansion of the receptive fields of VP neurons. This expansion was detected from 2 h ≤ 1 mo after the TRN lesions, and it was accompanied by an increase of the average response latency to the surround receptive field whiskers, probably resulting from an increased number of surround whiskers triggering responses at longer latencies than the principal whisker. The topographic relationships between the recording site and the lesioned site were not investigated since large parts of the somatosensory TRN were damaged.

Generally, the role of inhibitory inputs arriving onto MG cells is discussed either in terms of a control of neuronal excitability (e.g., Xiong et al. 2004; Yu et al. 2004) or in terms of sharpening of frequency tuning by lateral inhibitions (e.g., Suga 1995; Suga et al. 1997). The present results suggest that the inhibitory inputs arising from the TRN can control both the excitability and the receptive field size of MG cells. For 20 MGv cells the CFs of which matched the ones at the ejection...
sites, changing TRN activity modulated the strength of evoked responses and the acoustic threshold, two effects that likely reflect a change in neuronal excitability. For the same 20 cells, changing TRN activity also modulated the breadth of tuning. This effect was accompanied by a change (an increase or a decrease) in response threshold, indicating that the entire TC was shifted upward or downward. These results thus differ from the predictions based on the classical lateral inhibition concept. They rather suggest that, as demonstrated at the cortical level (Wehr and Zador 2003), the inhibitory input coming from TRN is tuned similarly to the excitatory input. Depending on the level of TRN activity, subthreshold acoustic inputs would trigger postsynaptic responses in MG cells, thus decreasing the threshold and increasing the tuning breadth, or suprathreshold inputs would become inefficacious, thus increasing the threshold and decreasing the tuning breadth.

**Functional implications**

Several recent experiments have suggested that the activity of TRN neurons can be modulated during behavioral tasks and that these changes in activity influence the animal’s performance. In monkey and rodent, shifting the animal’s attention from one sensory modality to another increased the neuronal activity in the TRN sector associated with the attended stimulus (McAlonan et al. 2000, 2006). Conversely, during an attentional task involving valid and invalid cues, unilateral TRN lesions abolished the processing advantage conferred by cues contralateral to the lesion (Weese et al. 1999). These results suggest that, as suspected by earlier studies ( Skinner and Yingling 1976; Yingling and Skinner 1976), the TRN may be an important component in the process of stimulus selection during attentional tasks. That this process involves modifications of the functional properties of thalamic relay cells remains to be demonstrated, but this is most likely given that TRN neurons exclusively project to their thalamic partners. Thus it is an important challenge for future studies to characterize how TRN activity controls the receptive field properties of thalamic cells in awake animals and how attentional tasks conjointly modulate the receptive fields of reticular and thalamic neurons.

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