Auditory Thalamus Bursts in Anesthetized and Non-Anesthetized States: Contribution to Functional Properties

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Massaux, A., G. Dutrieux, N. Cotillon-Williams, Y. Manunta, and J.-M. Edeline. Auditory thalamus bursts in anesthetized and non-anesthetized states: contribution to functional properties. J Neurophysiol 91: 2117–2134, 2004. First published January 14, 2004; 10.1152/jn.00970.2003. Over the last 10 years, high-frequency bursts of action potentials have been the subject of intense researches to understand their potential role in information encoding. Based on recordings from auditory thalamus neurons (n = 302) collected during anesthesia (pentobarbital, urethan, or ketamine/xylazine), waking (W), and slow-wave sleep (SWS), we investigated how bursts participate to frequency tuning, intensity-function, response latency (and latency variability), and stimulus detectability. Although present in all experimental conditions, bursts never dominated the cells mode of discharge: the highest proportion was found during ketamine/xylazine anesthesia (22%), the lowest during waking (4.5%). In all experimental conditions, bursts preferentially occurred at or around the cells best frequency (BF), thus increasing the frequency selectivity. This effect was observed at both the intensities producing the highest and the lowest evoked responses. Testing the intensity-functions indicated that for most of the cells, there was no systematic relationship between burst proportion and responses strength. Under several conditions (W, SWS, and urethan), when cells exhibited bursts >20%, the variability of their response latency was reduced in burst mode compared with single-spike mode. During W, this effect was accompanied by a reduction of the response latency. Finally, a receiver operating characteristic analysis indicated no particular relation between bursts and stimulus detectability. Compared with single-spike mode, which is present for broader frequency ranges, the prominence of bursts at the BF should contribute to filter information reaching the targets of medial geniculate cells at both cortical and subcortical levels.

INTRODUCTION

In various brain regions, neurons have the property to produce bursts, i.e., clusters of action potentials emitted at high frequency. Since the last decade, investigations have been carried out to elucidate the role of these bursts in integrative functions. For instance, in hippocampus, bursts increase the reliability of synaptic transmission compared with single action potentials (APs) and promote long-term synaptic modifications (see for review, see Lisman 1997). In sensory systems, several functional roles of bursts have also been envisioned (Krahe and Gabbiani 2004). At the cortical level, recordings obtained in awake monkeys indicate that bursts convey more information about the stimulus direction of motion than do single APs if bursts are considered to be individual events (Bair et al. 1994).

At the thalamic level, the most appealing hypothesis has been formulated by Sherman (2001b): thalamic bursts could act as “wake-up call” for cortical cells (but see Steriade 2001 for comments). Supporting this view, cross-correlation techniques revealed that the probability that cortical cells spontaneously fire an AP is higher after thalamic bursts than after single APs (Swadlow and Gusev 2001).

In contrast with cortical bursts, which display a large range of frequencies and patterns (for review, see Nowak et al. 2003), thalamic bursts are highly stereotyped due to the presence of an ubiquitous low-threshold calcium current, namely the \( I_{\text{CL}} \) current (Crunelli et al. 1989; Llinas and Jahnsen 1982). In vivo thalamic bursts have been particularly described in the later geniculate nucleus of anesthetized and unanesthetized animals (see for review Sherman 2001a). Criteria established during intracellular experiments were subsequently applied to detect bursts in extracellular experiments during which more functional aspects were investigated. Among the main findings of these studies, it appeared that burst responses better support signal detection than tonic responses (Guido et al. 1995), decrease the latency variability (Guido and Sherman 1998), introduce a nonlinearity in the visual responses (Guido et al. 1992), transform low-pass temporal tuning in band-pass temporal tuning (Mukherjee and Kaplan 1995), and are associated more closely than lone spikes with preceding microsaccades (Martinez-Conde et al. 2002).

So far, the functional role of bursts has not yet been investigated in the medial geniculate (MG) nucleus. In vitro studies have documented the presence in MG neurons of appropriate ionic conductances to fire stereotyped bursts (Bartlett and Smith 1999; Hu 1995; Tenuigiet et al. 1996), which were subsequently detected in anesthetized guinea pigs (He and Hu 2002). In a first approach to compare the burstiness of MG neurons between drugged and undrugged animals, we recently reported that the burst proportion was much higher under pentobarbital anesthesia than during waking (W) or slow-wave sleep (SWS) (Massaux and Edeline 2003).

In the present study, we will first provide a comparison of the burst proportions between three anesthetics and two undrugged states (W and SWS). Then we will describe the burst contribution to frequency selectivity and intensity function of MG cells in the different experimental conditions. The incidence of bursts on the response latency and its variability will also be evaluated. Finally, we will investigate the possibility that the proportions of bursts within the spike train influence
the signal detectability. So far, only one study performed in the visual thalamus has reported that bursts promote a better stimulus detection than single APs (Guido et al. 1995). To determine if this is also the case in the auditory thalamus, we constructed the receiver operating characteristic (ROC) curves for responses to constant auditory stimuli.

METHODS

All procedures were performed in conformity with national (J0887-848) and European (86/609/EEC) legislations on animal experimentation, which are similar to those described in the Guidelines for the Use of Animals in Neuroscience Research of the Society for Neuroscience.

Animal preparation

CHRONIC RECORDINGS. For 18 male guinea pigs (380–450 g), microelectrodes were implanted during an initial surgery performed under anesthesia (atropine sulfate: 0.08 mg/kg, diazepam: 8 mg/kg, pentobarbital sodium: 20 mg/kg) (see Evans 1979). All injections were intraperitoneal. Three silver-ball electrodes (400 μm) were placed between bone and dura: one served as reference during the recording sessions, the other two were set over the frontal and parietal cortices to record a cortical electroencephalogram (EEG). Two silver wires were inserted into the dorsal neck muscles to record the electromyogram (EMG). An array of five tungsten microelectrodes (1 MΩ at 1 kHz, spaced 200–300 μm rostrocaudally) was implanted in the MG nucleus: the electrodes were first lowered until a depth of 5 mm below pia, then they were advanced by steps of 100 μm while pure tone frequencies (100 ms, 70 dB, 0.1–10 kHz) were analyzed.

To SWS. No animal was sleep-deprived before the recording sessions. The signals from each electrode were checked daily.

ACUTE RECORDINGS. Recordings obtained under general anesthesia were from both adult Hartley guinea pigs and adult Sprague-Dawley rats.

For guinea pigs (n = 6; 400–450 g), the recording sessions took place under pentobarbital anesthesia [20 mg/kg with atropine sulfate (0.08 mg/kg) and diazepam (8 mg/kg)] (see Evans 1979). For rats, the recording sessions took place under pentobarbital anesthesia (n = 11; 300–400 g; 60 mg/kg), urethan anesthesia (n = 28; 290–450 g; 1.5 g/kg), or ketamine/xylazine anesthesia (n = 9; 300–500 g; ketamine: 120 mg/kg and xylazine: 20 mg/kg). During all the recording sessions, supplements of anesthetics were delivered to maintain an anrelexive state (pentobarbital: 4 mg · kg⁻¹ · h⁻¹; urethan: 0.5 g/kg; ketamine/xylazine: 5.4 and 0.8 mg · kg⁻¹ · h⁻¹, respectively). All injections were intraperitoneal.

After opening the scalp, a local anesthetic (xylocaine 2%) was injected in the wound. A hole was drilled in the skull above the MG nucleus, and single units were recorded using tungsten microelectrodes (5–10 MΩ) or glass micropipettes (5–15 MΩ). The recording session lasted from 7 to 10 h.

Experimental procedures

For undrugged animals, the EEG, HEG, and EMG were continuously monitored on a polygraph (band-pass 1–90 Hz, Grass, model 7P511). After each recording session, the polygraphic recordings were examined independently by at least two investigators. Only data obtained in an unambiguous state of vigilance (W or SWS) were analyzed.

The signal coming from the electrodes was amplified (band-pass: 0.6–10 kHz, gain: 5,000) and sent in parallel to an audio monitor and to a voltage window discriminator (Frederic Haer, model 74-60-1). As no waveform sorting system was used, only one clear single-unit waveform was isolated from a given electrode (signal/noise ratio ≥5:1) and continuously displayed on the screen of a digital oscilloscope with the corresponding TTL pulses generated by the discriminator. These TTL pulses were sent to the acquisition board (Pclab, PCL 720) of a Pentium II computer, their time of occurrence was known with a 50-μs resolution. Single-unit waveforms were digitized (Superscope, GW Instruments, 50-KHz sampling rate) and were constantly displayed on a computer screen. The data collection was stopped when the waveform was unstable or ambiguous. The stability of the waveform was also checked off-line, and the data were not further analyzed when the quality did not guarantee that only one cell was recorded. Examples of collected waveforms and of raw traces are presented in Fig. 3 and in Fig. 10A.

<table>
<thead>
<tr>
<th>Experimental Condition</th>
<th>Spontaneous, Spike/s</th>
<th>Mean, Spike/s</th>
<th>Best Frequency, Spike/s</th>
<th>First Spike Latency, ms</th>
<th>Variability of First Spike Latency, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>W guinea pigs (n = 100)</td>
<td>6.1 ± 5.6</td>
<td>17.4 ± 17.2</td>
<td>35.8 ± 29.6</td>
<td>26.8 ± 10.4</td>
<td>20.0 ± 7.0</td>
</tr>
<tr>
<td>SWS guinea pigs (n = 100)</td>
<td>4.2 ± 4.9</td>
<td>10.3 ± 11.7</td>
<td>22.3 ± 21.5</td>
<td>28.7 ± 11.7</td>
<td>20.5 ± 8.5</td>
</tr>
<tr>
<td>Urethan rats (n = 62)</td>
<td>2.1 ± 4.5</td>
<td>14.7 ± 11.6</td>
<td>40.4 ± 28.7</td>
<td>29.6 ± 19.5</td>
<td>13.0 ± 8.9</td>
</tr>
<tr>
<td>Pentobarbital</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guinea pigs (n = 39)</td>
<td>3.4 ± 3.8</td>
<td>14.6 ± 11.1</td>
<td>35.2 ± 26.1</td>
<td>34.0 ± 19.3</td>
<td>18.1 ± 9.8</td>
</tr>
<tr>
<td>Rats (n = 57)</td>
<td>3.7 ± 9.3</td>
<td>14.5 ± 12.9</td>
<td>34.6 ± 23.3</td>
<td>30.9 ± 13.6</td>
<td>16.0 ± 10.5</td>
</tr>
<tr>
<td>Ketamine/Xylazine rats (n = 44)</td>
<td>3.6 ± 3.1</td>
<td>15.9 ± 16.0</td>
<td>34.3 ± 29.5</td>
<td>30.2 ± 12.0</td>
<td>18.6 ± 9.6</td>
</tr>
</tbody>
</table>

Values are means ± SD. Ranges are in parentheses. W, waking; SWS, slow-wave sleep.
FIG. 1. Effects of changing the criteria for burst detection on the proportion of detected bursts. For 20 cells coming from all experimental conditions [n = 4 in waking, slow-wave sleep (SWS), pentobarbital, urethan, and ketamine] and having different rates of spontaneous activity, we evaluated the impact of changing 1 of the criteria while keeping the others at a constant value. Statistical comparisons were made with ANOVA and post hoc tests (Scheffe) when necessary. A: decreasing the interspike interval (ISI) to 3 ms led to lower proportion of bursts (P < 0.01 both in spontaneous and evoked activity), but there was no significant difference between the proportion of bursts detected with an ISI of 5, 7, or 10 ms (preburst silence period was 100 ms and duration of burst last ISI was 10 ms). The effects were similar in each condition (waking, SWS and anesthesia). No burst was detected with an ISI ≥ 2 ms. B: on average (B1), increasing the silence period before burst detection did not change significantly the proportion of detected bursts (lowest P value = 0.11), even between a 0- and a 200-ms delay (ISI was 5 ms and duration of burst last ISI was 10 ms). However, examination of data from each condition revealed that during waking the proportion of detected bursts was significantly decreased when a silence period was introduced (see B, 2 and 3). C: changing the duration of the last ISI within the bursts never changed the proportion of detected bursts (lowest P value = 0.12), even between a 5- and a 16-ms ISI (ISI was 5 ms and preburst silence period was 100 ms).
In addition to these careful on- and off-line verifications, an analysis performed on a subset of 20 cells indicated that there was no interspike-intervals ≤2 ms, thus indicating that our detected bursts were not the result of the co-firing of adjacent neurons (see the results of our parametric analysis).

The sound-delivery system has been previously described (Edeline et al. 2000). Pure tone frequencies were generated by a remotely controlled wave analyzer (Hewlett-Packard, model HP 8903B) and attenuated by a passive programmable attenuator (Wavetek, P557, maximal attenuation: 127 dB); both controlled by a computer via an IEEE bus. An earphone (Beyer DT-48) was placed close to the ear canal of adjacent neurons (see the results of our parametric analysis).

For each single unit, the spontaneous firing rate was quantified as the discharge rate during the 100 ms preceding each tone. The mean evoked response (mean) was defined as the discharge rate averaged across the eleven frequencies used to determine the frequency tuning curve at a given intensity.

Bursts were quantified during both spontaneous and evoked activity. Spontaneous bursts were quantified during a period beginning 100 ms after tone offset and lasting up to the end of the inter-tone interval (800 ms × 11 frequencies × 10 repetitions = 88 s). For 28 cells, we verified that quantifications of firing rate and of burst proportion during the inter-tone interval was equivalent to those obtained during a continuous silence period of 120 s. For on responses, evoked bursts were quantified over the 100 ms of tone duration and for off responses (n = 18/302 recordings) during the 100 ms after tone offset. In both cases, these periods extended over 11 s (100 ms × 11 frequencies × 10 repetitions).

During off-line analyses, bursts were isolated from single APs on

### TABLE 2. Burst internal structure

<table>
<thead>
<tr>
<th>Experimental Condition</th>
<th>Duration, ms</th>
<th>Internal Frequency, Hz</th>
<th>Percent Bursts Composed of 2APs</th>
<th>Percent Bursts Composed of 3APs</th>
<th>Percent Bursts Composed of 4APs</th>
<th>Percent Bursts Composed of &gt;5APs</th>
</tr>
</thead>
<tbody>
<tr>
<td>W guinea pigs (n=100)</td>
<td>3.9 ± 1.7</td>
<td>348 ± 86</td>
<td>79 ± 28</td>
<td>18 ± 26</td>
<td>2 ± 6</td>
<td>1 ± 2</td>
</tr>
<tr>
<td>SWS guinea pigs (n=100)</td>
<td>4.2 ± 1.5</td>
<td>358 ± 64</td>
<td>66 ± 30</td>
<td>25 ± 25</td>
<td>6 ± 8</td>
<td>2 ± 5</td>
</tr>
<tr>
<td>Urethan rats (n=62)</td>
<td>3.9 ± 1.8</td>
<td>342 ± 88</td>
<td>82 ± 28</td>
<td>11 ± 17</td>
<td>7 ± 20</td>
<td>0 ± 1</td>
</tr>
<tr>
<td>Pentobarbital guinea pigs (n=39)</td>
<td>4.5 ± 2.1</td>
<td>328 ± 66</td>
<td>72 ± 23</td>
<td>21 ± 16</td>
<td>4 ± 6</td>
<td>3 ± 8</td>
</tr>
<tr>
<td>Pentobarbital rats (n=57)</td>
<td>4.3 ± 1.8</td>
<td>363 ± 54</td>
<td>65 ± 28</td>
<td>22 ± 17</td>
<td>8 ± 12</td>
<td>4 ± 12</td>
</tr>
<tr>
<td>Ketamine/Xylazine rats (n=44)</td>
<td>4.9 ± 1.8</td>
<td>328 ± 59</td>
<td>62 ± 31</td>
<td>23 ± 19</td>
<td>11 ± 15</td>
<td>4 ± 7</td>
</tr>
</tbody>
</table>

### A. Spontaneous activity

| W guinea pigs (n=100)  | 4.4 ± 2.4   | 350 ± 107              | 72 ± 33                         | 22 ± 29                         | 6 ± 14                          | 1 ± 4                           |
| SWS guinea pigs (n=100)| 4.3 ± 2.2   | 360 ± 78               | 67 ± 29                         | 22 ± 25                         | 8 ± 16                          | 3 ± 8                           |
| Urethan rats (n=62)    | 3.8 ± 1.7   | 366 ± 98               | 78 ± 30                         | 20 ± 25                         | 4 ± 11                          | 0 ± 2                           |
| Pentobarbital guinea pigs (n=39)| 4.2 ± 2.0 | 354 ± 66               | 70 ± 28                         | 22 ± 23                         | 5 ± 8                           | 4 ± 11                          |
| Pentobarbital rats (n=57)| 5.0 ± 2.2 | 345 ± 78               | 59 ± 35                         | 22 ± 23                         | 11 ± 14                         | 8 ± 17                          |
| Ketamine/Xylazine rats (n=44)| 4.7 ± 1.4 | 336 ± 56               | 61 ± 32                         | 26 ± 25                         | 10 ± 19                         | 4 ± 10                          |

### B. Evoked activity

Values are means ± SD.
the basis of criteria established during in vivo intracellular recordings of thalamic relay cells (Lu et al. 1992). Groups of two or more APs were defined as being part of a burst only when the interspike interval (ISI) was <5 ms and when there was a preceding silent period of ≥100 ms. Such criteria have been applied to extracellular recordings in anesthetized or awake animals (Guido and Weyand 1995; Guido et al. 1992; Ramcharan et al. 2000; Swadlow and Gusey 2001 Swadlow et al. 2002). As previously noted (McCarley et al. 1983), on-line observations led us to consider, that when there was more than two APs in a burst, the last AP could be occasionally separated from the preceding one by ≤10 ms. A first step to extract bursts was to apply the ISI criteria: the second step was to apply the 100-ms silent period criterion. So groups of adjacent APs found in a 100-ms evoked activity period were defined as bursts when a period of 100-ms silence was detected before the beginning of the evoked activity. For each recording, the number of single APs, the number of bursts, and their characteristics (duration, internal frequency, number of APs per burst) were determined during spontaneous and evoked activities. For each cell, the spike train is composed of both burst events and of single APs events: the sum of all these events was considered as a proportion of 100%. The burst proportion—as events contributing to the spike train—was calculated as follows: [(number of bursts)/(number of bursts + number of single APs)] × 100.

The single APs proportion was simply the rest of the events: 100% – the burst proportion.

The original spike trains were split in two components: a spike train composed only of single APs and a spike train composed only of bursts. The frequency tuning curve (or the intensity function) of each cell was reconstructed using either the single APs component or the burst component. Based on the tuning curves, we calculated, for each cell, the relative weight of bursts in the mean evoked response and in the response obtained at the best frequency (BF; i.e., at the frequency providing the strongest excitatory drive). Based on the intensity functions, we calculated, for each cell, the relative weight of bursts in the response evoked at each tested intensity. Subsequently, we will refer to the relative weight of bursts as “the burst contribution.” The sharpness of the frequency tuning was assessed by a selectivity index: [response at the BF – mean evoked response]/response at the BF] × 100.

This index was computed both with the single APs component and with the burst component. With this index, a value approaching 100 means that the cell responds only at the BF; a value equal to zero means that the cell responds equally to the different frequencies.

The first spike latency and its variability (quantified by the SD of the mean value) were calculated over the 110 tone presentations (11 frequencies × 10 repetitions). The first spike latency and its variability were computed separately for the single APs spike train and for the burst spike train.

ANOVAs were used to assess if there was an effect of the experimental condition. Then if so, paired t-test were used to compare the variables obtained in W with those obtained in SWS; and unpaired t-test were used to compare the variables obtained under a given anesthetic with those obtained during W and SWS or under another anesthetic. The significance level was P ≤ 0.05.

ROC analysis

ROC curves for responses to constant auditory stimuli were built for 50 cells to assess whether the burst proportion was related to the detectability performance of MG neurons. Details concerning the ROC methodology can be found in Green and Swets (1966), Cohn et al. (1975), Macmillan and Creelman (1991), and Guido et al. (1995). ROC curves were derived from probability distributions of spike density counts obtained during a sampling period of evoked activity ($\tau_e$) and of spontaneous activity ($\tau_s$). Based on 110 repetitions of the same stimulus (a 100-ms pure tone or a 1-ms click), we determined the probability than a neuron fire a criterion number of spikes for $\tau_e$ and $\tau_s$. The ROC curve was built by plotting for all criterion levels $P(\text{false alarm})$ on the abscissa versus $P(\text{hit})$ on the ordinate: $P(\text{false alarm})$ is the probability of attaining a criterion response during $\tau_e$, and $P(\text{hit})$ is the probability of attaining a criterion response during $\tau_s$. The values of the periods $\tau_e$ and $\tau_s$ were either 25, 50, or 100 ms depending on the duration of evoked responses. ROC curves were plotted against a line of unity slope, which represents the locus of points of equal probabilities for $\tau_e$ and $\tau_s$. As classically used, analyses of ROC curves involved computing the area under the ROC curve, which provides a distribution-free estimate of proportion correct for an ideal observer in a two-alternative forced-choice procedure. There is a monotonic, but nonlinear, relationship between ROC area and signal detectability (Green and Swets 1966; Macmillan and Creelman 1991). In practice, the area under the ROC curve varies between 0.5 and 1.0: an area of 0.5 represents a signal detection not better than noise (as delimited by the line of unity slope).

For a subset of cells which exhibited a similar proportion of responses in single APs mode and in burst mode, a within-cell comparison was made between the ROC areas obtained from the single APs spike train and the burst spike train. The classification of the trials in single APs mode and burst mode was based on the type of response obtained during the sampling period of evoked activity ($\tau_s$). Trials where no spontaneous and no evoked activity was observed were not discarded.

Histology

At the end of the experiment, the animals received a lethal dose of pentobarbital (200 mg/kg). In the case of the chronic recordings, the animals were perfused intracardially with 0.9% saline (200 ml) followed by 2,000 ml of fixative solution (4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4). In the case of the acute recordings, the brains were removed from the skull and placed in the fixative solution for 2 weeks. In both cases, the brains were subsequently placed in a 30% sucrose solution for 3–4 days, then coronal sections were cut on a freezing microtome (50-μm thickness) and counterstained with cresyl violet. The analysis of histological material was always done “blind” of the electrophysiological results. The sections were examined under several microscopic magnifications to find the electrode penetration tracks.

In the case of chronic recordings, the recording sites were found unambiguously at the bottom of the electrode tracks, which were easy to follow because of the glial reaction around the electrodes (see Fig. 104A).

In the case of acute recordings, several attempts were made to determine the location of the recorded cells within the MG nucleus. First, for 62 cells, the pipette was filled with Pontamine sky blue that

<table>
<thead>
<tr>
<th>TABLE 3. Percentages of cells that exhibited no bursts and a burst proportion &gt;40%</th>
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</thead>
<tbody>
<tr>
<td>Experimental Condition</td>
</tr>
<tr>
<td>-------------------------</td>
</tr>
<tr>
<td>W guinea pigs (n = 100)</td>
</tr>
<tr>
<td>SWS guinea pigs (n = 100)</td>
</tr>
<tr>
<td>urethan rats (n = 62)</td>
</tr>
<tr>
<td>Pentobarbital guinea pigs (n = 39)</td>
</tr>
<tr>
<td>Pentobarbital rats (n = 57)</td>
</tr>
<tr>
<td>Ketamine/Xylazine rats (n = 44)</td>
</tr>
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</table>
was ejected after the last recording. However, the method was not satisfactory because the blue spot was often too small relative to the electrode track. In some cases, a blue line was observed but its curved shape did not help to localize the recording sites. Second, doing a systematic mapping with the same tungsten electrode (to keep track of the electrode coordinates) was not possible because after one or two penetrations, the electrode impedance always felt <1 MΩ (as measured in vivo during the experiment) and single units could not be isolated in these conditions. Last, we have tried to locate the recording sites using the electrode tracks for guidance, the point of entrance in
the thalamus, the estimated dorsoventral extent of the MG, and the depth coordinate read from the microdrive during the experiment. However, this analysis was not precise enough to allow clear assignment of the recordings with regard to the MG divisions.

RESULTS

Data presented here are from a total of 302 single units recorded in the MG nucleus of guinea pigs engaged in periods of W and SWS \((n = 100)\), rats under urethan anesthesia \((n = 62)\), guinea pigs and rats under pentobarbital anesthesia \((n = 39\) and \(n = 57\), respectively), and of rats under ketamine/xylazine anesthesia \((n = 44)\).

Both in drugged and undrugged animals, the analyses reported in the following text will focus on the data obtained at the intensity eliciting the strongest evoked response, but data obtained at the other intensities were also analyzed. For all the variables described in the results, no differences were detected between guinea pigs and rats anesthetized with pentobarbital \((P > 0.08\) in all cases; unpaired \(t\)-test). This suggests that no major distinctions exist in the bursting activity of MG cells for these two species and that comparisons can be made across the different experimental conditions.

Table 1 presents the mean values of spontaneous and evoked firing rates obtained under the various conditions, as well as the

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**FIG. 3.** Examples of frequency tunings collected in each experimental condition. A1–E1: the histograms (bin: 5 ms) and rasters display the evoked response during 10 repetitions of a sequence of 11 ascending frequencies for cells recorded in each experimental condition. These responses were obtained at the intensity providing the strongest excitatory drive. The short bars below the abscissa denote the 100 ms of tone duration, and only the 100 ms immediately preceding each tone are presented. The numbers below the short bars are the values of the frequencies in kHz. Circles and dotted lines represent the burst contribution (in spikes/s) to the response evoked over the 100 ms of each tone. Although the tuning curves derived from the burst contribution could notably differ from the total tuning curve, note that for each example, the highest burst contribution occurs at the frequency eliciting the strongest evoked response. **Insets:** the waveform of the recorded single units (30 sweeps, scale bars: 500 ms, 300 μV). A2–E2: averaged spontaneous and evoked firing rates corresponding to the cells depicted on the left side. The spontaneous firing rate (Spon) was quantified during the 100 ms preceding each tone, and the mean evoked response (Mean) was quantified across the 11 frequencies used to determine the tuning. Numbers, in or above the bars, represent the burst proportion during spontaneous and evoked activity.

**FIG. 4.** Examples of bursts and single action potentials (APs) contributions to frequency tuning. For these 6 cells, the frequency tuning was reconstructed using the total evoked response (●), the burst component of the response (○), and the single APs component of the response (● or ○). The burst contribution to the responses (mean value) is indicated by the number in the top right corner: cells displayed in A had a high burst contribution, cells in B a medium one, and cells in C a low one. Note that, in all but 1 case (B2), the best frequency derived from the bursts matched the one obtained from the total response (indicated by the dashed line).

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**TABLE 1.** Mean values of spontaneous and evoked firing rates obtained under the various conditions, as well as the
mean values for the latency and the latency variability. The highest spontaneous firing rate was found during W (all $P < 0.04$). The mean evoked response and the response at the BF were similar between W and anesthetics (mean: all $P > 0.26$; BF: all $P > 0.33$); these responses were the lowest during SWS (compared with W: mean and BF $P < 0.0001$; compared with anesthetics: mean all $P < 0.05$, BF all $P < 0.007$).

### Parametric investigation of burst detection

At the time this study was conducted because no in vivo intracellular study was available to select the appropriate criteria required to detect bursts from MG cells, we first evaluated whether applying those previously used in the visual thalamus was relevant. On a subset of 20 cells—coming from all experimental conditions (4 cells from waking, SWS, pentobarbital, urethan, ketamine anesthesia) and having different levels of spontaneous firing rate—we explored to what extent burst proportion is influenced by the values of the detection parameters. Figure 1 summarizes the results of this parametric study. As shown in Fig. 1A, applying a criterion of 3 ms for the ISI led to detect fewer bursts than with a criterion of 5, 7, or 10 ms both during spontaneous and evoked activity (in all cases $P < 0.01$ and $P < 0.02$, respectively). During spontaneous activity, increasing the ISI from 5 to 7 and 10 ms non-significantly increased the proportion of detected bursts ($P = 0.18$ for 5 vs. 7 ms and $P = 0.10$ for 7 vs. 10 ms), but no changes were obtained during evoked activity. Note that no burst was detected with an ISI of 2 ms.

Both during anesthesia and SWS, changing the duration of the silence period before the detected bursts did not significantly affect their proportion (Fig. 1B). Increasing the period of silence before the burst occurrence decreased the proportion of detected bursts, but this diminution never reached significance, even between a 0- and a 200-ms delay period (spontaneous activity: $P = 0.11$; evoked activity: $P = 0.08$). In contrast, during waking, introducing a period of silence significantly decreased the amount of detected bursts ($P = 0.03$ and 0.003). Nonetheless, increasing the silence from 100 to 150 or 200 ms did not change the proportion of detected bursts ($P > 0.11$; see Fig. 1B, 2 and 3).

Last, whatever the experimental condition, there was no effect of changing the delay allowed for detection of the burst last spike (Fig. 1C): when this delay was increased from 5 up to 16 ms, the proportion of detected bursts was similar during both spontaneous ($P = 0.11$) and evoked activity ($P = 0.09$).

Thus this analysis gave us some confidence in our criteria: an ISI of 5 ms seems adequate as longer ISIs did not capture...
more bursts from the spike trains, whereas a shorter one (3 ms) probably missed some; a silence period of 100 ms seems reasonable as there was no change when the duration of this period was longer; and the value of the delay allowed to catch the last spike has no consequence on burst detection.

Proportions of bursts in the different experimental conditions

Bursts were detected in all experimental conditions both during spontaneous and evoked activities. However, single APs always dominated the cells mode of discharge: as shown in Fig. 2, bursts never represented >30% of the events present in the spike trains. The burst characteristics are displayed in Table 2. The burst duration and the burst internal frequency were homogeneous over the experimental conditions for spontaneous and evoked activities. In all experimental conditions, the large majority of bursts were composed of two and three APs.

An ANOVA revealed that there was an effect of the experimental condition for the burst proportion both during spontaneous \( [F(3,97) = 22.7; P < 0.0001] \) and evoked \( [F(3,97) = 11.9; P < 0.0001] \) activities. The proportion of spontaneous and evoked bursts increased from W to SWS to the anesthetic conditions (Fig. 2, A and B, respectively). These proportions differed significantly between W and SWS (\( P < 0.0001 \) in both cases) and also between W and the anesthetics (\( P < 0.0005 \) in

**FIG. 6.** Distributions of the distances between BF obtained with the total evoked response and BF derived from bursts. Only cells that exhibited bursts at the BF are included in these distributions. The closer the 2 BFs are, the smaller the distance (expressed in 1/16th of an octave). Whatever the experimental condition, the large majority of cells had a match between the 2 BFs: these cells correspond to the central peak. The other cells had 2 distinct but close BFs: the mismatch is often <5/16th of an octave.
all cases). The burst proportions found during SWS were similar to those obtained under urethan anesthesia ($P > 0.08$ for both spontaneous and evoked activity). The proportion of spontaneous bursts found in the ketamine/xylazine condition was higher compared with W, SWS, urethan, and pentobarbital anesthesia ($P < 0.01$ in all cases, except for rats under pentobarbital: $P = 0.09$). Similarly, the proportion of evoked bursts was higher under ketamine/xylazine (compared with W, SWS, and urethan, all $P < 0.005$; pentobarbital $P = 0.09$ for rats and $P = 0.16$ for guinea pigs).

As shown in Table 3, a non-negligible percentage of cells never exhibited bursts, whether in spontaneous or in evoked activity. The highest percentage of non-bursting cells was found under urethan anesthesia (spontaneous and evoked activity) and during W (evoked activity). In addition, cells recorded under anesthesia—particularly under ketamine/xylazine—exhibited burst proportions $>40\%$, a value never reached during W or SWS.

To evaluate if burst proportions were related with discharge rates, linear regressions were carried out in each experimental condition. Whatever the condition was, the proportions of spontaneous and evoked bursts were not function of the spontaneous or evoked firing rate (all $r < 0.40$). For example, if we consider the cells obtained in guinea pigs under pentobarbital anesthesia ($n = 39$), there was no relationship either during spontaneous ($r = 0.26$, $P = 0.11$) or during evoked activity ($r = 0.04$, $P = 0.83$).

### Contribution of bursts to frequency tuning

As explained in METHODS, each original spike train was split based on the entire spike trains without being present at all the responses. Thus there is no strict correspondence between burst contribution and strength of evoked responses across frequencies. Figure 4 shows examples of tuning curves reconstructed using the whole spike train, the burst spike train and the single APs spike train for cells exhibiting a high, a medium, or a low contribution of bursts to frequency tuning. As illustrated in these tuning curves, in many cases, the highest burst contribution occurred at the BF. To go further, we calculated for each cell the burst contribution to the mean evoked response and to the BF. Figure 5A displays these quantifications across the whole cell population, i.e., including the non-bursting cells. In each experimental condition, the burst contribution to the BF was significantly larger than the one to the mean evoked response (Fig. 5A; $P < 0.04$ in all cases). Among the cells that exhibited bursts, very few, if any, displayed no bursts at the BF (W 1% of the cells; SWS 4%; guinea pigs under pentobarbital 5.1%; rats under pentobarbital or urethan or ketamine/xylazine 0%).

As depicted from the examples presented in Figs. 3 and 4, the BF obtained with the bursts often matched the one obtained from the total response (single APs plus bursts). In the other cases, the BF obtained with the bursts occurred at adjacent frequencies (e.g., Fig. 4B2). The distance (in octave) between these two BFs was quantified in each experimental condition. The distributions of these distances are displayed in Fig. 6 for the cells that exhibited bursts at the BF. There was a match between the two BFs for at least half of these cells as indicated by the central peak of these distributions. This peak represented 50.8% of the cells during W; 49.5% during SWS; 63.4% under urethan; 54.8% and 58% under pentobarbital (guinea pigs and rats, respectively); and 65.8% under ketamine/xylazine. For the cells exhibiting a mismatch, the distance between the two BFs was often <5/16th of an octave. Thus the highest contribution of bursts to frequency tuning was found at the BF, or at adjacent frequencies.

This preferential prevalence of bursts at the BF had consequences on the frequency tuning. When the frequency selectivity was calculated using the single APs component or using the burst component (see METHODS), the highest selectivity index was systematically found for the burst component (Fig. 5B; $P < 0.03$ in all cases).

As the results detailed in the preceding text came from the intensity that provided the highest excitatory responses, an obvious question was whether such results could also be obtained from the intensity that provided the lowest excitatory responses. Table 4 presents the burst contribution to frequency tuning for a subset of cells tested at least at four intensities. The results were qualitatively the same at the highest and at the lowest intensity: in all but one case (rat under pentobarbital anesthesia), the BF obtained with the bursts occurred at adjacent frequencies.

### TABLE 4. Bursts contribution to frequency tuning for cells tested at minimum 4 intensities

<table>
<thead>
<tr>
<th>Experimental Condition</th>
<th>Bursts contribution to the mean, %</th>
<th>Bursts contribution to the BF, %</th>
<th>Selectivity index based on single APs</th>
<th>Selectivity index based on bursts</th>
<th>Bursts contribution to the mean, %</th>
<th>Bursts contribution to the BF, %</th>
<th>Selectivity index based on single APs</th>
<th>Selectivity index based on bursts</th>
</tr>
</thead>
<tbody>
<tr>
<td>W guinea pigs ($n = 45$)</td>
<td>16.1</td>
<td>19.7</td>
<td>15.2</td>
<td>58.7**</td>
<td>9.5</td>
<td>14.6</td>
<td>49.9</td>
<td>75.6***</td>
</tr>
<tr>
<td>SWS guinea pigs ($n = 33$)</td>
<td>25.5</td>
<td>30.3*</td>
<td>41.5</td>
<td>60.3***</td>
<td>21.4</td>
<td>30.5**</td>
<td>43.0</td>
<td>73.5***</td>
</tr>
<tr>
<td>Pentobarbital rats ($n = 18$)</td>
<td>27.8</td>
<td>31.3</td>
<td>0.0</td>
<td>61.2*</td>
<td>30.9</td>
<td>28.3</td>
<td>58.7</td>
<td>70.1</td>
</tr>
<tr>
<td>Ketamine/Xylazine rats ($n = 5$)</td>
<td>57.1</td>
<td>64.6</td>
<td>6.0</td>
<td>60.5</td>
<td>38.5</td>
<td>47.2</td>
<td>57.6</td>
<td>74.7</td>
</tr>
</tbody>
</table>

Data are from the intensities providing the highest and the lowest excitatory drive. Paired $t$-tests for W, SWS, and rats under pentobarbital anesthesia. Wilcoxon signed-rank tests for rats under ketamine/xylazine anesthesia. Mean, mean evoked response; BF, best frequency. * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$. 

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anesthesia), the burst contribution was higher for the BF than for the mean evoked response. Also the selectivity index based on bursts was greater than the one based on single APs. Thus results described at the intensity that provided the highest excitatory drive seem also valid at the intensity that provided the lowest excitatory one.

**Contribution of bursts to intensity function**

Thirty-one intensity functions were tested (urethan: \( n = 2 \); pentobarbital: \( n = 14 \); ketamine/xylazine: \( n = 15 \)). The intensity function of each cell was reconstructed using the whole spike train, the burst spike train and the single APs spike train (see Fig. 7 for examples). Qualitatively, bursts often seemed to progress with the strength of evoked responses (Fig. 7, A–C). To confirm these observations, linear regressions were made for each cell between the burst contribution and the responses evoked at each intensity. Among the 31 tested cells, 7 did not exhibit bursts; 5 exhibited strong correlations (0.79 < \( r < 0.99 \)), 6 exhibited weak correlations (0.56 < \( r < 0.76 \)), and 13 exhibited no correlation (0.03 < \( r < 0.51 \)). Representative examples of these situations are presented in Fig. 7. Thus as it is the case for frequency tuning curves, on the whole population there was no systematic relationship between burst contribution and strength of evoked responses.

**Does the burst mode change the responses latency and its variability?**

It was shown in a recent study that MG bursting cells exhibited more variable, and delayed, first spike latencies that lagged non-bursting responses by \( \pm 7 \) ms (He and Hu 2002). To assess if such relation was present in our data, linear regressions were performed between the burst contribution to the mean evoked response and the first spike latency or its variability. Whatever the experimental condition, no correlations were obtained (all \( r < 0.39 \)). The same results were found (all \( r < 0.36 \)) when considering only the cells that had a burst contribution above 20% (W: \( n = 18 \); SWS: \( n = 44 \); urethan: \( n = 21 \); pentobarbital: \( n = 45 \); and ketamine/xylazine: \( n = 26 \)). Thus on the whole population, there was no relationship between the response latency (or its variability) and the burst contribution.

On the other hand, in a study performed in the lateral geniculate of anesthetized cats, each of the 11 tested cells showed significantly less latency variability to visual stimuli when responding in burst mode compared with tonic mode (Guido and Sherman 1998). Here, for the aforementioned cells exhibiting a burst contribution >20%, the first spike latency and its variability were computed separately for the single APs spike train and for the burst spike train. Significant differences

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**FIG. 7.** Examples of bursts and single APs contributions to intensity function. The intensity functions were reconstructed using the total evoked response (●), only the burst component of the response (●), and only the single APs component of the response (●). The burst contribution (mean value) is indicated by the number in the top right corner. The result of the linear regression performed between burst contribution and strength of evoked responses is indicated in the top left corner. Qualitatively, bursts often progressed with the strength of evoked responses (A–C), but no systematic relationships were found (strong relation in A; weak relation in B and lack of relation in C and D).
were found only during W, SWS, and under urethan anesthesia. During W, the response latency based on bursts was significantly lower than the one based on single APs (14.4 vs. 21.2 ms; \( P < 0.0001 \)) and so was the latency variability (4.7 vs. 16.3 ms; \( P < 0.0001 \)). The latency variability based on bursts was also lower during SWS (14.3 vs. 20.9 ms; \( P < 0.0001 \)) and under urethan anesthesia (6.8 vs. 12.4 ms; \( P = 0.02 \)). Indeed, these effects were observed for all the cells recorded during W and almost all the cells during SWS and under urethan anesthesia (only 14/44 and 3/21 cells, respectively, increased their latency variability in burst mode). Figure 8 depicts the behavior of the 12 cells that exhibited, both during W and SWS, a burst contribution \( >20\% \). All these cells decreased their latency and latency variability when in burst mode compared with single AP mode, whether during W (Fig. 8A) or during SWS (Fig. 8B; except 2 cells for the variability). Thus these data indicate that, during W, the occurrence of bursts allow faster responses and responses more time-locked to stimulus onset. The latter was also observed during SWS and under urethan anesthesia.

**ROC analysis**

Based on a population of 50 cells (pentobarbital: \( n = 16 \); ketamine/xylazine: \( n = 34 \)), we assessed the relationships between the proportion of evoked bursts and the cell capability to detect acoustic stimuli. For each cell, the area under the ROC curve was determined to detect acoustic stimuli. For each cell, the area under the ROC curve was determined to detect acoustic stimuli. For each cell, the area under the ROC curve was determined to detect acoustic stimuli.

A within-cell analysis was performed to determine whether the detection performance of cells was improved when the burst mode was present (see METHODS). This analysis was carried out only when the percentage of trials in burst mode and in single AP mode did not differ too much to base the ROC analysis on similar numbers of trials. For the 15 cells on which this analysis was conducted, the ROC area derived from the trials in single AP mode did not differ from the ROC area derived from the trials in burst mode [single AP mode: 0.639, burst mode: 0.576; \( n(14) = 1.61, P = 0.17 \)]. These unexpected results, compared with those of Guido et al. (1995), can be explained when looking at the percentage of trials where the cells responded to the stimulus: on average, they responded twice less often when in burst mode than when in single AP mode (32 vs. 64\% of the trials). Thus two opposite factors have to be considered when comparing the stimulus detectability performed by single APs and by bursts. First, burst responses occurring on a background of no (or very sparse) spontaneous activity obviously signaled the presence of stimuli more efficiently than tonic responses. But, second, the burst mode also promoted a higher proportion of trials where the cells did not respond to the stimulus.

**Location of the recorded neurons**

As mentioned in METHODS, the location of all recorded cells was estimated according to the electrode penetration tracks and the cells depth coordinate.

In the case of the chronic recordings, the electrodes tracks were easy to follow and their bottom could be determined unambiguously. Figure 10A presents microphotographs of recording sites in each of the three main divisions of the auditory thalamus. Of the 100 recorded cells during W and...
SWS, 26 were found in the dorsal part of the MG nucleus, 25 in the medial part, 39 in the ventral part (and 10 in the posterior thalamus; Po). An ANOVA revealed that there was an effect of the recording site for the burst proportion obtained during W, both during spontaneous \( F(3,97) = 3.64; \ P = 0.015 \) and evoked activities \( F(3,97) = 2.60; \ P = 0.056 \): the highest proportion was found in MGv. However, during SWS, no differences between divisions were found for spontaneous or evoked bursts [SWS: \( F(3,97) < 1, \ ns \) in both cases].

In the case of the acute recordings, the determination of the recording sites was not precise enough to assign each recording to a given anatomical division. However, it can be mentioned that, as shown in Fig. 10B, bursts were usually found at all recording depths and through the entire rostrocaudal extent of the MG nucleus.

**DISCUSSION**

Our results demonstrated that, both in drugged and undrugged conditions, auditory thalamus neurons can fire high-frequency bursts, although these bursts never dominated the cells mode of discharge. Burst proportion was higher in drugged states compared with undrugged ones (W and SWS) and was also higher during SWS than during W. In all conditions, even when they were rare events, as during W, bursts preferentially appeared at the BF, thus promoting a higher frequency selectivity than did single APs. Qualitatively, bursts often increased with response strength, but regression analyses performed on intensity functions did not reveal systematic relationships. During W, the latency of evoked responses and its variability were decreased when cells shifted to burst mode; during SWS and under urethan anesthesia, only the variability was decreased.
A1  A2  A3

B

<table>
<thead>
<tr>
<th>BF (kHz)</th>
<th>Latency (ms)</th>
<th>Bursts (%)</th>
<th>Depth (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>off</td>
<td>29</td>
<td>-5058</td>
</tr>
<tr>
<td>15</td>
<td>20.6±4.3</td>
<td>25</td>
<td>-5090</td>
</tr>
<tr>
<td>click</td>
<td>10.1±1.9</td>
<td>45</td>
<td>-5164</td>
</tr>
<tr>
<td>10</td>
<td>14.3±4.2</td>
<td>3</td>
<td>-5772</td>
</tr>
<tr>
<td>9</td>
<td>off</td>
<td>74</td>
<td>-5882</td>
</tr>
</tbody>
</table>
Methodological considerations

In the present study, burst detection employed criteria defined from in vivo intracellular recordings collected in visual thalamus. When a cluster of APs occurred with ISI ≤ 4 ms after a period of silence ≥ 100 ms, the probability that this cluster of APs resulted from the occurrence a low-threshold spike (LTS) was >98% (Lu et al. 1992). Reasons for this relationship rely on the properties of the thalamic I<sub>T</sub> current. When the membrane potential is hyperpolarized below −65 mV for ≥50–100 ms, the I<sub>T</sub> current is de-inactivated, and the next suprathreshold depolarization activates it: an influx of Ca<sup>2+</sup> produces a depolarizing waveform known as the LTS, which, in turn, usually activates a burst of conventional Na<sup>+</sup>/K<sup>+</sup>-dependent action potentials (Jahnsen and Llinas 1984a,b; Llinas and Jahnsen 1982).

The crucial question is then whether or not MG cells display the same ionic currents as do visual relay cells. In vitro several intracellular studies have demonstrated that auditory thalamus neurons exhibit bursts of two to seven APs riding on a LTS (Bartlett and Smith 1999; Hu 1995; Tennigkeit et al. 1996). A confirmation that short (<5–7 ms) ISIs correspond to LTS currents came from a recent intracellular study performed in MG nucleus: groups of APs with longer ISI (11.6 ms) were never accompanied by LTS current in pentobarbital-anesthetized guinea pigs (Xiong et al. 2003). Thus it seemed appropriate to use criteria previously defined from intracellular studies in visual thalamus. However, there are two obvious risks in detecting bursts from extracellular recordings: too stringent criteria lead to miss some of the bursts; too liberal criteria lead to classify as bursts the highest firing rate reached during the tonic mode of discharge.

On the one hand, our criteria (ISI ≤ 5 ms and 100 ms of silence) might be considered as too stringent regarding those selected by some authors (ISI ≤ 6 ms and ≤16 ms with a period of silence ≥50 ms) (Ramcharan et al. 2000; Weyand et al. 2001). It is important to remind here that, increasing the ISI from 5 up to 10 ms did not recruit more evoked bursts (24.9 vs. 25.9%). In contrast, reducing the ISI to 3 ms lowered the proportion of detected burst obviously because thalamic bursts can have internal frequency <333 Hz. The intraburst frequency observed here is in good agreement with most of the previous studies. In vivo the initial studies by the Steriade group have mentioned intraburst frequencies of 250–400 Hz during SWS (see for example Dohmich et al. 1986), and the intracellular study by Lu et al. (1992) reported intraburst frequencies of 286–429 Hz in visual thalamus neurons. In vitro such frequencies have also been reported both in the visual (e.g., 190–320 Hz in Leresche et al. 1991; 344 ± 18 Hz in Pirchio et al. 1997) and in the auditory thalamus [338 ± 51 Hz in MGd and 365 ± 86 Hz in MGv in Barlett and Smith (1999)].

On the other hand, our criteria can be considered as liberal if, as suggested by Hu (1995), the refractoriness of the I<sub>T</sub> current is 200 ms in MG cells. Two points argue against this later view. First, thalamic neurons can fire bursts at 10 Hz when submitted to particular sequences of hyperpolarization/depolarization (Fig. 3 in McCormick and Feuerer 1990). Second, based on our parametric study, it appeared that increasing the silence period before burst detection from 100 to 200 ms decreased the proportion of detected bursts by <3% (24.9% with 100 ms vs. 22.2% with 200 ms). Furthermore, because our study is mainly based on comparisons between experimental conditions, the major conclusions still hold, even if the overall proportions of detected bursts changed by a few percents.

Other methodologies than the ones based on the known properties of ionic conductances have been used to detect bursts of MG cells. In particular, some studies quantified thalamic bursts using deviations from a random Poisson process (Villa 1988; Villa et al. 1991; Zurita et al. 1994). Using this technique, the average duration of the “detected bursts” is −38 ms (calculated from Fig. 4 in Villa et al. 1991), the average number of APs is two (page 101 in Villa 1988), the average firing frequency is 68 Hz (page 309 in Zurita et al. 1994), and only 6/255 cells never fired bursts (Villa et al. 1991). Most likely, this method detects bursts underlined by LTS currents and also groups of single APs that correspond to the highest firing rate obtained during tonic discharges.

In the present study, comparisons were made between experimental conditions (W, SWS, urethan, pentobarbital, and ketamine/xylazine) based on neuronal recordings coming from both rats and guinea pigs. So, one can wonder if species differences did not bias our comparisons. Obviously, if auditory thalamus neurons of the two species do not share the same electrophysiological properties, our conclusions might be wrong. Arguing against this possibility, in vitro recordings performed in rats (Hu 1995; Tennigkeit et al. 1996) and in guinea pigs (McCormick and Feuerer 1990) revealed the presence of similar I<sub>T</sub> currents in thalamocortical neurons. In addition, the fact that under pentobarbital anesthesia, neuronal data coming from rat and guinea pig MG cells never significantly differed indicates that comparisons between experimental conditions are relevant.

Homogeneous or regional distribution of bursting cells in the MG nucleus?

Initially, an in vitro study has pointed out that the burstiness of MG cells is higher in the dorsal than in the ventral MG

2 Occasionally, differences were noted between rat and guinea pig MG cells. Application of muscarine induces hyperpolarization in guinea pig (McCormick and Prince 1987) whereas hyperpolarizing responses are uncommon in rat (Mooney et al. 1995).

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division: 95% of the rat MGd cells exhibited burst responses, whereas 50–70% of the MGv cells exhibited single- or dual-spike discharges in response to stimulation of the brachium of the inferior colliculus (Hu et al. 1994). However, a subsequent study from the same laboratory showed that 118/186 cells (63%) of the rat MGv exhibited bursts (Mooney et al. 1995). Also, Bartlett and Smith (1999) could not find differences between subdivisions in the proportion of cells displaying the $I_c$ current, a crucial actor underlying burst generation in thalamic relay cells. In the present study, our analysis of the histological material does not indicate differences in bursting proportions within the MG nucleus. Obviously, our analysis has intrinsic limitations because we did not performed lesions at the bottom of each track and we did not systematically ejected tracers via the recording glass pipette. In fact, only intracellular staining allows to assign a particular recording to a precise location in the MG.

In contrast with the present results, an in vivo study performed in ketamine/xylazine-anesthetized guinea pigs (He and Hu 2002) reported that bursting cells were prominent in MGd (77%, 33/43) but were rather rare in MGv (17%, 5/29). As previously explained (Massaux and Edeline 2003), the divergence between our results and those of He and Hu (2002) could mainly rely on the criteria used to define bursts. The criteria adopted by He and Hu (2002) were that bursting neurons should exhibit responses made of two to six spikes occurring in 15 ms in $\geq 50\%$ of the trials and that single-spike neurons should show no more than two spikes in a 15-ms period in $\geq 75\%$ of the trials. In fact, in their study, cells exhibiting frequent two-spike responses could not be easily classified. Here, as well as in all previous studies performed in the visual (Guido and Weyand 1995; Guido et al. 1992; Lu et al. 1992; Ramcharan et al. 2000; Weyand et al. 2001) or somatosensory thalamus (Swadlow and Gusev 2001; Swadlow et al. 2002), two-spike responses were treated as bursts.

Potential mechanisms affecting the burst proportions

The differences in burst proportions observed here between drugged and undrugged states, as well as between SWS and W, could mainly result from differences in membrane polarization. First, because in undrugged animals, intracellular studies demonstrated that thalamic neurons are slightly hyperpolarized during SWS compared with W (Hirsch et al. 1983), and second, because anesthetics are usually considered as having hyperpolarizing effects (see for a range of general anesthetics, see Nicoll and Madison 1982; and for effect of pentobarbital on MG neurons, see Wan and Pui 2002). However, this hypothesis should not be oversimplified: at the cortical level, it was shown that the membrane potential is permanently fluctuating over time both during W and SWS, reaching transiently, but frequently, hyperpolarized states during SWS (Steriade et al. 2001; Timofeev et al. 2001). The main consequence of these permanent fluctuations is that the single AP mode is also prominent during SWS. Furthermore, brief epochs of hyperpolarization could also be reached during W offering the possibility for LTS to be triggered. If similar fluctuations also occur in thalamic cells, they should promote the occurrence of LTS-related bursts during SWS and, to a lesser extent, during W. According to this view, the higher proportion of bursts in drugged states could be explained by the fact that these fluctuations take place at more hyperpolarized levels than during undrugged states, and/or that the epochs spent at hyperpolarized states last longer than during SWS and W.

Under anesthesia, burst proportion increased from urethan to pentobarbital to ketamine/xylazine. Explaining the differences observed here between anesthetics is a complex task which is out of the scope of this study. This requires to integrate the effects induced by each anesthetic on MG cells but also on the entire central auditory system, in particular at the three main afferences of MG cells: the inferior colliculus, the auditory cortex and the auditory sector of the reticular nucleus (RE). To illustrate this problem, one can mention that although it is widely accepted that barbiturates, like pentobarbital, enhance the actions of GABA at the ionotropic GABA_A receptors in the entire CNS (see for review Mehta and Ticku 1999), a recent in vitro study reported that the depressant effect of pentobarbital on MG cells did not involve GABA receptors (Wan and Pui 2002).

Finally, it is also important to mention that other factors than the level of somatic membrane polarization can be crucial for burst occurrence. Given that several studies (Destexhe and Sejnowski 2002; Destexhe et al. 1998; Williams and Stuart 2000) have shown that the $I_c$ current is prominent in proximal dendrites, a conductance shunt of voltage-dependent Na$^+$ and Ca$^{2+}$ currents could prevent the initiation of LSTs and/or their electrotonic propagation to the axon hillock. Last, burst proportions could also be a function of the amount of excitatory inputs that reaches MG cells and triggers the LTS events. The high burst proportion found with ketamine might result from an increase in the number of active cells in the inferior colliculus compared with urethan and pentobarbital (Astl et al. 1996).

Comparisons with previous studies

In all experimental conditions, bursts of auditory thalamic neurons emerged at, or around, the neurons’ BF thus contributing to increase the frequency selectivity. This is in good agreement with previous findings. In the lateral geniculate (LG) nucleus, an increased in bursting activity was related monotonically with the sharpening of temporal tuning, which shifted from low- to band-pass (Mukherjee and Kaplan 1995). This property was also found at the cortical level. In auditory cortex, the frequency tuning was more selective when responses were composed of bursts compared with single APs (Eggermont and Smith 1996). Also, in lightly anesthetized cats, increasing the burstiness of cortical cells by a mixture of ketamine/pentobarbital improved the frequency tuning (Zurita et al. 1994). Note, however, that these two studies quantified bursts using deviations from a random Poisson process. In a pioneering study performed in visual cortex, bursts were able to sharpen the orientation and spatial frequency tunings better than single APs (Cattaneo et al. 1981). Thus in various sensory modalities, bursts increased the neuron selectivity for a particular stimulus dimension. More importantly, burst-induced increase in frequency selectivity was observed here at both the intensity eliciting the highest and the lowest evoked responses. This indicates that bursts can appear at all sound intensities, as observed when testing the intensity functions.

The effect observed here on latency variability is in agreement with the one reported by Guido and Sherman (1998). In halothane-anesthetized cats, they showed that the burst mode contribu-
uted to decrease the latency variability of LG cells. Here, for cells exhibiting >20% of bursts, a similar effect was observed during W and SWS and under urethane anesthesia but, surprisingly, not under pentobarbital or ketamine/xylazine where bursts were abundant. Furthermore, burst mode also decreased the response latency during W. These results differ from those reported by He and Hu (2002) because all their bursting cells exhibited longer and more variable response latency. However, as already pointed out, important differences in burst-detection criteria could account for these discrepancies.

Our ROC analyses gave results at variance with those obtained by Guido et al. (1995) in the LG, where the higher the burst proportion the larger its ROC area. Our study is far to be as detailed as the aforementioned one, and caution has to be exerted when comparing the two results. First, the use of a different anesthetic (Halothane) in the study by Guido et al. 1995 should be seriously considered because this might favor higher spontaneous and evoked activity compared with pentobarbital or ketamine/xylazine. Other reasons could explain this discrepancy. Examination of Fig. 10 in the study by Guido et al. (1995) revealed that our cells apparently exhibited as much bursts as theirs but tended to have smaller ROC areas. One explanation for these lower ROC areas values might rely on the extremely phasic nature of evoked responses in MG nucleus. As illustrated in Fig. 3, auditory thalamus cells respond by a very brief excitatory response (25–50 ms), usually composed of 2–5 APs, which contrasts with the prolonged responses of LG cells (≥100 ms) composed of 7–15 APs. Because ROC analysis compares the probability of occurrence of n APs between two periods of identical duration (a period of evoked activity, \( \tau_e \), vs. a period of spontaneous activity, \( \tau_s \)), the number of APs falling within \( \tau_e \) is crucial. In fact, ROC analysis might not be appropriate to assess signal detection in the thalamocortical auditory system where neurons respond by very few APs (sometimes only 1) emitted with a precise timing after stimulus onset.

**Functional role of thalamic bursts**

Several lines of evidence suggest that bursts are far more efficient than single APs to activate postsynaptic cells (see for reviews Izhikevich et al. 2003; Krahe and Gabbiani 2004; Lisman 1997). This notion was recently confirmed in the somatosensory system: using cross-correlograms between thalamic and cortical cells, it was demonstrated that thalamic bursts were highly potent in activating cortical cells (Swadlow and Gusev 2001; Swadlow et al. 2002). If auditory thalamic bursts have the same impact on their postsynaptic targets—namely the auditory cortices, the auditory reticular nucleus, and the lateral amygdala (LA)—two functional roles can be proposed. Bursts might act as a physiological filtering device and as a plasticity inductor, both at the cortical and subcortical levels.

First, MG bursts could help focusing the spread of activation coming from thalamic inputs. At presentation of a complex sound (such as a vocalization with a wide frequency content), a large population of thalamic cells will fire action potentials and consequently will send inputs to a large amount of target cells. Only the thalamic cells that have their BF at (or near) the most represented frequency band in this sound will emit important burst proportions. Hence, although all the target cells will be bombarded by a vast amount of synaptic inputs, those made of bursts will mostly come from cells responding to the dominant frequency of the sound. Thus as already envisioned (Crick 1984), thalamic bursts may be a way to restrict the number of target cells activated at the cortical and subcortical levels during presentation of a complex stimulus.

Second, the high internal frequency of MG bursts should promote induction of synaptic plasticity. At the cortical level, the synchronized bursts that are naturally present during spontaneous or evoked spindles can induce an increase in responsiveness to single stimuli which last for several minutes; (Timofeev et al. 2002; see for review Steriade and Timofeev 2003). More importantly, bursts might also induce similar effect at the subcortical level. Indeed, nontonotopic regions of MG project to LA, a key structure involved in acoustic fear conditioning and that exhibits synaptic plasticity in response to high-frequency stimulation (see for review LeDoux 2000). Because thalamic bursts are in the same frequency range than the frequencies used to trigger synaptic plasticity in LA (300–400 Hz in Clugnet and LeDoux 1990; Rogan and LeDoux 1995), one can envision that thalamic bursts are natural events triggering plastic changes at presentation of a meaningful sound. Depending on the resonance frequency of the target cells, thalamic bursts might be more efficient at the cortical or subcortical level (Izhikevich et al. 2003).

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