Evoked Oscillations in the Thalamo-Cortical Auditory System Are Present in Anesthetized but not in Unanesthetized Rats

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Cotillon-Williams, Nathalie and Jean-Marc Edeline. Evoked oscillations in the thalamo-cortical auditory system are present in anesthetized but not in unanesthetized rats. J Neurophysiol 89: 1968–1984, 2003; 10.1152/jn.00728.2002. Over the last decade, a large number of studies have characterized stimulus-evoked oscillations in the visual cortex of anesthetized and unanesthetized animals. Comparative studies have been performed in auditory cortex. This study compared the tone-evoked oscillations detected from the same recording sites in the thalamo-cortical auditory system of unanesthetized and anesthetized rats. Simultaneous multiunit recordings were collected in auditory cortex, auditory thalamus, and the auditory sector of the reticular nucleus of restrained rats, which spontaneously shifted from waking (W) to slow-wave sleep (SWS) and paradoxical sleep (PS). Subsequently, the same recording sites were tested under pentobarbital anesthesia, then under high doses of diazepam, and finally under urethan anesthesia. Under these drugs, oscillations were detected in 54% of the recordings: one-half of them were stimulus-locked oscillations and were directly observed on peri-stimulus time histograms (PSTHs); one-half of them were non-stimulus-locked oscillations and were detected on autocorrelograms. Spontaneous oscillations were present for 17% of the recordings. During SWS, only non-stimulus-locked oscillations were observed for a small percentage of recordings (12%). This percentage did not differ significantly from the one of spontaneous oscillations obtained during SWS (8%). No oscillations were found in W and PS. Both under anesthesia and in SWS, the frequency range of the oscillations was 5–15 Hz, and there was no frequency difference between evoked and spontaneous oscillations. Although surprising, the absence of oscillations in awake animals may allow each neuron to process acoustic information independently of its neighbors and may in fact benefit auditory perception.

INTRODUCTION

Since their original description (Eckhorn et al. 1988; Gray and Singer 1989), stimulus-evoked neuronal oscillations have been an intense field of research. Initially, these oscillations have been studied in the visual system where their frequency was often found to be in the gamma range (30–90 Hz). In most of the cases, they were non–stimulus-locked (“induced oscillations”). Based on their characteristics, several authors have proposed that they could play a key role in synchronizing neuronal populations that respond to different properties (shape, color, direction of motion) of a given stimulus (for reviews see Eckhorn 2000; Engel et al. 1992; Frégnac et al. 1994; Singer 1990). These high-frequency oscillations were originally obtained under various anesthetics (mainly under N0/2 anesthesia: Eckhorn et al. 1993; Engel et al. 1990, 1991a,b; Ghose and Freeman 1992; Gray et al. 1989, 1990), and their presence in undrugged animals was questioned when some laboratories failed to detect them in these conditions (Bair et al. 1994; Tovee and Rolls 1992; Young et al. 1992). Nonetheless, several subsequent studies performed in awake animals described oscillations in proportions equal or superior to those obtained in anesthetized animals (Friedmann-Hill et al. 2000; Gray and DiPrisco 1997; Kreiter and Singer 1992; Maldonado et al. 2000).

In the auditory cortex, several studies using single unit or multiunit recordings have revealed oscillations that were stimulus-locked (“evoked oscillations”) and were in low frequency ranges. In several anesthetic conditions (ketamine, equithesin, pentobarbital, urethan), the oscillations frequency was between 6 and 15 Hz (Cotillon and Edeline 2000; Cotillon et al. 2000; Dinse et al. 1997; Eggermont 1992; Gaese and Ostwald 1995; Maldonado and Gerstein 1996). In contrast, using local field potentials (LFP) in halothane anesthetized rats, high-frequency “induced oscillations” have been reported (Barth and MacDonald 1996; Franowicz and Barth 1995; MacDonald and Barth 1995). These oscillations, already present during spontaneous activity, had a complex temporal decay after a brief (1 ms) click presentation: they disappeared at stimulus presentation, re-appeared 300 ms later, and intensified 600–800 ms after stimulus onset. High-frequency induced oscillations were also recently reported from LFP and multiunit recordings of ketamine anesthetized monkeys (Brosch et al. 2002).

So far, the only study that has looked for oscillations in the auditory cortex of undrugged animals has failed to detect both high- and low-frequency oscillations (Horikawa et al. 1994). The present study aimed at comparing the evoked oscillations observed in anesthetized and unanesthetized conditions in the thalamo-cortical auditory system. Multiunit recordings were obtained from auditory cortex, auditory thalamus [the medial geniculate body (MGB)], and the auditory sector of the thalamic reticular nucleus (RE) of undrugged, restrained rats. The same recordings were subsequently tested under pentobarbital and urethan anesthesia, as well as under high doses of diazepam.
Methods

Animal preparation

Twenty-two adult Sprague-Dawley rats (290–350 g) underwent a surgery under pentobarbital anesthesia (60 mg/kg, ip, preceded by an atropine injection 0.08 mg/kg). Three silverball electrodes 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). Two silver wires were inserted into the dorsal neck muscles to record the electromyogram (EMG).

The recording electrodes (tungsten 1.0 MOhm at 1 kHz) were slowly lowered in the target structures under electrophysiological control. For the recordings in the auditory thalamus (5.8 mm posterior to Bregma, 3.5 mm lateral, and 5–6.6 mm below dura) a set of four electrodes was used. For the recordings in the auditory cortex, a large craniotomy was made in the temporal bone (from 3.0 to 6.0 posterior to Bregma and 3–4 mm dorso-ventral) and an array of five or six electrodes (spaced 200–300 μm in the rostrocaudal axis) was lowered in the cortex. In each structure, the occurrence of short-latency responses (<25 ms) to tone bursts presentation was used as criteria for the final placement of the electrodes. The small size of the skull in rat prevented implantation of three arrays of electrodes on the same animal, and, as a consequence, electrodes were implanted in only two of the three investigated structures for a given animal.

A pedestal in dental acrylic cement including two cylindrical threaded tubes was built to allowatraumatic fixation of the animal’s head during the subsequent recording sessions. An antiseptic ointment (IAC, model AC2). Each animal was placed in a loose bag with the head directed into the pedestal, and an antibiotic (Terramycin, oxotetracycline, Pfizer) was administered during 5 days following surgery. All surgical procedures were performed in conformity with national (JO 887-848) and European (86/609/EEC) legislation on animal experimentation, which are similar to those described in the Guidelines for the Use of Animals in Neuroscience Research of the Society of Neuroscience.

From 3 days after surgery, each animal was adapted for several days to restrained conditions in an acoustically isolated chamber (IAC, model AC2). Each animal was placed in a loose bag with the head fixed for increasing periods of time starting from a few minutes per day to ≤2–5 h/day. It was also accustomed to hear brief (1 ms) clicks and sequences of pure tone bursts delivered via a calibrated speaker placed, from day to day, closer and closer from the entrance of the external ear. At the end of this period of adaptation, spontaneous alternations of waking (W), slow-wave sleep (SWS), and paradoxical sleep (PS) were obtained even when delivering tones of 80 dB SPL.

Recording procedures

The signal coming from each electrode 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 to select the largest spikes (signal-to-noise > 3/1) from the multunit signal. The TTL pulses generated by the voltage window discriminators were sent to the acquisition board (PCLab, PCL 720) of a Pentium II CPU microcomputer. The time of occurrence of the TTL pulses corresponding to each action potential was known with a resolution of 50 μs. During most of the recording sessions, the raw signals coming from three electrodes were sent to a magnetic tape recorder (TEAC, model R-61D). The EEG and the EMG were sent to a polygraph (Grass model 79D, band-pass 1–90 Hz) and digitized (1-kHz sampling rate, MacAdios board, GW Instruments).

Acoustic stimuli

The sound generating system was the same as in previous studies (Edeline et al. 1999, 2000; Manunta and Edeline 1997, 1999). Pure tone frequencies (tone duration: 100 ms, rise/fall time: 5 ms) were generated by a remotely controlled wave analyzer (model HP 8903B, Hewlett-Packard) and attenuated by a passive programmable attenuator (Wavetek, PS57, maximal attenuation 127 dB), both controlled by a computer via an IEEE bus. Contralateral tones were delivered through a calibrated earphone mounted in a small stainless steel container filled with foam. The opening of the container was fitted into the ear canal to deliver the stimuli close to the tympanic membrane. Although the intensities used were calibrated with respect to the SPL scale, the intensities expressed here are best viewed as relative values, given that a sealed sound system cannot be used in awake animals.

Experimental protocol

Out of 22 animals that underwent the initial surgery, only 14 animals were submitted to the following protocol. For the other eight animals, either the recordings were never of good enough quality to run the experimental protocol (n = 4), the neurons exhibited only poor auditory responses (n = 3), or the animal did not adapt enough to the restrained conditions (n = 1).

At the beginning of each recording session, all the electrodes exhibiting correct multunit activity (signal-to-noise ratio >3) were tested: sets of tones of ascending frequency were presented from threshold ≤80 dB. Based on these tests, we selected the frequency and intensity that provided the best excitatory drive for the different electrodes that were simultaneously recorded. In some occasions, a 1-ms click was also used as acoustic stimulus. Five inter-tone intervals (ITIs) were used here: 10, 2.5, 1, 0.7, and 0.5 s. To keep the testing time to about 2 min, the number of tone presentations varied as a function of the ITI: 110 tone presentations were used for the ITIs of 0.5, 0.7, and 1.0 s; 55 tone presentations were used with the 2.5-s ITI, and 11 tone presentations were used with the 10-s ITI. Sequences of tones were regularly presented while the animal was spontaneously shifting from one state of vigilance to another (see Fig. 1 for a schematic diagram of the protocol). A “trial” was defined as a 2-min period of tone presentation with the same ITI. During a recording session, the ITI was kept the same for 20–30 successive trials. As the animal vigilance states always fluctuated in an unpredictable manner, the probability that the 2 min corresponding to a given trial belonged entirely to a stable vigilance state was quite low. Thus in a given session, only 2–10 trials were usually collected in W and SWS and only 1 or 2 trials in PS. The other trials were classified as “mixed state of vigilance” (transitions from W to SWS, SWS to W, or PS to W). About the same amount of trials was collected for each ITI. The order of presentation of the ITIs was changed from 1 day to another. A “group of trials” was defined as all the trials that were collected for a given recording session in a given state of vigilance (W, SWS, and PS) and with the same ITI.

For nine animals, after 3–6 days of testing in unanesthetized conditions, the same recording sites were tested under anesthesia. The recording sessions were conducted under pentobarbital anesthesia (30 mg/kg, ip), under high doses of diazepam (15 mg/kg, ip), and finally under urethan anesthesia (1 g/kg, sc). The animals were allowed to recover for a few days between each session in drugged conditions. All electrodes could not be tested in all drugged conditions because the evoked responses at some recording sites were not strong enough. For the sound generating system, a “trial” was defined as in undrugged conditions, and a “group of trials” was defined as the set of trials collected with a given ITI under a given drug (pentobarbital, diazepam, urethan).
Data analysis

A first analysis of the oscillations was made by visual inspection of the rasters and PSTHs (10-ms bin, from 500 ms before tone onset to \( \pm 2 \) s after tone onset) obtained from each trial. To determine whether or not an oscillation was present on a PSTH, we used the same criteria as in our previous studies (Cotillon and Edeline 2000; Cotillon et al. 2000). We considered that a trial exhibited an oscillation if, and only if, 1) there were \( \geq 3 \) distinct peaks on the PSTH after the “on” phasic response, and 2) the rasters showed regularly spaced discharges at each tone presentation. This analysis only revealed oscillations that were “stimulus-locked” to tone onset.

A second analysis was based on autocorrelation histograms (duration, 600 ms; bin, 1 ms, occasionally a 2-ms bin was used), which were computed for the evoked activity (during and immediately after tone presentation) and allowed to detect both stimulus-locked and non–stimulus-locked oscillations. This analysis was also performed during spontaneous activity (between tone presentations) to detect spontaneous oscillations.

A group of trials was considered as oscillatory when at least one-half of the trials displayed oscillations. Potentially, a “group of trials” can be made of 1) trials with only stimulus-locked oscillations, 2) trials with only non–stimulus-locked oscillations, 3) trials with both stimulus-locked and non–stimulus-locked oscillations, and 4) trials without any oscillation.

Quantification of the oscillations was made by computing a power spectrum of the autocorreglograms (home-made program written under Labview 6.0) for each trial constituting a “group of trials.” On every trial, the power was systematically computed in three frequency bands (5–15, 15–30, and 30–90 Hz) that have been used in previous studies (Frien and Eckhorn 2000; Frien et al. 2000; Schanze and Eckhorn 1997). The peak areas obtained in the power spectrum were delimited by a digital filter using optimal filtering methods with FFT (Press et al. 1988) and a Hanning window (band-pass 4 Hz). For each power spectrum, the largest value among \( P_{5–15 \text{ Hz}} \), \( P_{15–30 \text{ Hz}} \), and \( P_{30–90 \text{ Hz}} \) will be called \( P_{\text{max}} \), and the frequency of the peak that provided \( P_{\text{max}} \) will be called \( P_{\text{max}}^{\text{freq}} \). The values of \( P_{\text{max}}^{\text{freq}} / P_{\text{max}}^{\text{max}} \), \( P_{5–15 \text{ Hz}} / P_{15–30 \text{ Hz}} \), and \( P_{30–90 \text{ Hz}} / P_{15–30 \text{ Hz}} \) were obtained from each trial were compared between spontaneous and evoked activity as well as between conditions (i.e., between anesthesia and SWS) and between types of oscillations (stimulus-locked or not) using unpaired \( t \)-test (\( P < 0.05 \)). The distributions of the power and of the frequency of the oscillations detected in each condition were compared using \( \chi^2 \). In all but one case, the differences were confirmed using Kolmokorov-Smirnov (KS) tests.

Histology

At the end of the last recording session in drug conditions, the animal received a lethal dose of pentobarbital (120 mg/kg) and was perfused (intracardiac perfusion with 250 ml of saline followed by 500 ml of paraformaldehyde; pH 7.4). After 2 days of postfixation, the brains were placed for 3 days in a 30% sucrose solution in 0.1 M phosphate buffer; and after they were sunk, they were cut on a freezing microscope (50 \( \mu \text{m} \) thick). After Nissl staining, the sections were examined under several microscopic magnifications to determine the electrodes placements in the three investigated structures.

RESULTS

Data are from 45 recording sites in the auditory cortex, 14 recording sites in the MGB, and 6 recording sites in the auditory RE. Of them, 24 recording sites were tested under drugs in the auditory cortex, 6 were tested in the MGB and 4 were tested in the auditory RE. In the cortex, the recording sites were in all cortical layers; they were in the dorso-caudal part of the RE, and they were mostly (10/14) from the dorsal division of the MGB (3 sites were found in the ventral MGB and 1 in medial MGB).

Whatever the experimental conditions, detection of evoked oscillations from the PSTHs was very unusual with the 0.5-, 0.7-, and 1.0-s ITIs, whereas it was common with the ITIs of 2.5 and 10 s, which were already found to be optimum to trigger oscillations in anesthetized animals (Cotillon et al. 2000). Therefore the presentation of the results will be based on data obtained with the 2.5- and 10-s ITI. As the results obtained with these two ITIs were the same, the statistical analyses will be presented only for the 10-s ITI (unless specified in 1 case). Illustrations of the findings with individual examples will use both the 2.5 and 10 s ITIs.

PSTHs revealed oscillations only in drugged conditions

Table 1 presents the number of recordings exhibiting stimulus-locked oscillations observed on PSTHs in undrugged and drugged conditions. Out of a total of 689 “tests” (i.e., groups of trials with an ITI of 2.5 s or with an ITI of 10 s) that have been analyzed in the three structures over the different states of vigilance, none exhibited oscillations during W, SWS, or PS. In contrast, when the same recording sites were subsequently tested under pentobarbital, diazepam or urethan, tone-evoked oscillations were often observed from the raw PSTHs in the auditory cortex and in the auditory RE. Figure 2 presents raw traces of these oscillations for two recordings from the auditory RE under urethan anesthesia (Fig. 2A) and from two cortical recordings under urethan or pentobarbital anesthesia (Fig. 2, B and C). Figure 3 shows the PSTHs of a cortical recording that exhibited clear stimulus-locked oscillations (ITI 2.5 s) under the three drugs, but never displayed such oscillations in undrugged conditions.
TABLE 1. *Number of recordings sites and of recordings sessions that exhibiting phase-locked oscillations in undrugged and drugged conditions*

<table>
<thead>
<tr>
<th>Structure</th>
<th>Nb Recording Sites</th>
<th>Nb of Recordings</th>
<th>W</th>
<th>SWS</th>
<th>PS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undrugged Conditions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Auditory cortex</td>
<td>45</td>
<td>82</td>
<td>169 tests (0 oscillation)</td>
<td>173 tests (0)</td>
<td>142 tests (0 oscillation)</td>
</tr>
<tr>
<td>MGB</td>
<td>14</td>
<td>18</td>
<td>38 tests (0 oscillation)</td>
<td>40 tests (0)</td>
<td>37 tests (0 oscillation)</td>
</tr>
<tr>
<td>RE auditif</td>
<td>6</td>
<td>20</td>
<td>27 tests (0 oscillation)</td>
<td>33 tests (0)</td>
<td>30 tests (0 oscillation)</td>
</tr>
<tr>
<td>Drugged Conditions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Auditory cortex</td>
<td>24</td>
<td>9</td>
<td>18 tests (6 oscillations)</td>
<td>20 tests (2)</td>
<td>23 tests (8)</td>
</tr>
<tr>
<td>MGB</td>
<td>6</td>
<td>6</td>
<td>6 tests (0 oscillation)</td>
<td>3 tests (0)</td>
<td>3 tests (0 oscillation)</td>
</tr>
<tr>
<td>RE auditif</td>
<td>4</td>
<td>3</td>
<td>4 tests (2 oscillations)</td>
<td>4 tests (2)</td>
<td>4 tests (4)</td>
</tr>
</tbody>
</table>

Undrugged conditions: in each structure, 1–4 electrodes were tested during a given session, and each recording was tested 1–14 days in the various states vigilance. Drugged conditions: within each session 1–5 electrodes were recorded. Each recording site that was recorded in one or several drugs has been previously recorded in undrugged conditions during 1–4 sessions. Both in undrugged and drugged conditions, a “test” corresponds to a “group of trials” (i.e., 2–10 trials from the same recording site, collected with the same ITI, in the same state of vigilance within the same recording session) for an ITI of 10 or 2.5 s. A “test” is considered as oscillatory when at least one trial is oscillatory (i.e., when it showed 3 peaks after the “on” evoked response, see METHODS).

drugged conditions whatever the animal state of vigilance. Figure 4 shows a similar example for a recording obtained in the auditory RE (ITI 10 s). No oscillation was found in the MGB (see Table 1). Although surprising, this lack of oscillations in MGB is in fact in good agreement with the very low proportions of oscillations obtained from this structure in previous studies (Cotillon and Edeline 2000; Cotillon et al. 2000).

**Autocorrelograms revealed oscillations in drugged conditions and in SWS, but not in waking or in PS**

Examples of autocorrelograms obtained from one recording site tested in the different states of vigilance and under the different drugs are presented on Fig. 5. In all drug conditions, the autocorrelograms exhibited regularly spaced large peaks, which clearly signed the presence of oscillations. In contrast, during W or PS, there were no such peaks on the autocorrelograms, whereas during SWS at least three small, but distinct, peaks were observed on the autocorrelograms. On the whole population (Table 2), oscillatory patterns were commonly observed under the three drugs: they represented 66% of the cases under urethan, 60% of the cases under pentobarbital, and 23% of the cases under diazepam. In contrast, they were only occasionally observed during SWS (12% of the cases), and were never observed during W and PS. Oscillations were never observed in transition trials (W to SWS, SWS to PS, or PS to W).

**Comparison between stimulus-locked and non–stimulus-locked oscillations**

**QUALITATIVE COMPARISONS DURING DRUGGED AND UNDRUGGED CONDITIONS.** Parallel analyses of the autocorrelograms and the PSTHs revealed that, in drugged conditions, the oscillations could be either stimulus-locked or non–stimulus-locked. Figure 6, A–C, display examples of stimulus-locked and of non–stimulus-locked oscillations under each drugged condition. As illustrated in this figure, the fact that the oscillations were stimulus-locked, or not, was neither function of the strength of the evoked response nor of the level of spontaneous activity. As illustrated on Fig. 6D, the only type of oscillations that was detected during SWS was the non–stimulus-locked oscillations.

Group data are summarized on Fig. 7A. During drugs conditions, the proportion of stimulus-locked oscillations was a larger (32%) than the one of non–stimulus-locked oscillations (8%; Fig.

![Figure 2](image-url)
We also observed that, for some recordings, groups of trials (10%) can be composed of trials displaying stimulus-locked oscillations and of trials displaying non-stimulus-locked oscillations, suggesting that at the same recording site, oscillations can be stimulus-locked or not. In contrast, during SWS, the small percentage (12%) of groups of trials exhibiting oscillations was only composed of non-stimulus-locked oscillations (Fig. 7A2).

**POWER OF THE STIMULUS-LOCKED AND NON-STIMULUS-LOCKED OSCILLATIONS IN DRUGGED CONDITION.** As stimulus-locked oscillations were only observed in drugged conditions, quantita-
tive comparisons (power and frequency) were only performed for these conditions.

As expected, the values of $P_{\text{max}}$ and those obtained in each frequency band (i.e., $P_{5-15 \text{ Hz}}$, $P_{15-30 \text{ Hz}}$, and $P_{30-90 \text{ Hz}}$) were higher for the trials exhibiting oscillations than those obtained for the nonoscillating trials. This was true for the stimulus-locked oscillations, as well as for the non-stimulus-locked oscillations (unpaired $t$-test, $P < 0.0001$ in all cases).

Figure 8 displays examples of PSTHs, autocorrelograms, and their corresponding power spectra for recordings exhibit-
ing stimulus-locked oscillations (Fig. 8A), non–stimulus-locked oscillations (Fig. 8B), or no oscillation (Fig. 8C). Clear peaks in the power spectra were observed for both stimulus-locked and non–stimulus-locked oscillations. The comparison between the mean values indicates that there was a tendency for the stimulus-locked oscillations to have a higher power than the non–stimulus-locked oscillations ($t(73) = 1.61; P = 0.10$).

The distribution of the frequency for the stimulus-locked and the non–stimulus-locked oscillations obtained in drugged conditions is presented in Fig. 9, A1 and B1. Although the two distributions overlap, they differ significantly ($\chi^2 = 129.36; P < 0.0001$).

**FREQUENCY OF THE STIMULUS-LOCKED AND NON-STIMULUS-LOCKED OSCILLATIONS IN DRUGGED CONDITIONS.** Recall that the frequency of an oscillation was defined as the frequency of the largest peak obtained in the entire power spectrum (5–90 Hz) calculated from the autocorrelogram. The distribution of the frequency obtained for the two types of oscillation is presented in Fig. 9, A2 and B2; the frequencies of the stimulus-locked oscillations were from 6 to 19 Hz (mean, 12.9 Hz; median, 13.4 Hz), and the frequencies of the non–stimulus-locked oscillations were from 10 to 17 Hz (mean, 14.1 Hz; median, 11.7 Hz). The two distributions differed ($\chi^2 = 15.84; P = 0.03$), but the mean values did not differ [unpaired $t$-test, $t(73) = 1.05; P = 0.30$].

**Comparison of evoked oscillations between drugged conditions and SWS**

**QUALITATIVE COMPARISONS BETWEEN DRUG AND SWS.** When a recording exhibited non–stimulus-locked oscillations on auto-
correlogram under natural SWS, it also exhibited oscillations in the following session performed in drugged conditions. The reverse was not true; many recording sites which did not display oscillations during SWS exhibited oscillations in drug conditions (see Fig. 7A). Nevertheless, if we considered only the non–stimulus-locked oscillations, the percentage did not differ significantly between drug conditions and SWS (8% vs. 12%; \( \chi^2 = 1.00; P = 0.32 \)).

**POWER OF THE NON–STIMULUS-LOCKED OSCILLATIONS OBTAINED IN DRUGGED CONDITIONS AND IN SWS.** As the only oscillations obtained during SWS were non–stimulus-locked, the between states comparisons were based on them. When the mean values of the \( P_{9-15 \text{ Hz}} \), \( P_{15-30 \text{ Hz}} \) and \( P_{30-90 \text{ Hz}} \) obtained from SWS and from the drug conditions were compared, there was no significant difference \( [t(26) = 1.51, P = 0.15 \text{ for the } P_{9-15 \text{ Hz}}; \ t(26) < 1 \text{ for } P_{15-30 \text{ Hz}} \text{ and } P_{30-90 \text{ Hz}}] \). Also, there was no significant difference between the \( P_{\text{max}} \) value obtained in SWS and in drug conditions \( [t(26) = 1.36, P = 0.18] \). As shown in Fig. 9, the distributions of the power values obtained in SWS and in drug conditions largely overlapped and did not differ (compare Fig. 9, B1 and C1; \( \chi^2 = 1.72; P = 0.89 \)).

**FREQUENCY OF THE NON–STIMULUS-LOCKED OSCILLATIONS OBTAINED IN DRUGGED CONDITIONS AND IN SWS.** As for the power, the comparisons between SWS and drug conditions were based on the non–stimulus-locked oscillations. The mean frequency obtained in SWS was 14.4 Hz, whereas it was 14.1 Hz in drug conditions (unpaired 1-t-test, \( t(26) < 1 \), ns). The distribution of the frequencies obtained during SWS and in drug conditions are presented in Fig. 9, B2 and C2. The frequency range was broader during SWS; the difference between the two distributions almost significantly differed (\( \chi^2 = 13.85; P = 0.09 \); KS: \( P = 0.053 \)).

**Comparison between spontaneous and evoked oscillations**

**QUALITATIVE COMPARISON BETWEEN SPONTANEOUS AND EVOKED OSCILLATIONS IN DRUGGED CONDITIONS AND SWS.** The relationship between the presence of spontaneous and evoked oscillations is illustrated by the examples presented in Fig. 10. In drug conditions, when oscillations were present during spontaneous activity (16/168 trials), they were also present in evoked activity (Fig. 10, A1 and A2). However, oscillations can be observed in evoked activity without any spontaneous oscillations (Fig. 10, A3 and A4). During natural SWS, oscillations can be observed either both during spontaneous and evoked activity (Fig. 10, B1 and B2), only during evoked activity (Fig. 10, B3 and B4), or only during spontaneous activity (Fig. 10, B5 and B6).

Spontaneous oscillations were observed only for a low percentage of recordings both under drugs and during SWS. As shown in Fig. 7B, during drugged conditions, 14% of the recordings displayed spontaneous oscillations, a percentage that did not differ from the 8% of recordings that displayed nonstimulus locked evoked oscillations in these conditions (\( \chi^2 = 2.1, P = 0.15 \)). During SWS, there was 8% of spontaneous oscillations, a percentage that did not differ from the one of non–stimulus-locked evoked oscillations (12%). This suggests that both during drug conditions and during SWS stimulus presentation did not trigger more nonstimulus locked oscillations than those already present during spontaneous activity. Last, in contrast with the evoked oscillations, the distributions of spontaneous oscillations obtained under drugs and during SWS did not differ significantly (14% vs. 8%, \( \chi^2 = 2.1, P = 0.15 \)).

As oscillations were mainly observed in drugged conditions, the quantitative comparisons between spontaneous and evoked oscillations were only performed under these conditions.

**POWER OF THE OSCILLATION OBTAINED IN SPONTANEOUS AND EVOKED ACTIVITY.** Figure 11A shows the relationship between the power of the oscillations during spontaneous and evoked activity. When both stimulus-locked and non–stimulus-locked oscillations were considered, the values obtained in evoked activity were higher than during spontaneous activity \( [t(15) = 2.75, P = 0.02] \). This relation was also true when only the non–stimulus-locked oscillations were considered \( [t(7) = 3.68, P = 0.008] \). This suggests that, in drugged conditions, stimulus presentation intensified oscillations that were already present during spontaneous activity.

**FREQUENCY OF THE SPONTANEOUS AND EVOKED OSCILLATIONS IN DRUGGED CONDITIONS.** Figure 11B shows the relationships between the frequency of the oscillations during spontaneous and evoked activity. When both the stimulus-locked and the non–stimulus-locked oscillations were considered, the values obtained in evoked activity were similar to those obtained during spontaneous activity \( (t < 1 \text{, ns}) \). When only the non–stimulus-locked oscillations were considered, the frequencies obtained in evoked activity and in spontaneous activity were also similar \( [t(7) = 1.6, P = 0.15] \). This indicates that there was no change in the oscillation frequency at stimulus presentation.
Comparisons between the different drugs

QUALITATIVE COMPARISONS BETWEEN DRUGS. Over the three structures, the percentage of tone-evoked oscillations observed on the autocorrelograms was higher under anesthesia than under diazepam (urethan vs. diazepam: \( \chi^2 = 6.56; P = 0.01 \); pentobarbital vs. diazepam: \( \chi^2 = 3.66; P = 0.06 \), but was not different between the two anesthetics (\( \chi^2 = 0.31; P = 0.58 \)). In the auditory cortex, the percentage of stimulus-locked oscillations tended to be lower under diazepam (\( \chi^2 = 3.10, P = 0.08 \) and \( \chi^2 = 3.68, P = 0.06 \) with pentobarbital and urethan, respectively). In the auditory RE, the stimulus-locked oscillations were present in 100% of the cases under urethan, whereas they were observed in only 50% of the case under pentobarbital and diazepam. The percentage of spontaneous oscillations was low, whatever the drug used, and was not different between the drugs (lowest \( P \) value, \( P = 0.48 \)).

POWER OF THE OSCILLATIONS BETWEEN THE DIFFERENT DRUGS. Using the 10-s ITI, the values obtained under pentobarbital were significantly higher from those obtained under urethan and under diazepam [unpaired \( t \)-test, \( t(64) = 2.71, P < 0.009 \) and \( t(45) = 3.24, P < 0.002 \), respectively], and the distributions of the power values differed between pentobarbital and diazepam (\( \chi^2 = 20.63; P = 0.04 \)).

FREQUENCY OF THE OSCILLATIONS BETWEEN THE DIFFERENT DRUGS. The frequency of oscillations obtained under diazepam (13–17 Hz; mean, 15 Hz; median, 15 Hz) was slightly higher than that observed under urethan (12–16 Hz; mean, 13.5 Hz).

**FIG. 6.** PSTHs and autocorrelograms of stimulus-locked oscillations (left) and of non-stimulus-locked oscillations (right) obtained under the different drugs (A–C) and in slow-wave sleep (D). In A–C, the PSTHs (10-ms bin size) and rasters present the responses based on 55 tone presentations (ITI: 2.5 s). Note that in each drug condition, the “on” phasic response was followed by regularly spaced discharges observed on the rasters and PSTHs. Note that there was only non-stimulus-locked oscillations in slow-wave sleep (D). Conventions are as in Figs. 3 and 4 for the PSTHs and as in Fig. 5 for the autocorrelograms.
Evoked Oscillations in Drugged and Undrugged Conditions

Different types of oscillations were triggered by acoustic stimuli in the thalamo-cortical auditory system of drugged and unanesthetized animals. In drugged animals (under anesthesia or high doses of Valium), the oscillations were composed of both stimulus-locked and non-stimulus-locked oscillations, whereas in unanesthetized animals they were only composed of non-stimulus locked oscillations. In undrugged animals, these oscillations were only observed during SWS episodes. In drugged and in undrugged animals, the oscillations were mainly in the low-frequency range (5–15 Hz). Both in drugged and undrugged conditions, a small percentage of recordings displayed spontaneous oscillations.

Comparisons with previous findings in the thalamo-cortical auditory system of anesthetized animals

Tone-evoked oscillations in the low-frequency range (about 10 Hz) have long been noted from single unit recordings in MGB (Aitkin et al. 1966; Galambos et al. 1952). Later, similar oscillations have been reported in the auditory sector of the reticular nucleus (Shosaku and Sumitomo 1983) and at the cortical level (Dinse et al. 1997; Maldonado and Gerstein 1996). Thus the dominant frequency range observed here is largely in agreement with previous studies performed in anesthetized animals. The low percentage of oscillations in the MGB is probably the consequence of a sampling bias in our experiment: our recordings mostly came from the dorsal MGB, an area where rhythmic responses were observed for only 4% of the cells, whereas they were observed for 42% of the cells in the ventral MGB (Bordi and Leduc 1994).

While we did not detect high-frequency oscillations from multiunit recordings, such oscillations have been described in the auditory cortex of halothane anesthetized rats using LFP recordings (Barth and MacDonald 1996; Franowicz and Barth 1995; MacDonald and Barth 1995). It is crucial to mention that the 40-Hz oscillations were detected with trains of clicks presented at 40 Hz (Franowicz and Barth 1995) but not with single clicks. In fact, presentation of a single click suppressed the spontaneous 40-Hz oscillations which re-appeared a few hundreds of milliseconds later (Franowicz and Barth 1995; MacDonald and Barth 1995). Obviously, observation of oscillations in LFP does not guarantee their presence in multiunit or single-unit recordings. In the visual cortex, a much larger proportion of oscillations was detected from LFP than from multiunit or single-unit recordings. For example, Gray and Singer (1989) described 97% of oscillations based on LFP recordings, but only 47% are based on multiunit recordings. Nonetheless, in the auditory cortex Brosch et al. (2002) reported about the same percentage (76% and 60%) of high-frequency oscillations using LFP and multiunit recordings in ketamine anesthetized monkeys. These oscillations seem to differ from those observed in rat by their frequency (40 Hz in rat vs. 45–80 Hz in monkey) and their latency (350 ms in rat vs. 100 ms in monkey).

Last, two other methodological factors have to be considered when comparing the results obtained among studies. First, we should mention that Barth and MacDonald (1996) as well as Brosch et al. (2002) used anesthetic agents that were not utilized in our experiments (halothane and ketamine). Second, it is important to remind that the criteria used to determine what is an oscillatory recording can lead to different results (Young et al. 1992). In other sensory modalities, two techniques have been used to determine if an autocorrelogram was oscillatory. Some laboratories used the fit of the autocorrelogram by a Gabor function as a decision criterion, while others used a Fourier Transform of the autocorrelogram. No consensus exists in that domain. Adjusting the autocorrelograms by a Gabor function and setting up three or four criteria to decide if the fit corresponds to an oscillation can still lead to classify as oscillatory autocorrelograms that are weakly oscillatory using visual criteria (e.g., the discussion in Young et al. 1992). When using a Fourier Transform of the autocorrelogram, one has to face the most appropriate criterion to decide what is indeed a peak in the power spectrum. Here too, there is no general agreement. In some cases, the authors computed a ratio between the power at a particular frequency and the power in the 250–500 Hz range, a ratio > 1.5 being considered as significant (Ghose and Freeman 1992). In other cases, the “background spectral” was subtracted by adjusting a curve between 20 and 90 Hz (Bauer et al. 1995; Kruse and Eckhorn 1996). The use of a signal-to-noise ratio > 1.5 was accused of leading to overestimations of the percentage of oscillations (Gray and Di
Prisco 1997, but for information against such a claim see Molotchikoff and Shumikhina 2000). In a previous study, (Cotillon et al. 2000), we tried to develop objective criteria to separate "reliable" and "labile" oscillations based on several measurements taken from power spectrum. We used the power of the main peak ($P_{\text{peak}}$), the "relative" power of the peak ($P_{\text{rpeak}}$; relative to the entire power spectrum), and a signal-to-noise ratio ($P_{\text{peak}}/P_{\text{noise}}$), where $P_{\text{peak}}$ was the power at the main peak and $P_{\text{noise}}$ was the power in the background spectrum. It was never possible to set systematic cut-off values for any of these parameters. In all cases, including the signal-to-noise ratio, the distributions of the parameter overlapped between reliable and labile oscillations (see Fig. 2 in Cotillon et al. 2000). Here, each power spectrum (and each autocorrelogram) was examined independently by the two co-authors, and it was only in case of agreement that the recordings were classified as oscillatory or not. This procedure was drastic, as attested by the fact that a large difference exists between the power values obtained for the oscillatory and for the nonoscillatory recordings.

**FIG. 8.** Examples of power spectra in the case of stimulus-locked oscillations (A), non–stimulus-locked oscillations (B), and no oscillation (C). A: recording collected in the auditory RE under urethan anesthesia. Oscillations can be observed both on the raster and PSTH (click of 1 ms; ITI of 10 s), as well as on the autocorrelogram. The power spectrum of the autocorrelogram reveals an important peak at 12 Hz. B: recording collected in the auditory cortex under pentobarbital anesthesia. The PSTH shows the response to a 9-kHz tone (100 ms; ITI of 2.5 s) and does not reveal any oscillation. The autocorrelogram shows regularly spaced peaks that indicated a non–stimulus-locked oscillation. The power spectrum of the autocorrelogram reveals a peak at 11 Hz. C: recording collected in the auditory cortex under pentobarbital anesthesia. The PSTH shows the response to a click (1 ms; ITI of 2.5 s) and does not reveal any oscillation. There was no particular peak both in the autocorrelogram and in the power spectrum of the autocorrelogram. The unit for the power spectrum is spikes$^2$. 

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Comparisons with previous findings in other sensory systems of anesthetized animals

Initially, most of the studies concerning stimulus-evoked oscillations have been performed in the visual cortex of anesthetized animals (see for reviews Engel et al. 1992; Frégnac et al. 1994; Singer 1990). They have mainly reported non–stimulus-locked oscillations in the gamma range (30–90 Hz). Low-frequency oscillations have been reported in a few studies in anesthetized cat (stimulus-locked: Dinse et al. 1997; non–stimulus-locked: Bringuier et al. 1992, 1997), but not in monkey. In fact, in the cat visual cortex, slow movements of visual stimuli can induce changes from low- to high-frequency (Kruse and Eckhorn 1996). In the monkey visual cortex, low-frequency oscillations have been reported during spontaneous activity, but these oscillations were largely attenuated at stimulus presentation (Eckhorn 2000; Schanze and Eckhorn 1997). Nevertheless, the proportion of low-frequency oscillations in the visual cortex of cat or monkey might have been underestimated. Indeed, these studies were interested in high-frequency oscillations and not in low-frequency oscillations. In fact, an important degree of temporal overlap exists between the different frequency ranges: when power bispectra were used, 70% of the oscillatory events were found to occur simultaneously in the different frequency ranges (Schanze and Eckhorn 1997).

Comparisons with previous findings in unanesthetized animals

Obviously, several factors can account for the differences observed between the results from the visual literature and our data: besides the difference in sensory modality, species differences should also be considered; high frequencies were more often observed in cat and in monkey than in rat where low frequencies were often reported. Also, the anesthetic conditions could participate to the differences in oscillatory patterns (see Frégnac et al. 1994); in the cat visual cortex, all the studies that reported high-frequency oscillations have used NO/O2 anesthesia, whereas lower oscillations were obtained under alfathesine anesthesia (Bringuier et al. 1992, 1997). However, arguing against this factor, one can point out that low-frequency (stimulus-locked) oscillations have also been observed in cat anesthetized with NO/O2 (Dinse et al. 1997).

Conflicting results have been obtained from awake subjects in various sensory modalities.

In the cat visual cortex, all the studies using LFP, multiunit, and single-unit recordings have described high-frequency oscillations (Gray and Di Prisco 1997; Kruse and Eckhorn 1996; Molotchnikoff and Shumikhina 1996, 2000). Gray and Di Prisco (1997) even mentioned a higher percent of oscillations in unanesthetized cat compared with anesthetized one (26.4%...
FIG. 10. Examples of autocorrelograms illustrating the relationship between spontaneous and evoked oscillations. In drug conditions, oscillations were detected either on spontaneous and evoked activity (A1 and A2) or only in evoked activity (A3 and A4). During natural SWS, the oscillations were detected either on spontaneous and evoked activity (B1 and B2), only on evoked activity (B3 and B4), or only on spontaneous activity (B5 and B6).
The situation is different in human and nonhuman primates. In monkey, some studies have found a high percentage of oscillations in the area IT (61% in Friedman-Hill et al. 2000; 71% in Eckhorn et al. 1993), whereas others did not find any (Tovee and Roll 1992; Young et al. 1992). A similar discrepancy also exists in the area MT: Kreiter and Singer (1992) reported 58% of oscillations, whereas Bair et al. (1994) did not detect any. In human, oscillations in the gamma range have often been reported on EEG recordings (Lachaux et al. 2000; Lutzenberger et al. 1995; Tallon-Baudry et al. 1996–1998). Nevertheless, cautions have to be exercised: when EEG power spectra were compared in human and monkey, visual stimulation elicited an increase in gamma range in the monkey EEG, but not in the human EEG (Juergens et al. 1999).

In the somatosensory cortex, spontaneous 30-Hz oscillations have been observed from single-unit recordings in monkeys performing a reaction-time task, but these oscillations disappeared at stimulus presentation (Ahissar and Vaadia 1990). In rat, LFP recordings have revealed an opposite pattern: spontaneous oscillations were not observed, but moving the vibrissae induced oscillations (Jones and Barth 1997).

In the auditory system, the only study looking for oscillations in awake animals was performed in the auditory cortex of mustached bat (Horikawa et al. 1994). Multiunit and single-unit recordings did not reveal any oscillation on PSTH and only 2% on autocorrelogram. Nevertheless, this study used a 1-s ITI, an interval that we found to be too short to evoke low-frequency oscillations in anesthetized rats (Cotillon et al. 2000). In human, oscillations of evoked potentials (mostly in the gamma range) have been reported at stimulus presentation (Galambos et al. 1981; Jacobson and Fitzgerald 1997; Yordanova and Kolev 1997; Yordanova et al. 1997).

Thus, except in the cat visual cortex, the presence of oscillations in the thalamo-cortical system of unanesthetized animals remains a matter of conflicts. In the case of the auditory system, further investigations should be done in animals, using both units and LFP recordings, to determine if their unambiguous presence in human is species-specific or is due to the type of recordings.

Potential mechanisms and functional implications

Previously, we characterized the low-frequency stimulus-locked oscillations under urethan anesthesia (Cotillon and Edeline 2000; Cotillon et al. 2000). Striking similarities were noted between these oscillations and the spontaneous spindles: 1) they are in the same frequency range (5–15 Hz), 2) they occur with an inter-tone (or an inter-event) interval of ≥2 s (Bal and McCormick 1996; Cotillon et al. 2000), and 3) they both seem to origin from interactions between thalamic relay cells and thalamic reticular cells, without a dominant contribution of the cortical level (Bal et al. 1995; Cotillon and Edeline 2000). Nonetheless, an important difference exists between these oscillations and the spindles. Stimulus-locked oscillations were rarely synchronized between the thalamic and cortical levels, as well as between the two hemispheres (Cotillon et al. 2000), whereas spindles are highly synchronized over the cortex and the thalamus (Contreras et al. 1997a), as well as between the two hemispheres (Contreras and Steriade 1996). The involvement of cortical activity was shown to explain the long-range synchronization of spindles (Contreras et al. 1996, 1997b; Destexhe et al. 1999); the absence of cortical involvement in tone-evoked oscillations could explain their lack of long-range synchronization.

The major finding of the present study is that stimul-
locked oscillations are only present in drug conditions and never during undrugged conditions, whatever the animal state of vigilance. Their presence can be detected under different drugs acting on GABAergic receptors (pentobarbital, valium) or not (urethan). A possibility is that this type of oscillations requires important levels of hyperpolarization. It is well known that the presence of bursts, underlying the low-frequency oscillations, requires an hyperpolarized state of the thalamic cells (Contreras and Steriade 1996; Curro-Dossi et al. 1991, 1992; Deschenes et al. 1984; Lo et al. 1991; Lu et al. 1992). During SWS, only modest hyperpolarizations were observed at the thalamic level (only 4 mV in the cat LGd, see Hirsch et al. 1983). One can argue that this moderate level of hyperpolarization is, however, enough for the presence of a small percentage of spontaneous spindles. Again, the cortical waves of activity, which synchronize massively the discharge of both RE and thalamic cells during spindles, can play a key role; by acting more strongly onto RE cells than onto thalamic cells (Golshani et al. 2001), spindles can be triggered by cortical activity even with relatively modest levels of hyperpolarization. In contrast, sensory stimuli \( I \) activate thalamic cells before the RE cells and \( 2 \) only activate a small population of thalamic cells (those responding to a particular frequency or to click presentation). Thus because of the small size of the cell populations and because the triggering event starts by an excitation of thalamic cells (and not by an excitation of RE cells), the thalamo-reticular network probably needs to be more strongly hyperpolarized for the occurrence of stimulus-locked oscillations than for the spontaneous spindles.

As the spontaneous spindles, the non–stimulus-locked oscillations might require only moderate levels of hyperpolarization, and as a consequence, they were detected during SWS. In line with this view, the non–stimulus-locked oscillations were found in the same proportion than spontaneous oscillations both in SWS and in drugged conditions. They were in the same frequency range (5–15 Hz), and as spontaneous oscillations, they were observed in similar proportions in SWS and under drugs. Note that in drugged or undrugged conditions the spontaneous and the non–stimulus-locked oscillations were present at a lower percentage and with a lower power than the stimulus-locked oscillations.

According to this scheme, the lack of oscillations during W and PS is simply the consequence of a more depolarized level during these states of vigilance. Obviously, the large literature that has involved the oscillations in cognitive functions (particularly in the visual system) will argue that the lack of oscillations during waking is due to the absence of an attentional demand, and that training the animals in behavioral tasks can reveal a higher proportion of oscillations. However, an alternative view is that the presence of evoked oscillations might not be an advantage for processing auditory information and might even set the temporal limits of auditory perception. For example, in anesthetized cats, the ability of neurons to follow a train of clicks was inversely correlated with the duration of spontaneous oscillations (Kenmochi and Eggermont 1997). Also, a relationship was found between the frequency of these oscillations and the best modulation frequency of units in the auditory cortex of anesthetized rats (Gaese and Ostwald 1995). Thus the lack oscillations in awake animals might, in fact, increase the temporal performance of auditory neurons and should allow detection of higher periodicity in complex stimuli. Because neuronal oscillations have often been viewed as a way to synchronize neuronal populations involved in perceptive processes, the present hypothesis clearly challenges the notion that visual perception and auditory perception rely on common mechanisms. By essence, auditory perception is temporal, and the need to rapidly respond to changes in the auditory stream is probably more crucial than in the visual system. Thus the absence of oscillations in awake animals might allow each neuron to process the auditory inputs independently of its neighbors, and should allow a fine grain analysis of auditory messages.

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