Anesthesia Changes Frequency Tuning of Neurons in the Rat Primary Auditory Cortex

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INTRODUCTION

To date, most studies on sensory processing in the mammalian brain have been performed at the single neuron level in anesthetized animals. This approach is based on the assumption that response properties of central neurons are not changed by anesthetic agents. However, in the auditory system not much is actually known about anesthetic influences on neural responses along the central pathway. Although some investigators have been aware of anesthetic influences on neural processing for a long time (Erulkar et al. 1956), only few attempts were made to quantify these effects. Several studies have described a predominantly depressant effect of different anesthetic agents on spontaneous activity (chloral hydrate, Erulkar et al. 1956; pentobarbitone, chloralose, and halothane, Evans and Nelson 1973; pentobarbital, Kuwada et al. 1989; ketamine and pentobarbital, Zurita et al. 1994). Neurons in the ventral cochlear nucleus of the cat showed only moderate inhibitory influences during Equithesin anesthesia. Thus when describing response properties of central auditory neurons, the animal’s anesthetic state has to be taken into account.

METHODS

The effects of Equithesin anesthesia were quantified in single neurons from layers II–IV of primary auditory cortex (Paxinos and Watson 1986). Either standard tungsten electrodes (F. Haer, 7–12 MΩ) or, in most cases, two-channel electrodes (stereotrodes, 25-μm nichrome wires) were used. Usually three movable electrodes per animal, each mounted in a separate miniature microdrive (Wilson et al. 1991), were chronically implanted 1 wk prior to the experiments. In each recording session (21 sessions from 6 animals), a set of single neurons (5 neurons on average) was recorded. Electrodes were moved to a new position at the beginning of each recording session. Experimental procedures were according to federal regulations and approved by the local animal care committee.

After recording, each electrode track was marked with two or three electrolytic lesions. The brain was sectioned, and the 40 μm-sections were stained in two series: Nissl and myelin staining (Schmued 1990). Recording tracks were reconstructed from the lesions, and it was determined whether recording sites were located in the primary auditory cortex (area Te 1) (Zilles 1985). Neural signals were buffered with a miniature eight-channel pre-
amplifier (Buzsáki et al. 1989), amplified, filtered (500 Hz – 5 kHz), and fed into a computer. Single units were defined by a spike-sorting algorithm based on clustering of parameter values calculated from the spike waveform. In case of stereotrodes, waveforms of action potentials are sampled through two closely spaced recording tips. The relative amplitudes of unit signals on the two channels, which are in part determined by the spatial locations of the spike generators relative to the two recording surfaces, are used to aid unit isolation (McNaughton et al. 1983). Much care was taken to include only stable units as determined by carefully inspecting spike waveforms on-line and again during off-line analysis (Fig. 2). The recording technique used allows for very stable recording over several hours (Wilson and McNaughton 1993, 1994), therefore only few units had to be removed from the dataset.

Rats were trained to sit quietly during recordings on a post in a shielded, sound-attenuating room and keep their head in a forward position. To determine frequency response areas, pure tones (100 ms duration) with intensities of 30, 50, 60, and 70 dB SPL and 22 different frequencies between 2 and 76 kHz (1/4-octave spacing) were delivered in a random sequence with recalculated randomization for each repetition of all stimulus combinations. Repetition rate of stimuli was around 1 Hz (randomly varied up to 30%). Twenty repetitions of each stimulus always were delivered (Fig. 1). Because unrestrained animals cooperate for only a limited amount of time, the testing at additional sound pressure levels or of frequencies at higher resolution was not possible. Therefore we tested the sound pressure range of normal communication and additionally 30 dB SPL as the minimal threshold of a majority of neurons under anesthesia was at this level (Sally and Kelly 1988). Following the characterization of responses in the awake animal, we injected Equithesin [chloral hydrate (4.26%), sodium pentobarbital (0.96%), and magnesium sulfate (2.12%) in aqueous solution with 9.5% ethanol]. The dosage level used (2.5 ml/kg ip) is about two-thirds of that required for surgical anesthesia. To simulate the state of “light anesthesia,” as it is often described for electrophysiological recording, we waited for 30–45 min. after injection until first reflexes (cornea, paw withdrawal reflex) had reappeared (Kuwada et al. 1989).

Frequency response areas were determined by evaluating the stimulus-evoked activity on a trial-by-trial basis using Wilcoxon’s matched-pairs signed-rank (MPSR) test (Siegel and Castellan 1988). It was tested whether a significant number of trials showed an increase (for excitation) or decrease (for inhibition) of activity in the first 20 ms of stimulus-driven activity relative to spontaneous activity (20 ms before stimulus onset). The minimal neuronal latency was always taken into account. Results from all stimulations were combined into one plot, and the level of significance was coded in dot size ($P < 0.05$, $P < 0.01$, $P < 0.001$; evaluation of Fig. 1 is shown in Fig. 2B). Minimal size of a “significant frequency response area” or a separate subregion of it was at least one large dot ($P < 0.001$), two adjacent dots with one at least medium size ($P < 0.01$), or three adjacent small dots ($P < 0.05$). For further analysis, we determined the response area’s size (number of dots), characteristic frequency (CF), and minimal threshold (lowest level with significant activity). Tuning sharpness was measured by an index relating minimal threshold to the size of the response area (i.e., number of dots): $S$ (tuning sharpness) = 100/(size of the response area * minimal threshold [dB]).

To compare response variability under awake and Equithesin-anesthetized conditions, we calculated the coefficient of variance (CV) as (variance/mean firing rate), assuming a Poisson statistics for the underlying spiking process. The CV was calculated at two frequency/intensity combinations inside the response area, one in its center at 70 dB SPL, and one at minimal threshold and characteristic frequency. Calculation of CV was based on the same stimulus-induced activity as was used for statistical evaluation of response areas.

FIG. 1. Effect of pentobarbital/chloral hydrate anesthesia (Equithesin) on the activity of a single neuron in the rat auditory cortex. Responses are shown under awake (left) and anesthetized conditions (right). Depicted is single trial activity (dot display) and summed activity [perstimulus time histogram (PSTH)] determined with pure tone stimulation at 7 different stimulus frequencies (between 19.0 and 53.8 kHz, around the response area of this neuron) and at 4 different sound intensities. Dot displays (20 trials, 1st trial at top) and PSTHs (binwidth 5 ms) for each frequency/intensity-combination show activity 50 ms before stimulus onset (vertical bar in dot display) and 100 ms during the stimulus. Spontaneous and stimulus-induced activity is reduced under Equithesin anesthesia, in this case more than average. Dot displays indicate the typical degree of variability in responses of neurons in the rat auditory cortex. Statistical evaluation of these responses is shown in Fig. 2B.
RESULTS

All neurons \((n = 108, \text{ recorded from 21 recording sites in 6 animals})\) were responsive to clicks and/or white noise stimuli in the awake state. All cells were from middle cortical layers (II–IV) of area Te 1, the presumed primary auditory cortex (Sally and Kelly 1988). Most neurons were recorded in cortical layers II/III. We found no obvious layer-specific differences in the effects of Equithesin anesthesia.

As expected, Equithesin anesthesia reduced spontaneous neural activity. The distribution of spontaneous activity rates was strongly shifted (Fig. 3), resulting in an average reduction to 20.5\% of the awake activity level (Wilcoxon MPSR, \(n = 108, S = 2791.5, P = 0.0001\)). However, all units still showed some low rate activity under light anesthesia. Stimulus-induced activity was reduced under anesthesia to a similar extend and response patterns were altered. Most of the phasic-tonic response characteristics were changed to a phasic response (Fig. 1). Of 15 neurons that responded phasic-tonically in the awake state, only one showed excitation and two inhibition after the initial on response (1st 20 ms of stimulus duration) in the anesthetized state.

The effect of Equithesin anesthesia on frequency response areas was strong in several respects. In the awake state, 80 neurons (74\%) had tuned frequency response areas. Under anesthesia, however, most neurons lost their response area, as shown in Fig. 2A. Only 23 neurons (29\% of the tuned ones) were still significantly tuned. Most of those still responded in the same frequency range but with a smaller response area (reduction of response area: Wilcoxon MPSR test, \(n = 23, S = 115.5, P < 0.0001\); Figs. 2B and 3B). Characteristic frequency (CF, frequency at minimal threshold) did not change significantly (paired \(t\)-test: \(n = 23, t = 1.86, P = 0.077\)). Some neurons, however, shifted their response areas to a different frequency range compared with the awake state (Fig. 2C).

Even rather broad response areas, or response areas with two or three separate subregions in the awake state, disappeared or were reduced to one narrow area under anesthesia (Fig. 3B).

Note that the measure we used for response evaluation is rather independent of absolute firing rate; therefore these effects on the frequency response areas occurred in addition to the anesthesia-induced strong reduction of spontaneous and stimulus-evoked activity under anesthesia. Although the latency of responses to pure tone stimulation was significantly increased (paired \(t\)-test: \(n = 23, t = -2.79, P = 0.011\)), this did not effect the analysis above. The analysis window still included responses with increased latency.

Response variability, as measured with CV (coefficient of variance), was not changed by Equithesin anesthesia. This was the case for trial-by-trial variability of mean firing rate in the center of the response area (paired \(t\)-test: \(n = 23, t = 1.3, P = 0.17\) and close to minimal threshold (paired \(t\)-test, \(n = 23, t = -1.06, NS\). Therefore increased variability can be largely ruled out as an additional factor causing the reduction of response areas as noted in the preceding text.

This general reduction in the size of response areas was a change toward narrower, i.e., sharper tuning. Despite a strong reduction in the size of response areas, minimal threshold did not change significantly (Wilcoxon MPSR test, \(n = 23, S = -7.5, P = 0.45\)). This still holds true for the subset of neurons with thresholds higher than 30 dB under anesthesia (paired \(t\)-test: \(n = 11, t = 0.32, P = 0.32\), which was investigated to ensure that the lower limit of sound pressure levels tested had no effect. Sharpness of tuning is usually described by a \(Q\) value that relates CF to the width of the response area at some level above threshold. The stimulation procedure used here, namely fixed frequency/intensity combinations with a logarithmic spacing of frequency, does not allow for the measurement of \(Q\) values comparable to those in the literature. We used a measure, instead, that is reciprocal to the size of the response area and minimal threshold. As shown in the preceding text (Fig. 2, B and C), tuning sharpness was increased under anesthesia by a factor of 1.6 on the average (paired \(t\)-test: \(n = 23, t = 4.47, P = 0.0002\); Fig. 3C). This corresponds to a reduced bandwidth of the response areas at 50 dB.

FIG. 2. Anesthesia-induced changes of frequency response areas in the rat auditory cortex. Depicted are plots of response areas for 3 neurons (A–C) together with waveform samples of spikes, each for the awake (left) and anesthetized state (right). Each plot shows the statistical evaluation of activity after stimulation with pure tones of 22 different frequencies and 4 different intensities. Dots indicate significant excitation (filled circle) or inhibition (open circle) at a given frequency/intensity combination [Wilcoxon matched-pairs signed-rank (MPSR) test]. The level of significance is coded in dot size (small: \(P < 0.05\), medium: \(P < 0.01\), large: \(P < 0.001\)). Dashes indicate nonsignificant results. Statistical evaluation of the neuronal activity in Fig. 1 is depicted in B, right part (19.0–53.8 kHz) of each panel (awake, anesthesia). Most neurons completely lost their response area under Equithesin anesthesia as shown in A. Response areas of single neurons under anesthesia were narrower but stayed mostly in the same frequency range as in the awake state (B). Some response areas decreased and, in addition, were shifted in frequency (C). Waveform samples are shown for each neuron for both the awake and anesthetized state [window size: 1 ms (horizontal) and 250–300 \(\mu\)V (vertical, fix value for all windows of a given neuron)]. One window per plot is shown for single electrode recordings (as in C), 2 windows are shown for stereotrode recordings, one for each recording channel (as in A and B).
dB SPL under Equithesin anesthesia, measured by a “$Q$ at 50 dB” (CF/bandwidth at 50 dB SPL; Wilcoxon MPSR test, $n = 19$, $S = 54.5$;  $P = 0.016$). Median $Q$ at 50 dB values in the awake state were increased from 2.14 (25% quartile = 1.12, 75% quartile = 5.69, $n = 20$) to 4.36 (25% quartile = 1.77, 75% quartile = 6.09, $n = 19$).

Some neurons seemed to be more susceptible to Equithesin anesthesia than others and loose their response area in the anesthetized state. We tested if these had special characteristics already under awake conditions. Neurons that lost their response areas under anesthesia showed lower spontaneous activity rates in the awake state compared with neurons showing response areas both in the awake and anesthetized state (Wilcoxon test: $N_1 = 57$, $N_2 = 23$, $z = 3.23$, $P = 0.0013$; Fig. 4). Their response areas were smaller (Wilcoxon test: $z = 2.99$, $P = 0.0028$) and their minimal thresholds were higher (Wilcoxon test: $z = -2.72$, $P = 0.0028$). All three facts indicate possibly stronger inhibitory influences in the neurons more susceptible to anesthesia.

**DISCUSSION**

The observed changes are consistent with the hypothesis of an overall enhancement of inhibition. As neurons with small response areas and low spontaneous activity (in the awake state) are most susceptible, the effects seem to be a general reduction of activity and not some action on neuronal thresholds. The observed sharpening of response areas together with an unchanged minimal threshold is best explained by increased inhibitory sideband activity under anesthesia.

It is very likely that both drugs included in Equithesin, pentobarbital and chloral hydrate, participate in this general inhibitory effect. Both drugs increase GABA_A-mediated chloride currents. Other than the directly acting pentobarbital (Tanelian et al. 1993), it is not chloral hydrate itself, but its main active metabolite, trichlorethanol, that activates GABA_A receptors (Peoples and Weight 1994). The site of action in the ascending auditory pathway cannot be localized easily. Effects of chloral hydrate or pentobarbital on spontaneous activity or auditory processing have been described at most levels peripheral to the auditory cortex (Kuwada et al. 1989; Young and Brownell 1976; Zurita et al. 1994). The especially strong effects described here for the auditory cortex might be caused by a combination of anesthetic actions at multiple levels of the ascending pathway.

The effects of chloral hydrate and pentobarbital on neuronal activity observed here are comparable to those reported for auditory structures in other mammalian species. Besides a strong reduction of spontaneous activity, as noted in the preceding text (see INTRODUCTION), changes of response patterns induced by pentobarbital anesthesia were mainly described for the auditory midbrain and the cortical level. As shown here, often only the phasic parts of excitatory response components were still visible (Kuwada et al. 1989; Zurita et al. 1994). Effects on inhibitory components were less pronounced. In the
cat auditory cortex, sustained inhibition disappeared to a high percentage (Zurita et al. 1994) but not in the rabbit inferior colliculus (Kuwada et al. 1989). Response latency was generally increased, as it was described here for the rat auditory cortex. Anesthesia-induced changes of response areas, as presented here, were indicated in earlier data from the cat auditory cortex. They included reduced frequency range of response areas but also shifted excitatory or inhibitory subregions (Zurita et al. 1994). The latter was also reported for the cat dorsal cochlear nucleus (Young and Brownell 1976). More recent reports from the cochlear nucleus found only minor differences between awake and anesthetized state, besides a reduced dynamic range under anesthesia (May and Sachs 1992; May et al. 1998). Response trial-to-trial variability (on the assumed basis of a Poisson statistics) during Equithesin anesthesia was not significantly changed compared with the awake state. A modulation of response variability of click-evoked potentials along the depth of ketamine/xylazine anesthesia, on the other hand, has recently been described for the rat auditory cortex (Kisley and Gerstein 1999). However, one should keep in mind the possible different actions of the different anesthetics used and the different levels of description.

Taken together, the data presented here indicate that it is necessary to carefully select an experimental procedure when investigating central auditory neurons. Some neuronal properties might be influenced by anesthesia, others not. Properties shaped by inhibitory influences are more likely to be affected by pentobarbital or chloral hydrate.

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