Brain Struct Funct
DOI 10.1007/s00429-014-0862-1

ORIGINAL ARTICLE

Stimulus-specific adaptation in the inferior colliculus of the mouse: anesthesia and spontaneous activity effects

Daniel Duque · Manuel S. Malmierca

Received: 3 May 2014 / Accepted: 29 July 2014

Abstract Rapid behavioral responses to unexpected events in the acoustic environment are critical for survival. Stimulus-specific adaptation (SSA) is the process whereby some auditory neurons respond better to rare stimuli than to repetitive stimuli. Most experiments on SSA have been performed under anesthesia, and it is unknown if SSA sensitivity is altered by the anesthetic agent. Only a direct comparison can answer this question. Here, we recorded extracellular single units in the inferior colliculus of awake and anesthetized mice under an oddball paradigm that elicits SSA. Our results demonstrate that SSA is similar, but not identical, in the awake and anesthetized preparations. The differences are mostly due to the higher spontaneous activity observed in the awake animals, which also revealed a high incidence of inhibitory receptive fields. We conclude that SSA is not an artifact of anesthesia and that spontaneous activity modulates neuronal SSA differentially, depending on the state of arousal. Our results suggest that SSA may be especially important when nervous system activity is suppressed during sleep-like states. This may be a useful survival mechanism that allows the organism to respond to danger when sleeping.

D. Duque · M. S. Malmierca
Auditory Neurophysiology Unit, Laboratory for the Neurobiology of Hearing, Institute of Neuroscience of Castilla Y León, University of Salamanca, C/Pintor Fernando Gallego, 1, 37007 Salamanca, Spain

M. S. Malmierca (✉)
Department of Cell Biology and Pathology, Faculty of Medicine, University of Salamanca, Campus Miguel de Unamuno, 37007 Salamanca, Spain
e-mail: msm@usal.es

Published online: 13 August 2014

Keywords Auditory · SSA · Anesthesia · Awake animal · Inferior colliculus · Change detection · Spontaneous activity

Introduction

The inferior colliculus (IC) is important for integrating all information arising from the auditory brainstem (Malmierca 2003; Malmierca and Ryugo 2011), but it may also play a previously unknown role in gating thalamic activity, and hence controlling cortical activation (Winer et al. 1996; Peruzzi et al. 1997; Ito et al. 2009). In recent years, significant progress has been made in the understanding of auditory subcortical plasticity (Chandrasekaran and Kraus 2010), and evidence against a strict and simple input–output role for the IC is abundant (Skoe et al. 2013a, b; Skoe and Kraus 2010; Malmierca et al. 2014). Some authors even suggest that the IC is analogous to the main visual cortical region in processing complexity (King and Nelken 2009).

How anesthesia may alter plasticity in the IC is an important issue since there is little doubt that anesthesia may alter some neuronal properties. For example, degradation of temporal coding (Tollin et al. 2004; Song et al. 2011) and ITD sensitivity (Fitzpatrick et al. 1995) by anesthesia has been reported in the IC. Likewise, a much higher level of spontaneous activity has been reported in the IC of awake rabbits compared with anesthetized cats (Kuwada et al. 1989) and, recently, Chung and colleagues (2014) observed that unmasking of spontaneous activity in the awake state revealed suppressive responses to electric stimulation that were rarely observed in anesthetized animals. Moreover, a reduction in response adaptation kinetics has been shown in the IC of the awake gerbil (Ter-Mikaelian et al. 2007).

Stimulus-specific adaptation (SSA; Ulanovsky et al. 2003), the decrease that some auditory neurons show in response to repetitive sounds while maintaining responsiveness to uncommon sounds, has been extensively studied in anesthetized preparations. While SSA was originally described in the auditory cortex (AC; Ulanovsky et al. 2003), it has been shown to also occur at the levels of the midbrain and thalamus (for a review see Ayala and Malmierca 2013; Antunes and Malmierca 2014). However, most SSA experiments have been performed under anesthesia. Interestingly, the levels of SSA reported in the few experiments on awake animals (von der Behrens et al. 2009; Fishman and Steinschneider 2012; Thomas et al. 2012; Nir et al. 2013; Richardson et al. 2013) are considerably lower than those found in anesthetized animals (Malmierca et al. 2009; Antunes et al. 2010; Antunes and Malmierca 2011; Zhao et al. 2011; Duque et al. 2012, 2014; Pérez-González et al. 2012; Ayala et al. 2013). Thus, one might argue that SSA may be profoundly affected by anesthetic state or even represent an artifact due to anesthesia (Jones et al. 2012). But studies comparing SSA in awake and anesthetized using the same preparation are missing. Here we investigate what effect, if any, urethane anesthesia has on SSA in the IC of the mouse.

In the present account, we compared single-unit IC responses in awake and anesthetized mice. Our results demonstrate that SSA in the two conditions is qualitatively similar. The only significant difference between the two preparations was a much higher rate of spontaneous activity in the awake animals. Therefore, we conclude that SSA is a genuine property of some IC neurons and not simply the result of anesthesia.

Materials and methods

Animals

Experiments were performed on 28 animals, 16 awake and 12 anesthetized young-adult, male CBA/J mice (*Mus musculus*) aged between 2 and 6 months. The CBA/J strain exhibits normal hearing sensitivity during the first 2 years of life (Willott et al. 1988). All experimental procedures were carried out at the University of Salamanca with the approval of, and using methods conforming to the standards of, the University of Salamanca Animal Care Committee.

Surgical procedures: anesthetized preparation

Anesthesia was induced (1.5 g/kg, i.p., 20 % solution) and maintained (0.5 g/kg, i.p. given as needed) with urethane as routinely done in our lab (e.g., Malmierca et al. 2005, 2008, 2009; Hernández et al. 2005, 2006; Pérez-González et al. 2005, 2012; Izquierdo et al. 2008; Duque et al. 2012; Ayala et al. 2013). The animal was placed in a stereotaxic frame inside a sound-attenuated room and body temperature was maintained at 38 ± 1 °C by a heating blanket. The head was stabilized and leveled with ear bars; care was taken not to damage the eardrums. An incision was made in the scalp along the midline, and the skin was reflected laterally. A duralumin headpost (1 g) was glued to the skull with a one-component self-etching light-cured adhesive (G-Bond™, GC Corporation, Tokyo, Japan) and secured using a light-curing hybrid composite (Charisma®, Heraeus Kulzer, Hanau, Germany). A silver ground wire was glued onto the skull over the left cerebral cortex and a craniotomy was performed to expose the right IC. After the craniotomy, the ear bars were removed and sound was delivered to the left ear canal via polypropylene tubing.

Surgical procedures: awake preparation

Awake surgical procedures followed those of Bryant and colleagues (2009) and Portfors and collaborators (Portfors et al. 2009, 2011; Holmstrom et al. 2010; Muniak et al. 2012). Animals were anesthetized with isoflurane inhalation and placed in a stereotaxic frame. The surgical procedure was the same as described above: the head was stabilized and a headpost was cemented on the skull with the same light-cured adhesives. The analgesic buprenorphine (Buprex®, RB Pharmaceuticals, Berkshire, UK) was injected ip (0.03 mg/kg) and topical triple antibiotic (Dermisone®, Novartis, Barcelona, Spain) was applied to the wound. The animal was returned to its cage to recover from surgery for 2 days prior to starting electrophysiological recordings. Before the recording sessions began, the animals were acclimated to the recording chamber and were given a food reward (sweetened full cream condensed milk diluted 50:50 with water) at the end of every habituation session. During recordings, the head was immobilized by fixing the headpost to a custom-made clamp. In order to limit body movements the body was inserted in a loose fitting plastic tube. The mild sedative acepromazine (Equipromacina, Fatro Ibérica, Barcelona, Spain) was given if the mouse struggled excessively during the recording sessions (2 mg/kg, ip). If the animal continued struggling, we terminated the daily recording session.

Electrophysiological recording

Extracellular single unit responses were recorded using a tungsten electrode (1–2 MΩ, Merrill and Ainsworth 1972) lowered by means of a piezoelectric microdrive (Burleigh 6000 ULN). Acoustic stimuli were delivered through a sealed acoustic system (Malmierca et al. 2009) using two electrostatic loudspeakers (TDT-EC1: Tucker Davis

Technologies) driven by two TDT-ED1 modules. Search stimuli were pure tones (75 ms with a 5 ms rise/fall time) monaurally delivered under computer control using TDT System II hardware and custom software (Faure et al. 2003; Malmierca et al. 2009). The output of the system at the left ear was calibrated in situ using a free-field ½″ condenser microphone (model 4133, Brüel & Kjær) and a dynamic signal analyzer (Photon+, Brüel & Kjær). The maximum output of the TDT system was flat from 1 to 40 kHz (~89 ± 4.3 dB SPL). The highest frequency produced by this system was limited to 40 kHz. The proportion of neurons responding to frequencies above this range is relatively low (~15 %, Ehret 1979; Stiebler and Ehret 1985; cf. Figure 3B in Portfors et al. 2011). The second and third harmonic components in the signal were at least 40 dB lower than the level of the fundamental at the highest output level (Malmierca et al. 2009). Action potentials were recorded with a BIOAMP amplifier (TDT), the 10× output of which was further amplified and bandpass-filtered (TDT PC1; f_c, 500 Hz and 3 kHz) before passing through a spike discriminator (TDT SD1). Spike times were logged with a resolution of ≈150 μs on a computer by feeding the output of the spike discriminator into an event timer (TDT ET1) synchronized to a timing generator (TDT TG6). Generation of acoustic stimuli was exactly as described by Malmierca et al. (2009). From each isolated neuron, we measure the frequency response area (FRA) by presenting pure tones using an automated procedure with 5 stimulus repetitions at each frequency (from 1 to 40 kHz, in 25 logarithmic steps, presented randomly) and intensity (10 dB steps, presented from lower to higher intensities). The spike counts evoked at each combination of frequency and intensity were then plotted using MATLAB®. We used the FRA to calculate the minimum threshold and the best frequency of response, i.e., the sound frequency that can elicit a response at the minimum intensity.

Data analysis

For the acoustic stimuli, we chose pairs of frequencies (f_1 and f_2) that were 10–20 dB above the best frequency threshold for evaluating SSA using an oddball paradigm (Näätänen et al. 1978; Ulanovsky et al. 2003). Briefly, a train of 400 stimuli containing both frequencies f_1 and f_2 was presented under the oddball paradigm: one frequency (f_1) was presented as the standard (90 % occurrence) while, interspersed randomly among the standards, the deviant stimuli (10 % occurrence) were presented at the second frequency (f_2). After obtaining one data set, the relative probabilities of the two stimuli were reversed, with f_2 as the standard and f_1 as the deviant. Dot raster plots are used to illustrate the responses obtained to the oddball paradigm, plotting individual spikes (e.g., Fig. 1; red dots indicate responses to the deviant; blue dots indicate responses to the standard). Stimulus presentations are marked along the vertical axis. The responses to the standard and deviant stimuli were also expressed as spikes per stimulus in the peri-stimulus histogram (PSTH), to account for the different number of presentations in each condition, due to the different probabilities.

The amount of SSA was quantified by two indices: (1) the frequency-specific index SI(f_i), where $i = 1$ or 2, defined for each frequency as $\mathrm{SI}(f_i) = [d(f_i) - s(f_i)]/[d(f_i) + s(f_i)]$ and (2) the common-SSA index (CSI) defined as $\mathrm{CSI} = [d(f_1) + d(f_2) - s(f_1) - s(f_2)]/[d(f_1) + d(f_2) + s(f_1) + s(f_2)]$, where $d(f)$ and $s(f)$ are responses to each frequency f_1 or f_2 when they were the deviant (d) or standard (s) stimulus (Ulanovsky et al. 2003; Malmierca et al. 2009). The SI reflects the extent to which the response to a given frequency as standard is suppressed respect to the same frequency as deviant, i.e. is a frequency-specific index for SSA. CSI reflects the same effect but for a pair of frequencies, demonstrating that the SSA levels observed in the SI are extensible to other frequencies of the neuron, i.e. provides an approximate neuronal level of SSA. The values of both indices range from −1 to +1, being positive if the response to the deviant stimulus is greater. The same paradigm was repeated varying the interstimulus interval [ISI = 125 ms (8 Hz), 250 ms (4 Hz) and 1000 ms (1 Hz)] and the frequency contrast (Δf) between the standard and deviant stimulus [$\Delta f = 0.04$ (0.057 octaves), 0.10 (0.141 octaves) and 0.37 (0.526 octaves)]; where $\Delta f = (f_2 - f_1)/(f_2 \times f_1)^{1/2}$.

Since the levels of spontaneous activity (SR) in the awake animal are notably high, we considered two different approaches for evaluating SSA. At first, we analyzed the response, adjusting the limits of the response analysis window based on the shape of the PSTH—considering only the evoked response to the sound—and trying to avoid the inclusion of the SR in the response window used for the analysis of the SSA. In the second approach, considering that the SR was constant even during the presentation of the tones, we computed the SSA indices, but directly subtracting the SR. To do so, SR was estimated with a fixed window established 50 ms before the presentation of the stimuli while we presented the oddball paradigm (50 ms × 400 trials = 20 s sample window). Then, a driven rate was obtained by subtracting the averaged SR from the evoked firing rate in the same time windows used before to evaluate SSA (driven rate = firing rate − SR). The subtraction of the SR was directly done bin by bin (evoked responses in spikes/s minus spontaneous activity in spikes/s). The obtained driven rate was then used to calculate again the levels of SSA in each condition. It should be noticed that because the calculation of SR is not intended to include evoked activity (especially at repetition rates of 8 Hz), cases that showed a likely rebound-off

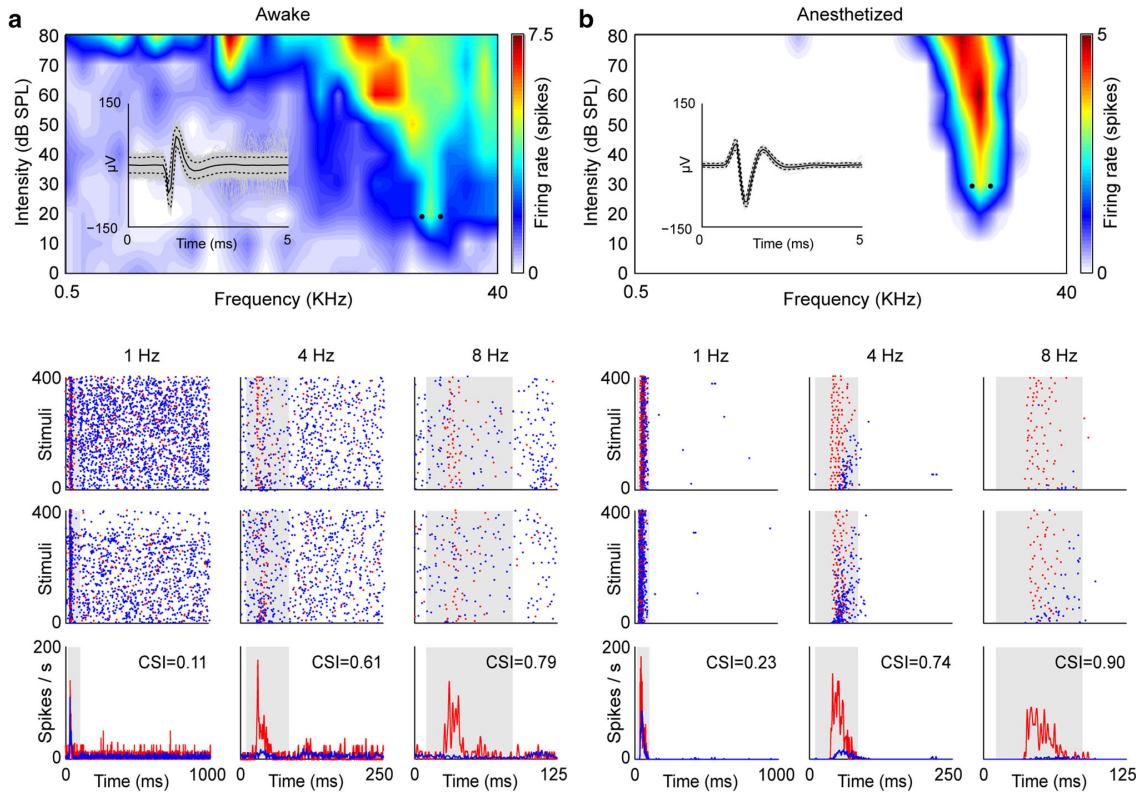

Fig. 1 Single-unit responses in the IC of the awake (**a**) and the anesthetized (**b**) mouse. *Top panels* show FRAs of neurons in the IC that exhibited high CSI values. *Black dots* over the FRAs indicate the pair of frequencies selected for analyzing SSA ($\Delta f = 0.37$). *Insets* in the FRAs show waveforms of the spikes recorded from these single units. Below the FRAs, *dot raster plots* are illustrated for the oddball paradigm under three different repetition rates: 1 Hz (left column), 4 Hz (middle column) and 8 Hz (right column). In the *top row* f_1 and f_2 are the standard and deviant stimuli, respectively (awake: 18.86 and 27.28 kHz; anesthetized: 15.80 and 22.87 kHz). In the middle row, in the reverse condition, f_2 and f_1 are the standard and deviant stimuli, respectively (awake: 27.28/18.86 kHz; anesthetized: 22.87/15.80 kHz). In the bottom row, PSTHs show averaged responses for both conditions: deviant (*red*) and standard (*blue*). CSI values obtained in each condition are shown as *insets* in the PSTHs. *Shaded backgrounds* indicate the duration of the stimulus

activity (i.e., post-stimulus suppression or forward masking) were not included in the analysis.

Classification of the type of response was based on previous reports (Rees et al. 1997; Duque et al. 2012): on, long-latency on, on-sustained, sustained, pauser and on–off. On responses show a robust response confined to the first 40 ms of the stimulus. Long-latency on responses started 50–80 ms after the beginning of the tone. On-sustained responses exhibit a clear on response followed by a sustained portion, with at least a 50 % decrease of firing rate compared with the on portion. Sustained responses show a constant response that lasts 50 ms or more. Pauser responses show a cessation of firing rate between a clear on and a sustained portion. On–off responses had two different components, with an on and off portion (after the end of the stimulus).

Histology

Neuron location was assigned to each of the main IC subdivisions based on stereotaxic coordinates (Franklin and Paxinos 2007), response properties (Egorova et al. 2001; Portfors et al. 2011; Malmierca and Ryugo 2011) and by reconstruction of the electrode tracks from the position of electrolytic lesions (14 μA for 10 s) in Nissl-stained brain sections. Histological procedures were as described by Duque et al. (2012).

Results

To characterize SSA in the mouse, we recorded responses from 93 well-isolated single neurons in the IC under an

oddball paradigm. Fifty-four units were recorded from 16 awake mice and 39 units from 12 anesthetized mice. For both populations, we determined the basic temporal and spectral response properties of each neuron and chose a pair of frequencies within the FRA to evaluate SSA under different conditions (i.e., variations in frequency contrast and repetition rate). Our major goal was to study the effect of anesthesia on SSA sensitivity in the IC of the mice. In the following, we will compare the levels of SSA, the neuronal response properties and the levels of spontaneous activity of IC neurons in the awake and the urethane-anesthetized mouse.

SSA and response properties in the awake and anesthetized preparation

Since there were no available SSA data for the IC in mouse, and for purposes of comparison, we performed the same experiments and analysis in two different preparations: awake and anesthetized. Figure 1a shows a FRA of an individual neuron in the awake preparation while Fig. 1b shows a FRA of an individual neuron in the anesthetized preparation. The black dots over the FRAs indicate the frequencies and the intensity chosen for the oddball paradigm (Fig. 1a: 18.86 and 27.28 kHz at 20 dB SPL; Fig. 1b: 15.80 and 22.87 kHz at 30 dB SPL). The neurons illustrated exhibit significant SSA, showing a cessation of the responses to the repetitive stimuli (Fig. 1a, b: blue dots in the dot rasters, especially at 4 and 8 Hz) while the responses to the deviant stimuli are maintained (Fig. 1a, b: red dots in the dot rasters). The normalized response in each condition is depicted by the corresponding PSTHs (Fig. 1a, b, bottom row); the average response to the deviant stimuli (red traces) is higher than the average response to the standard stimuli (blue traces) in all conditions. The only readily visible difference in the dot rasters (and also in the FRAs) between the awake and anesthetized preparation is the great amount of spontaneous activity (SR) observed in the awake preparation (Fig. 1a). Despite the SR, the levels of SSA depicted by the common SSA index (CSI, insets in the PSTHs) are comparable between the awake and the anesthetized preparation.

We quantified and compared the degree of neuronal adaptation in the awake and the anesthetized preparation by computing the CSI and the frequency-specific SSA index (SI, Fig. 2) under several experimental conditions. The overall degree of SSA was characterized at a standard condition with a frequency contrast (Δf) of 0.37 and a repetition rate of 4 Hz (awake: $n = 31$; anesthetized: $n = 29$). Under this condition, CSI levels in the awake preparation range from -0.017 to 0.854 with an average of 0.43 ± 0.24 (mean $\pm$ SD). In the anesthetized preparation the levels show a comparable range from -0.043 to 0.985 with a similar average level (Table 1, mean $\pm$ SD 0.48 ± 0.33). A CSI cut-off value of $+0.18$ was defined as significant SSA based on previous data (Antunes et al. 2010; Duque et al. 2012, 2014). Using this criterion, in the awake preparation 25 neurons (81 %) showed significant SSA, while the remaining 6 (19 %) did not. In the anesthetized preparation, 21 neurons (73 %) showed significant SSA, while the remaining 8 (27 %) lacked significant SSA (Table 1). Twenty-nine of the 39 neurons recorded in the anesthetized preparation were histologically localized. Anatomical analysis revealed that 6 neurons were located in the central nucleus of the IC (lemniscal), while 23 were located in the collicular cortices (non-leminscal): 4 in the dorsal, 5 in the rostral and 14 in the lateral cortex (data not shown). Neurons located in the collicular cortices showed the largest degree of SSA (mean $CSI_{non\text{-}lemniscal}$: 0.70 ± 0.24; mean $CSI_{lemniscal}$: 0.36 ± 0.27). Four of the seven neurons that lacked SSA were from the central nucleus, supporting previous findings that SSA is biased towards the non-lemniscal IC regions. On the other hand, recordings from awake animals limit the ability to localize individual recorded neurons. However, we recovered lesions from 7 of the 16 mice at the end of the last recording session, which allowed us to confirm that we were also biased towards the non-lemniscal regions of the IC.

To quantify the adaptation at the population level we compared the SSA indices across different conditions and between the two preparations. The SI is depicted in Fig. 2 for different conditions (awake: burgundy dots and crosses; anesthetized: green dots and crosses). These scatter plots show the individual SI values for each frequency pair and demonstrate significant SSA when the values are located in the upper right quadrant. Crosses represent the median CSI values while the mean CSI values are also indicated as insets in each condition. As expected from previous studies (Ulanovsky et al. 2003; Malmierca et al. 2009; Antunes et al. 2010), SSA values are positively correlated with the frequency contrast and the repetition rate in both preparations (two-way ANOVA; awake: $F_{\Delta f} = 6.62$, $p = 0.002$ $F_{\mathrm{Hz}} = 14.85$, $p < 0.001$; no interaction effect: $F = 0.72$, $p = 0.58$; post hoc Tukey test confirmed differences between $\Delta f = 0.37$ and $\Delta f = 0.04 - 0.1$ and between 1 and 4–8 Hz; $p < 0.05$; anesthetized: $F_{\Delta f} = 9.37$, $p < 0.001$; $F_{\mathrm{Hz}} = 11.32$, $p < 0.001$; no interaction effect: $F = 0.30$, $p = 0.88$; post hoc Tukey test confirmed differences between $\Delta f = 0.37$ and $\Delta f = 0.04 - 0.1$ and between 1 and 4–8 Hz; $p < 0.05$). However, at both preparations, SSA is still present at a small frequency contrast ($\Delta f = 0.04$) and at a repetition rate as low as 1 Hz. No differences in CSI were found at any condition between the awake and the anesthetized preparation (Mann–Whitney rank sum test, $p > 0.05$ for all conditions). Despite not

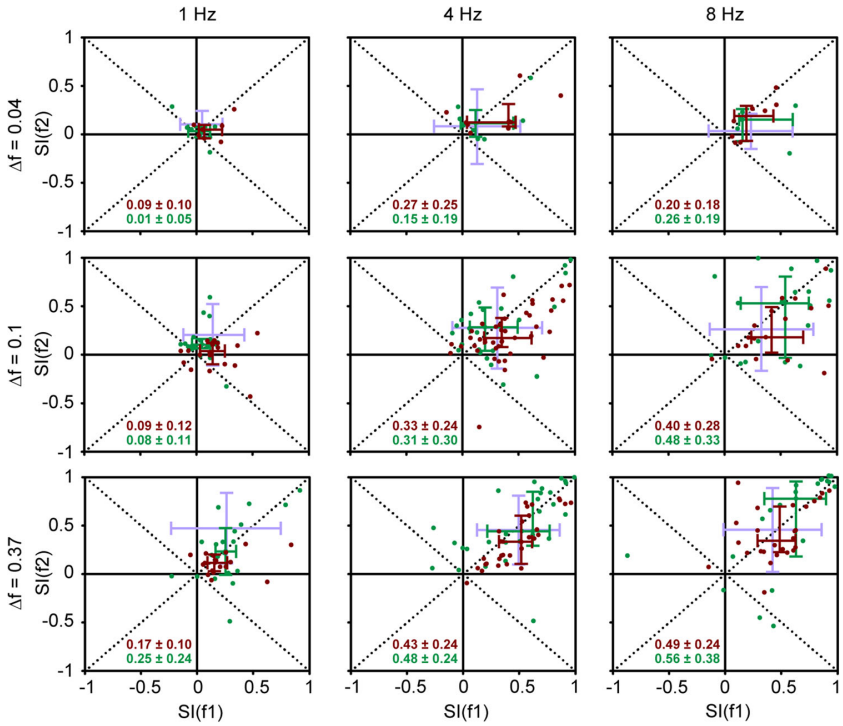

Fig. 2 Population SSA comparison between awake and anesthetized mouse. *Scatter plots* of the SI(f_1) versus SI(f_2), for the different Δf (*top row*: 0.04; *middle row*, 0.1; *bottom row*: 0.37) and ISIs (*left column*: 1 Hz; *middle column*: 4 Hz; *right column*: 8 Hz). Pairs of frequencies obtained in the awake (*burgundy dots*) and anesthetized (*green dots*) preparations are shown. [For comparison, we also show SSA data values from previous experiments in the anesthetized rat (light violet crosses) from Malmierca et al. 2009, and Zhao et al. 2011]. The *burgundy* (awake) and *green* (anesthetized) *crosses* indicate the median and the 25th–75th interquartile range for each axis. As each neuron was tested using different combinations of parameters, individual neurons may be represented in more than *one panel*. Median CSI values for each condition are shown as *insets* in each scatter plot

Table 1 Neuronal responses in the awake and the anesthetized preparations

Preparation	Mice		Rat
	Awake	Anesthetized	Anesthetized
n (all neurons)	79	46	95[b]
Type of FRA			
V-shaped (%)	12 (15)	13 (28)	20 (21)[b]
Non-V-shaped (%)	50 (63)	33 (72)	75 (79)[b]
'Inhibitory' (%)	17 (22)	0 (0)	0 (0)[b]
Mean firing rate (spk/s)[a]	94.27 ± 71.19	70.12 ± 43.6	69.22 ± 52.72[b]
Mean spontaneous activity (spk/s)	7.16 ± 9.29	0.98 ± 1.86	1.47 ± 5.01[b]
n ($\Delta f = 0.37$ at 4 Hz)	31	29	69[c]
SSA (CSI ≥0.18) (%)	25 (81)	21 (73)	52 (75)[c]
Non-SSA (CSI <0.18) (%)	6 (19)	8 (27)	17 (25)[c]
Mean CSI ± SD	0.43 ± 0.24	0.48 ± 0.33	0.46 ± 0.32[c]
Median CSI (25th–75th percentile)	0.40 (0.27–0.61)	0.49 (0.14–0.77)	0.47 (0.17–0.80)[c]
Mean latency difference (ms)	4.34 ± 6.59	9.04 ± 10.34	7.54 ± 14.44[c]
Adaptation values (mean ± 95 % c.i.)			
$\tau(r)$ (trial)	0.86 ± 0.38	0.58 ± 0.33	0.92 ± 0.13[d]
A_r (spk/trial)	5.00 ± 2.77	7.68 ± 7.47	9.48 ± 1.56[d]
τ(s) (trial)	34.27 ± 8.58	34.71 ± 14.23	44.54 ± 6.26[d]
A_s (spk/trial)	0.64 ± 0.11	0.34 ± 0.10	0.68 ± 0.07[d]
A_{stst} (spk/trial)	1.13 ± 0.02	1.04 ± 0.02	0.36 ± 0.01[d]

[a] Average firing rate response 20 dB above the best frequency threshold

[b] Data from Duque et al. (2012)

[c] Data from Malmierca et al. (2009)

[d] Data from Perez-Gonzalez et al. (2012)

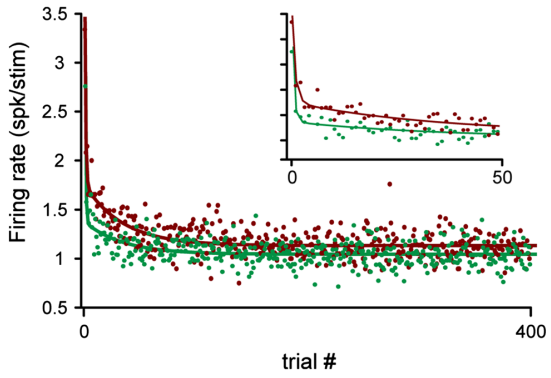

Fig. 3 Time course of adaptation in the awake and the anesthetized mouse. Averaged population firing rate responses (spikes/stimulus) to the standard stimulus at the condition with a $\Delta f = 0.37$ at 4 Hz for the awake (*burgundy*, $n = 62$) and the anesthetized (*green*, $n = 58$) preparations. The *upper right inset* shows the first 50 trials extended

being statistically different, we found slightly different values at $\Delta f = 0.1$ at 8 Hz; $\Delta f = 0.37$ at 4 Hz and $\Delta f = 0.37$ at 8 Hz (Fig. 2).

To better understand the dynamics of adaptation over time, we plotted the responses to the standard tone in the condition with $\Delta f = 0.37$ at 4 Hz over the consecutive trials (Fig. 3, awake preparation in burgundy trace: $n = 62$; anesthetized preparation in green trace: $n = 58$). We fitted the responses with a double exponential function defined as $f(t) = A_{\text{stst}} + A_r \cdot e^{-t/\tau(r)} + A_s \cdot e^{-t/\tau(s)}$ (Perez-Gonzalez and Malmierca 2012; Perez-Gonzalez et al. 2012; Duque et al. 2014). The responses to the deviant tones, because of minimal adaptation, do not fit this function and for this reason they were not depicted in the figure. The response to the standard stimulus is reduced immediately after the first stimulus trials in both preparations (Fig. 3). This double exponential function contains both a rapid (r) and a slow (s) component, after which the response reaches a steady state (A_{stst}). In terms of magnitude, the fast component, which occurs during the first trials, presents the largest degree of adaptation. The slow component defines the final steady-state response. Comparing the time course of adaptation to the standard stimuli between the awake and the anesthetized preparation, only the magnitude of the slow component (A_s) and the asymptote of the curve (A_{stst}) were statistically different (Table 1).

Since these higher time course of adaptation indices could imply that the firing rate was different in the awake and the anesthetized preparation, we compared the basic temporal and spectral responses of the recorded units in both conditions. We found that the firing rate, the PSTH response type and the response latencies were similar to those recorded in the awake preparation. No differences in firing rate at a common point in the FRA (we calculate the average firing rate response 20 dB above the best frequency threshold) were found between the awake and the anesthetized preparation (Table 1; Mann–Whitney rank sum test, $T = 2151$, $p = 0.13$). Next, we characterized the basic temporal and spectral responses of the recorded units in an attempt to determine whether there is a correlation between SSA and any of these features, including PSTH response type and response latency. We classified the response types of the units according to the PSTH shape in response to the standard condition $\Delta f = 0.37$ at 4 Hz as: on, long-latency on, on-sustained, sustained, pauser and on–off responses (see "Materials and methods"). In the awake preparation, the most common type found in our sample was the on response (15 units, 48 %), followed by the on-sustained (9 units, 29 %) and the pauser (5 units, 16 %) types. One sustained unit (3 %) and one on–off response (3 %) completed our data set. The highest levels of SSA were seen in the on and on-sustained units, while pauser and sustained units showed the weakest levels of SSA (one-way ANOVA; $p = 0.031$). In the anesthetized preparation, on responses (16 units, 55 %) were also the most common type of response, followed by the on-sustained (7 units, 24 %), sustained (3 units, 10 %), pauser (2 unit, 7 %), and long-latency on responses (1 units, 4 %). No on–off responses were found in our anesthetized data set. Thus, the proportion of response types do not vary between the awake and the anesthetized preparation (Chi square test for sampling distributions, $\chi^2 = 4.5$, $p = 0.479$), although it is difficult to make definitive conclusions due to the small number of neurons in our sample. Finally, we also evaluated the effects on spike latency by computing the latency difference, defined as the difference between the median first spike latency to the standard and the deviant condition for each frequency. Across the population, a high SSA level is correlated with a large latency difference (Spearman rank order correlation coefficient: 0.36, $p < 0.001$). Interestingly, no differences in latency difference were found as a function of repetition rate or frequency contrasts in the awake preparation (two-way ANOVA; $F_{\Delta f} = 0.82$, $p = 0.44$; $F_{\text{Hz}} = 2.09$, $p = 0.13$; no interaction effect: $F = 1.09$, $p = 0.36$) while they were evident in the anesthetized preparation (two-way ANOVA; $F_{\Delta f} = 10.42$, $p < 0.001$; $F_{\text{Hz}} = 3.01$, $p = 0.05$; no interaction effect: $F = 0.93$, $p = 0.45$; Tukey test confirmed differences between $\Delta f = 0.37$ and $\Delta f = 0.04 - 0.1$ and between 1 and 8 Hz; $p < 0.05$). At some conditions, latency difference was larger in the anesthetized mice compared with the awake ones (e.g. Table 1; Mann–Whitney rank sum test for $\Delta f = 0.37$ at 1 and 4 Hz, $p = 0.001$), but those differences did not generalize to all the conditions (Mann–Whitney rank sum test,

$p > 0.05$). Such small latency difference in the awake animal is likely due to the random integration of spontaneous spikes into the analysis window.

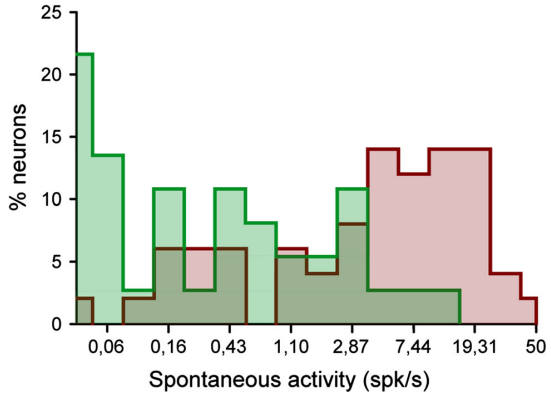

Fig. 4 Histogram of the SR in the awake and the anesthetized mouse. Note that the *x*-axis is logarithmic to account for the great difference in SR between the anesthetized (*green histogram*) and the awake preparation (*burgundy histogram*)

Urethane anesthesia decreases spontaneous activity

Even simple visual inspection suggested a high SR for the majority of units during the recording sessions in the awake mouse (68 % of the neurons recorded showed a SR larger than 2 spikes/s, Fig. 4, burgundy histogram). At a population level, the range of SR was from 0.11 to 28.31 spikes/s and the mean SR was 7.16 ± 9.29 spikes/s (Table 1). On the other hand, the mean SR in the anesthetized preparation was very low or negligible: 0.98 ± 1.86 spikes/s (Fig. 4, green histogram; SR range from 0 to 2.51 spikes/s). Only 16 % of the neurons recorded showed a SR larger than 2 spikes/s and 46 % of the neurons showed no SR at all. Thus, median SR in the awake preparation was significantly higher than that seen in the anesthetized mouse (Anesthetized: 0.222 spikes/s; Awake: 3.8 spikes/s; Mann–Whitney rank sum test, $p < 0.001$). Moreover, for some units in the awake preparation the SR was so high that no sound-evoked response could be distinguished out of the SR. In those cases, which account for almost a quarter of our sample (Table 1, 17/79; 22 %), the SR allowed us to detect suppression of responses to different combinations of frequencies and intensities, resulting in a distinct and

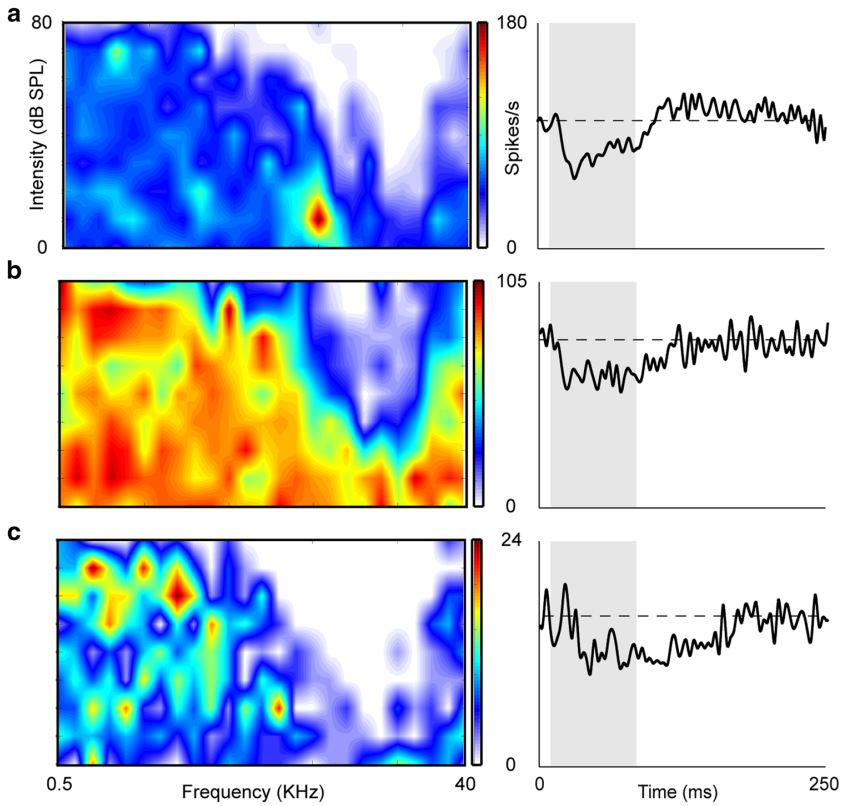

Fig. 5 Examples of iFRAs in the IC of the awake mouse. Three examples of inhibitory frequency response areas (iFRA) are shown in the *left panel*. Averaged PSTHs obtained for each neuronal example are shown in the *right panel* next to each iFRA to show that no excitatory response was elicited before the suppression of the SR. *Dotted lines* indicate the mean averaged SR for each neuronal example. Firing rate values for each iFRA shared the *y*-axis of the averaged neuronal PSTH

unambiguous 'inhibitory' FRA (iFRA: Fig. 5). Unfortunately, due to the lack of driven activity, we could not test those neurons showing iFRAs with the oddball paradigm, nor they did show any sign of SSA in the SR region. The significant difference in SR may explain why we did not find iFRAs in the anesthetized preparation (Table 1, 0/45).

Since the high SR rates in the awake preparation might have affected the levels of SSA in some conditions (Fig. 2, e.g. $\Delta f = 0.37$ at 4 Hz), we next proceeded to extract the SR and reevaluate the levels of CSI of the neurons recorded for the awake preparation. To do that, we subtracted the SR (see "Materials and methods"), in an attempt to mimic the anesthetized preparation. Figure 6a shows the same frequency pair example as in Fig. 1a, with a $\Delta f = 0.37$ at 4 Hz. The depicted PSTHs are shown in Fig. 6b, c, with and without SR, respectively. Interestingly, the subtraction of the SR demonstrates a distinct increase in the CSI level. In order to shed light on the role, if any, of the SR in the modulation of SSA, we plotted the CSI values of each neuron as a function of its SR and found that they were inversely correlated (Fig. 6d, $\Delta f = 0.37$ at 4 Hz, Spearman rank order correlation coefficient: -0.58, $p < 0.001$). The same analysis was performed to the neurons in the anesthetized preparation, and a similar trend occurred. However, here the correlation was not significant, most probably because many neurons in this group totally lacked of SR and the sample of neurons with SR turned very small ($n = 9$; Spearman rank order correlation coefficient: -0.45, $p = 0.204$, data not shown). Moreover, despite the large variability in SR across the population, the computation of the PSTH grand average showed a clear increase of the deviant to standard response ratio after the SR subtraction (Fig. 6e, $\Delta f = 0.37$ at 4 Hz). At a population level, this trend is maintained. Figure 7a shows the scatter plots for the same pairs of neurons recorded in the awake preparation, before and after the SR subtraction (burgundy and orange, respectively). The neurons that showed low levels of SSA (CSI <0.4) remain mostly unaltered when the SR was subtracted, while the neurons with higher levels (CSI ≥0.4) showed an increase of SSA (data not shown). That happened regardless of the condition, but was most clearly noticeable when the levels of CSI were consistently high (for that reason in Fig. 7 we only show 4 of the 9 conditions shown in Fig. 2; i.e., $\Delta f = 0.1$ and $\Delta f = 0.37$ at 4 and 8 Hz). Interestingly, the qualitative non-significant differences found between the awake and the anesthetized preparation (Fig. 2) seem to disappear when the SR is subtracted (Fig. 7b).

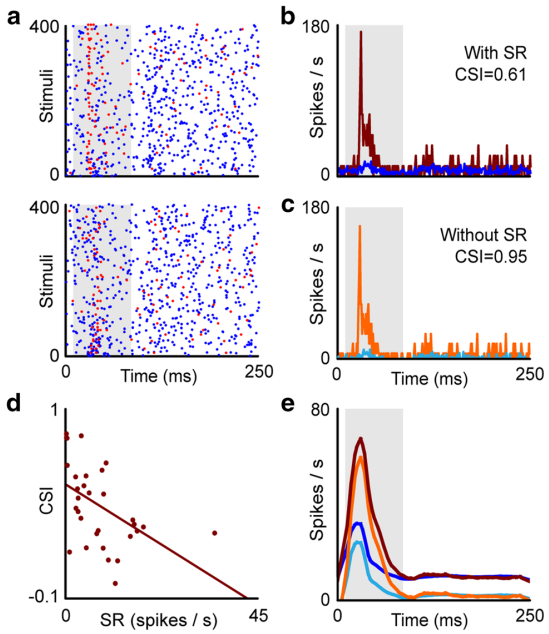

Fig. 6 Relation between spontaneous activity and SSA. **a** *Dot raster plots* for the same frequency pair illustrated in Fig. 1a, with a $\Delta f = 0.37$ at 4 Hz. **b** PSTHs for the averaged responses for the frequency pair before subtracting the spontaneous rate (SR). **c** PSTHs for the averaged responses for the frequency pair after subtracting the SR. **d** Correlation between the SR and the level of CSI in the standard condition, at $\Delta f = 0.37$ and 4 Hz ($n = 31$). **e** Grand average for the standard condition, with a $\Delta f = 0.37$ at 4 Hz ($n = 31$). Deviant and standard responses for the neurons before the subtraction of the SR are shown as *burgundy and blue lines*, respectively. Deviant (*orange*) and standard (*light blue*) responses after the subtraction of the SR are also shown

Discussion

The present study demonstrates high levels of SSA in the mouse IC and, more importantly, it shows that SSA in the awake mouse is comparable to that seen in the urethane-anesthetized preparation. The SSA observed in mice is equivalent in most respects to that seen in the rat IC (Malmierca et al. 2009; Duque et al. 2012; Ayala and Malmierca 2013). Our results further reveal that urethane anesthesia does affect other response properties in the mouse IC, most notably the SR. Although previous studies have shown SSA in awake preparations in different species or brain regions, our study is the first to directly compare the effect of anesthesia in the IC. Overall, the results demonstrate that SSA is a genuine property of some IC neurons and not an artifact attributable to anesthesia, but that it is dynamically modulated by the animal's state through the neuronal SR.

A detailed examination of SSA at the population level revealed no major differences between the anesthetized and the awake conditions in the proportion of neurons showing SSA and in the overall level of SSA that the

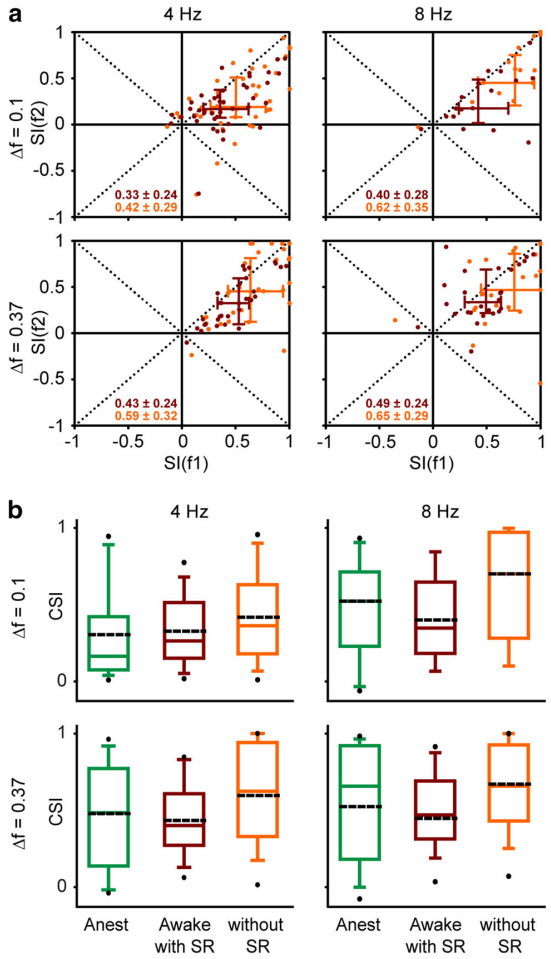

Fig. 7 Population comparison between the awake neurons before and after the subtraction of the spontaneous activity. **a** *Scatter plots* of the SI(*f1*) versus SI(*f2*), for different Δ*f* (*top row* 0.1; *bottom row* 0.37) and ISIs (*left column* 4 Hz; *right column*: 8 Hz). Pairs of frequencies obtained in the awake mouse before (*burgundy dots*; median value showed as a *burgundy cross*) and after (*orange dots*; median value showed as an *orange cross*) the subtraction of the spontaneous activity. Median CSI values for each condition are shown as *insets* in each *scatter plot*. **b** *Box plots* showing the median value of CSI (*straight line*) for the anesthetized (*green*), awake (*burgundy*) and awake without spontaneous activity (*orange*) neurons of the mice IC. Mean values are showed as a *dashed line*. The *box plots* are for the same conditions showed in Fig. 7a. The *box* delimits the 25th and the 75th percentiles, *whiskers* indicate the 10th and 90th percentiles, and *dots* indicate the 5th and 95th percentiles

neurons exhibited (Table 1). However, we did find some differences in other properties, including a much larger mean SR in the awake preparation (Table 1) as well as an increase in the slow component (A_s) and the steady-state (A_{stst}) indices in the dynamics of adaptation (Fig. 3; Table 1). Urethane anesthesia produces a decrease of spontaneous glutamate output in rat cerebral cortex (Moroni et al. 1981) and could have a similar effect on the IC. The high rates of SR allowed us to uncover a high incidence of inhibition in the IC and revealed a large proportion of purely inhibitory response maps (iFRAs). To the best of our knowledge, iFRAs have not been previously documented in the mouse IC, either in the anesthetized or the awake preparation. Similar inhibitory receptive fields have been described in the dorsal cochlear nucleus of the unanesthetized decerebrate cat (Young and Brownell 1976), the anesthetized guinea pig (Stabler et al. 1996), the awake mouse (Roberts and Portfors 2008) and, very rarely, in the IC of the anesthetized rat (Hernandez et al. 2005). Previous studies have shown higher evoked and spontaneous firing rates in the awake rabbit IC (Kuwada et al. 1989; Chung et al. 2014). We did not find higher evoked firing rates in the awake mice, but higher SR does seem to slightly decrease the levels of SSA (Figs. 6, 7). Although, theoretically, subtraction of SR will necessarily increase SSA based on the equation for CSI (i.e., without SR numerator will remain constant while denominator will become smaller by 4 times the SR), in practice, we observed a full range of changes. As a rule, neurons showing low levels of SSA also had the lowest variations of CSI levels (median variation: 0.02) while neurons with higher levels of SSA presented higher variations (median variation: 0.10; Mann–Whitney rank sum test, $p = 0.027$). These results are in accordance with our theoretical approximation, as we subtracted the SR according to the evaluation of the difference in the response with respect to a basal level. A recent study (Klein et al. 2014) discussed the possibility that spontaneous activity might be responsible for the low levels of SSA in the auditory cortex. The present data show that in the IC the SR has some effect on SSA sensitivity. Our data are in accord with previous elegant work that suggests a basal adaptive state due to high spontaneous activity rates (Abolafia et al. 2011). However, these potassium currents suggested by Abolafia and colleagues do not completely explain SSA (because they would affect all inputs and thus, will not be stimulus-specific). Such pre-adaptive situation will produce less adaptation to both the standard and deviant stimuli, decreasing the ratio between the deviant and the standard response, therefore reducing the levels of SSA. Indeed, since high SR in the awake behaving animals are related to a reduction of adaptation (Chung et al. 2002; Castro-Alamancos 2004), it is likely that attention during task engagement modulates both the evoked and the spontaneous firing rate (Buran et al. 2014). In this respect, we found a clear correlation between the CSI levels and the SR such that the higher the CSI level, the lower the SR (Fig. 6d). Thus, it is tempting to speculate that the attentional state might be dynamically

increasing the SR and, consequently, affecting the levels of SSA. One plausible mechanism would be through GABAergic inhibition. We have previously demonstrated that $GABA_A$ mediated inhibition acts as a gain control system that sharpens and enhances SSA (Perez-Gonzalez et al. 2012; Duque et al. 2014) and thus, might suppress the SR to increase SSA.

Since most reports on SSA from IC neurons have been in the anesthetized rat (Pérez-González et al. 2005, 2012; Malmierca et al. 2009, Lumani and Zhang 2010; Zhao et al. 2011; Duque et al. 2012; Perez-Gonzalez and Malmierca 2012; Ayala et al. 2013), it is pertinent to compare our current results with those in the rat. Table 1 and Fig. 2 not only compare the anesthetized and the awake mouse recordings, but they also include previous data from the anesthetized rat IC. All but one of these experiments employed urethane anesthesia (Lumani and Zhang 2010, used ketamine-xylazine). Urethane has been the anesthetic agent of choice in many electrophysiological recordings, since it produces (1) minimal disruption of breathing, heart rate and reflexes (Maggi and Meli 1986), (2) small changes in the receptor system by non-selectively affecting both the excitatory and inhibitory currents (Hara and Harris 2002) and (3) little effect on the time-course and amplitude of inhibitory and excitatory responses (Sceniak and MacIver 2006). As stated before, the greater amount of inhibition observed in the awake preparation allowed us to confirm the presence of iFRAs compared with the absence of these type of response areas in anesthetized preparations (Chi square test for sampling distributions, $\chi^2 = 34.271$, $p < 0.001$). No significant differences were found between previous data obtained in rat IC and the current work in the awake and the anesthetized mouse in the level of SSA (Table 1, Kruskal–Wallis ANOVA on ranks test: $p = 0.895$) or in the proportion of neurons showing SSA (Chi square test for sampling distributions, $\chi^2 = 0.586$, $p = 0.746$). In fact, the levels of SSA in the awake mice are unexpectedly similar to the ones in the anesthetized animals considering that anesthetic and inter-specific differences affect other features such as latency difference, firing rate and, particularly, spontaneous activity (Table 1, Kruskal–Wallis test: $p = 0.012$, $p = 0.042$, $p < 0.001$, respectively). For example, the latency difference is smaller for some conditions in the awake preparation compared with the anesthetized ones. We usually interpreted the delay in the response latency to the standard stimuli as a consequence of an independent input processing of the deviant and the standard frequencies. In such scenario, the latency of the response to the standard sound will be progressively delayed up to a point where the response to the repeated sound is no longer strong enough to evoke a response. Such a latency difference reduction in the awake preparation could be the result of a decrease in the general level of adaptation due to the pre-adaptive state (Abolafia et al. 2011). Moreover, the time course of adaptation shows slightly higher values in rat, but probably because the population used in that experiment presented an intentional bias towards highly adapting neurons (Perez-Gonzalez et al. 2012: median CSI: 0.78).

Our study is the first to compare the effect and influence of anesthesia on SSA by directly comparing awake and anesthetized responses in the IC in the same preparation. We were initially surprised by the similarity of the results we obtained in the awake and anesthetized preparations, as previous studies found SSA levels in awake experiments considerably lower than those found in anesthetized preparations. Several differences in the experimental paradigms may help to explain the discrepancies. First, some experiments were carried out at relatively high intensities (Von der Behrens et al. 2009) or employed fixed tones to assess SSA (Nir et al. 2013). Both of these factors may have influenced the level of SSA, as we have previously demonstrated that SSA is both level and frequency dependent (Duque et al. 2012). Further, the anatomical location of the recording sites may also have significantly influenced the reported values of SSA. For example, it is likely that the majority of the recordings in the IC of a recent study in an awake bat preparation (Thomas et al. 2012) were from the central nucleus, as bats possess a hypertrophied central nucleus and very small cortical regions compared to rats and mice (Zook et al. 1985). Similarly, studies in the medial geniculate body in the awake rat included a large number of units from ventral and dorsomedial geniculate neurons (Richardson et al. 2013) where SSA is weak (Antunes et al. 2010; Antunes and Malmierca 2011). Further, while the use of multi electrodes allowed for a large-scale examination of SSA in monkey A1 (Fishman and Steinschneider 2012), they might also result in averaging out high SSA values. Interestingly, the current IC data from mice contrasts with a previous report on the medial geniculate body in mice (Anderson et al. 2009), as the levels of SSA found in the IC are markedly higher. Most likely, the different anesthesia regime (ketamine-medetomine), anatomical sampling and stimulation protocols used in the medial geniculate body study may explain the low levels of SSA reported there, since a degradation of the SSA sensitivity along the auditory pathway is unlikely (Perez-Gonzalez et al. 2012; Dhruv and Carandini 2014; Duque et al. 2014; Escera and Malmierca 2014). Moreover, it is generally accepted that anesthesia can have a more pronounced effect on the auditory cortex than on the IC (Maggi and Meli 1986; Sloan 1998; laboratory unpublished observations), since more indirect input pathways—as the auditory cortex—are more likely to be affected by anesthesia (Goldstein and Abeles 1975). Therefore, it would be very interesting to directly compare how SSA is in the

auditory cortex in awake and anesthetized animals as we have done here in the IC in further experiments. Finally, it should be emphasized that the current experiments only provide an unequivocal demonstration that SSA is not generated by urethane anesthesia; the effect of other anesthetic agents remains unknown.

SSA has been associated with important sensory tasks, namely novelty detection or change detection (Pérez-González and Malmierca 2014) and its electrophysiological correlates, i.e. the mismatch negativity auditory evoked potential or the middle-latency response studied in detail in both humans (Näätänen et al. 1978; Slabu et al. 2010; Grimm et al. 2011) and small rodents using both the anesthetized (Tikhonravov et al. 2008; Astikainen et al. 2011; Shiramatsu et al. 2013) and awake (Nakamura et al. 2011; Jung et al. 2013) preparations. Recent studies have shown that the moderate urethane effects on neurotransmission seem to perfectly mimic natural sleep (Clement et al. 2008; Pagliardini et al. 2012, 2013), a state that implies a reduction of behavioral responsiveness (Rechtschaffen et al. 1966). While mismatch negativity shows some attenuation during natural sleep (Loewy et al. 1996; Ruby et al. 2008), our study is in agreement with a recent account (Nir et al. 2013) that showed that natural sleep does not affect the generation of SSA in the auditory cortex. Nevertheless, while urethane anesthesia closely mimics the alternations of forebrain rhythms found in the EEG components during sleep (Clement et al. 2008), the overall SR is dramatically affected by urethane, at least in the IC. Such a reduction of the SR could be related to a general tendency for high levels of SSA during sleep-like states. As we demonstrate, SSA is similar but certainly not identical, in the awake and anesthetized preparations. This suggests that during sleep we may need more robust resources to react to danger than in an awake state in which attention is already preactivated. Attenuation of SR during sleep could improve this essential response.

In conclusion, our study shows that while SSA is not due to urethane anesthesia, the awake preparation is different in that neurons show a much higher SR. These findings, neglected so far for technical reasons, should be considered in future studies, especially in view of recent work (Buran et al. 2014) demonstrating the importance of modulating the SR in attention and behavior. Our results also confirm the validity and the importance of studying SSA in the IC of the mouse because these results provide the baseline for future studies and open new avenues to the study genetic modifications affecting the auditory brain in transgenic mice. Moreover, because the IC acts as a computational center in the auditory midbrain that may gate thalamic activity (Winer et al. 1996; Peruzzi et al. 1997; Ito et al. 2009), the simple and old-fashioned IC as a relay center can be ruled out (Skoe et al. 2013a, b; Skoe and Kraus 2010; Malmierca et al. 2014), hence supporting the idea of the IC is a key controller of cortical activation, including cortical SSA.

Acknowledgments We are most grateful to Drs. Flora Antunes, Nell Cant, Douglas Oliver, Alan Palmer and Christine Portfors for their critical and valuable comments on a previous version of this manuscript. We also thank Mr. Javier Nieto and Dr. David Pérez-González for their help and assistance in the analysis of the data. Financial support was provided by the Spanish MEC (BFU2013-43608-P), to MSM; DD held a fellowship from the Spanish MEC (BES-2010-035649). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

Abolafia JM, Vergara R, Arnold MM, Reig R, Sanchez-Vives MV (2011) Cortical auditory adaptation in the awake rat and the role of potassium currents. Cereb Cortex 21:977–990. doi:10.1093/cercor/bhq163

Anderson LA, Christianson GB, Linden JF (2009) Stimulus-specific adaptation occurs in the auditory thalamus. J Neurosci 29:7359–7363. doi:10.1523/JNEUROSCI.0793-09.2009

Antunes FM, Malmierca MS (2011) Effect of auditory cortex deactivation on stimulus-specific adaptation in the medial geniculate body. J Neurosci 31:17306–17316. doi:10.1523/JNEUROSCI.1915-11.2011

Antunes FM, Malmierca MS (2014) An overview of stimulus-specific adaptation in the auditory thalamus. Brain Topogr. doi:10.1007/s10548-013-0342-6

Antunes FM, Nelken I, Covey E, Malmierca MS (2010) Stimulus-specific adaptation in the auditory thalamus of the anesthetized rat. PLoS One 5:e14071. doi:10.1371/journal.pone.0014071

Astikainen P, Stefanics G, Nokia M, Lipponen A, Cong F, Penttonen M, Ruusuvirta T (2011) Memory-based mismatch response to frequency changes in rats. PLoS One 6:e24208. doi:10.1371/journal.pone.0024208

Ayala YA, Malmierca MS (2013) Stimulus-specific adaptation and deviance detection in the inferior colliculus. Front Neural Circuits 6:89. doi:10.3389/fncir.2012.00089. eCollection 2012

Ayala YA, Perez-Gonzalez D, Duque D, Nelken I, Malmierca MS (2013) Frequency discrimination and stimulus deviance in the inferior colliculus and cochlear nucleus. Front Neural Circuits 6:119. doi:10.3389/fncir.2012.00119. eCollection 2012

Bryant JL, Roy S, Heck DH (2009) A technique for stereotaxic recordings of neuronal activity in awake, head-restrained mice. J Neurosci Methods 178:75–79. doi:10.1016/j.jneumeth.2008.11.014

Buran BN, von Trapp G, Sanes DH (2014) Behaviorally gated reduction of spontaneous discharge can improve detection thresholds in auditory cortex. J Neurosci 34:4076–4081. doi:10.1523/JNEUROSCI.4825-13.2014

Castro-Alamancos MA (2004) Absence of rapid sensory adaptation in neocortex during information processing states. Neuron 41:455–464

Clement EA, Richard A, Thwaites M, Ailon J, Peters S, Dickson CT (2008) Cyclic and sleep-like spontaneous alternations of brain state under urethane anaesthesia. PLoS One 3:e2004. doi:10.1371/journal.pone.0002004

Chandrasekaran B, Kraus N (2010) The scalp-recorded brainstem response to speech: neural origins and plasticity. Psychophysiology 47(2):236–246. doi:10.1111/j.1469-8986.2009.00928.x

Chung S, Li X, Nelson SB (2002) Short-term depression at thalamocortical synapses contributes to rapid adaptation of cortical sensory responses in vivo. Neuron 34:437–446

Chung Y, Hancock KE, Nam SI, Delgutte B (2014) Coding of electric pulse trains presented through cochlear implants in the auditory midbrain of awake rabbit: comparison with anesthetized preparations. J Neurosci 34:218–231. doi:10.1523/JNEUROSCI.2084-13.2014

Dhruv NT, Carandini M (2014) Cascaded effects of spatial adaptation in the early visual system. Neuron 81:529–535. doi:10.1016/j.neuron.2013.11.025

Duque D, Malmierca MS, Caspary DM (2014) Modulation of stimulus-specific adaptation by GABAA receptor activation or blockade in the medial geniculate body of the anaesthetized rat. J Physiol 592:729–743. doi:10.1113/jphysiol.2013.261941

Duque D, Perez-Gonzalez D, Ayala YA, Palmer AR, Malmierca MS (2012) Topographic distribution, frequency, and intensity dependence of stimulus-specific adaptation in the inferior colliculus of the rat. J Neurosci 32:17762–17774. doi:10.1523/JNEUROSCI.3190-12.2012

Ehret G (1979) Quantitative analysis of nerve fibre densities in the cochlea of the house mouse (Mus musculus). J Comp Neurol 183(1):73–88. doi:10.1002/cne.901830107

Escera C, Malmierca MS (2014) The auditory novelty system: an attempt to integrate human and animal research. Psychophysiology 51:111–123. doi:10.1111/psyp.12156

Egorova M, Ehret G, Vartanian I, Esser KH (2001) Frequency response areas of neurons in the mouse inferior colliculus. I. Threshold and tuning characteristics. Exp Brain Res 140:145–161

Faure PA, Fremouw T, Casseday JH, Covey E (2003) Temporal masking reveals properties of sound-evoked inhibition in duration-tuned neurons of the inferior colliculus. J Neurosci 23:3052–3065

Fishman YI, Steinschneider M (2012) Searching for the mismatch negativity in primary auditory cortex of the awake monkey: deviance detection or stimulus specific adaptation? J Neurosci 32:15747–15758. doi:10.1523/JNEUROSCI.2835-12.2012

Fitzpatrick DC, Kuwada S, Batra R, Trahiotis C (1995) Neural responses to simple simulated echoes in the auditory brain stem of the unanesthetized rabbit. J Neurophysiol 74:2469–2486

Franklin K, Paxinos G (2007) The Mouse Brain in Stereotaxic Coordinates, 3rd edn. Academic Press, New York

Goldstein MH Jr, Abeles M (1975) Note on tonotopic organization of primary auditory cortex in the cat. Brain Res 100(1):188–191

Grimm S, Escera C, Slabu L, Costa-Faidella J (2011) Electrophysiological evidence for the hierarchical organization of auditory change detection in the human brain. Psychophysiology 48:377–384. doi:10.1111/j.1469-8986.2010.01073.x

Hara K, Harris RA (2002) The anesthetic mechanism of urethane: the effects on neurotransmitter-gated ion channels. Anesth Analg 94:313–318 (table of contents)

Hernandez O, Espinosa N, Perez-Gonzalez D, Malmierca MS (2005) The inferior colliculus of the rat: a quantitative analysis of monaural frequency response areas. Neuroscience 132:203–217

Hernández O, Rees A, Malmierca MS (2006) A GABAergic component in the commissure of the inferior colliculus in rat. Neuroreport 17:1611–1614

Holmstrom LA, Eeuwes LB, Roberts PD, Portfors CV (2010) Efficient encoding of vocalizations in the auditory midbrain. J Neurosci 30:802–819. doi:10.1523/JNEUROSCI.1964-09.2010

Ito T, Bishop DC, Oliver DL (2009) Two classes of GABAergic neurons in the inferior colliculus. J Neurosci 29(44):13860–13869. doi:10.1523/JNEUROSCI.3454-09.2009

Izquierdo MA, Gutiérrez-Conde PM, Merchán MA, Malmierca MS (2008) Non-plastic reorganization of frequency coding in the inferior colliculus of the rat following noise-induced hearing loss. Neuroscience 154:355–369. doi:10.1016/j.neuroscience.2008.01.057

Jones S, Mill R, Denham S, Klump G (2012) Stimulus-specific adaptation in the inferior colliculus of the awake gerbil in response to Markov chain tone sequences. ARO Midwinter Meeting, abstr 795, San Diego, USA

Jung F, Stephan KE, Backes H, Moran R, Gramer M, Kumagai T, Graf R, Endepols H, Tittgemeyer M (2013) Mismatch responses in the awake rat: evidence from epidural recordings of auditory cortical fields. PLoS One 8:e63203. doi:10.1371/journal.pone.0063203

King AJ, Nelken I (2009) Unraveling the principles of auditory cortical processing: can we learn from the visual system? Nat Neurosci 12(6):698–701. doi:10.1038/nn.2308

Klein C, von der Behrens W, Gaese BH (2014) Stimulus-specific adaptation in field potentials and neuronal responses to frequency-modulated tones in the primary auditory cortex. Brain Topogr. doi:10.1007/s10548-014-0376-4

Kuwada S, Batra R, Stanford TR (1989) Monaural and binaural response properties of neurons in the inferior colliculus of the rabbit: effects of sodium pentobarbital. J Neurophysiol 61:269–282

Loewy DH, Campbell KB, Bastien C (1996) The mismatch negativity to frequency deviant stimuli during natural sleep. Electroencephalogr Clin Neurophysiol 98:493–501

Lumani A, Zhang H (2010) Responses of neurons in the rat's dorsal cortex of the inferior colliculus to monaural tone bursts. Brain Res 1351:115–129. doi:10.1016/j.brainres.2010.06.066

Maggi CA, Meli A (1986) Suitability of urethane anesthesia for physiopharmacological investigations in various systems. Part 1: general considerations. Experientia 42:109–114

Malmierca MS (2003) The structure and physiology of the rat auditory system: an overview. Int Rev Neurobiol 56:147–211

Malmierca MS, Ryugo DK (2011) Auditory system. The mouse nervous system, 1st edn. Academic Press, San Diego, pp 607–645

Malmierca MS, Hernandez O, Rees A (2005) Intercollicular commissural projections modulate neuronal responses in the inferior colliculus. Eur J Neurosci 21(10):2701–2710. doi:10.1111/j.1460-9568.2005.04103.x

Malmierca MS, Cristaudo S, Perez-Gonzalez D, Covey E (2009) Stimulus-specific adaptation in the inferior colliculus of the anesthetized rat. J Neurosci 29:5483–5493

Malmierca MS, Izquierdo MA, Cristaudo S, Hernández O, Pérez-González D, Covey E, Oliver DL (2008) A discontinuous tonotopic organization in the inferior colliculus of the rat. J Neurosci 28:4767–4776. doi:10.1523/JNEUROSCI.0238-08.2008

Malmierca MS, Sanchez-Vives MV, Escera C, Bendixen A (2014) Neuronal adaptation, novelty detection and regularity encoding in audition. Front Syst Neurosci 8:111. doi:10.3389/fnsys.2014.00111

Merrill EG, Ainsworth A (1972) Glass-coated platinum-plated tungsten microelectrodes. Med Biol Eng 10:662–672

Moroni F, Corradetti R, Casamenti F, Moneti G, Pepeu G (1981) The release of endogenous GABA and glutamate from the cerebral cortex in the rat. Naunyn-Schmiedeberg's Arch Pharmacol 316:235–239

Muniak MA, Mayko ZM, Ryugo DK, Portfors CV (2012) Preparation of an awake mouse for recording neural responses and injecting tracers. J Vis Exp. doi:10.3791/3755

Näätänen R, Gaillard AW, Mantysalo S (1978) Early selective-attention effect on evoked potential reinterpreted. Acta Psychol (Amst) 42:313–329

Nakamura T, Michie PT, Fulham WR, Todd J, Budd TW, Schall U, Hunter M, Hodgson DM (2011) Epidural auditory event-related

potentials in the rat to frequency and duration deviants: evidence of mismatch negativity? Front Psychol 2:367. doi:10.3389/fpsyg.2011.00367. eCollection 2011

Nir Y, Vyazovskiy VV, Cirelli C, Banks MI, Tononi G (2013) Auditory responses and stimulus-specific adaptation in rat auditory cortex are preserved across NREM and REM sleep. Cereb Cortex. doi:10.1093/cercor/bht328

Pagliardini S, Funk GD, Dickson CT (2013) Breathing and brain state: urethane anesthesia as a model for natural sleep. Respir Physiol Neurobiol 188:324–332. doi:10.1016/j.resp.2013.05.035

Pagliardini S, Greer JJ, Funk GD, Dickson CT (2012) State-dependent modulation of breathing in urethane-anesthetized rats. J Neurosci 32:11259–11270. doi:10.1523/JNEUROSCI.0948-12.2012

Perez-Gonzalez D, Malmierca MS (2012) Variability of the time course of stimulus-specific adaptation in the inferior colliculus. Front Neural Circuits 6:107. doi:10.3389/fncir.2012.00107. eCollection 2012

Pérez-González D, Malmierca MS (2014) Adaptation in the auditory system: an overview. Front Integr Neurosci 8:19. doi:10.3389/fnint.2014.00019

Pérez-González D, Malmierca MS, Covey E (2005) Novelty detector neurons in the mammalian auditory midbrain. Eur J Neurosci 22:2879–2885

Perez-Gonzalez D, Hernandez O, Covey E, Malmierca MS (2012) GABA(A)-mediated inhibition modulates stimulus-specific adaptation in the inferior colliculus. PLoS One 7:e34297. doi:10.1371/journal.pone.0034297 [Erratum in: PLoS One. 2012;7(8). doi:10.1371/annotation/883c90e9-2108-449f-a652-2cbf25ba6456]

Peruzzi D, Bartlett E, Smith PH, Oliver DL (1997) A monosynaptic GABAergic input from the inferior colliculus to the medial geniculate body in rat. J Neurosci 17(10):3766–3777

Portfors CV, Roberts PD, Jonson K (2009) Over-representation of species-specific vocalizations in the awake mouse inferior colliculus. Neuroscience 162:486–500. doi:10.1016/j.neuroscience.2009.04.056

Portfors CV, Mayko ZM, Jonson K, Cha GF, Roberts PD (2011) Spatial organization of receptive fields in the auditory midbrain of awake mouse. Neuroscience 193:429–439. doi:10.1016/j.neuroscience.2011.07.025

Rechtschaffen A, Hauri P, Zeitlin M (1966) Auditory awakening thresholds in REM and NREM sleep stages. Percept Motor Skills 22:927–942

Rees A, Sarbaz A, Malmierca MS, Le Beau FE (1997) Regularity of firing of neurons in the inferior colliculus. J Neurophysiol 77:2945–2965

Richardson BD, Hancock KE, Caspary DM (2013) Stimulus-specific adaptation in auditory thalamus of young and aged awake rats. J Neurophysiol 110:1892–1902. doi:10.1152/jn.00403.2013

Roberts PD, Portfors CV (2008) Design principles of sensory processing in cerebellum-like structures. Early stage processing of electrosensory and auditory objects. Biol Cybern 98:491–507. doi:10.1007/s00422-008-0217-1

Ruby P, Caclin A, Boulet S, Delpuech C, Morlet D (2008) Odd sound processing in the sleeping brain. J Cogn Neurosci 20:296–311. doi:10.1162/jocn.2008.20023

Sceniak MP, Maciver MB (2006) Cellular actions of urethane on rat visual cortical neurons in vitro. J Neurophysiol 95:3865–3874

Shiramatsu TI, Kanzaki R, Takahashi H (2013) Cortical mapping of mismatch negativity with deviance detection property in rat. PLoS One 8:e82663. doi:10.1371/journal.pone.0082663. eCollection 2012

Skoe E, Chandrasekaran B, Spitzer ER, Wong PC, Kraus N (2013a) Human brainstem plasticity: the interaction of stimulus probability and auditory learning. Neurobiol Learn Mem 109C:82–93. doi:10.1016/j.nlm.2013.11.011

Skoe E, Kraus N (2010) Auditory brain stem response to complex sounds: a tutorial. Ear Hear 31(3):302–324. doi:10.1097/AUD.0b013e3181cdb272

Skoe E, Krizman J, Spitzer E, Kraus N (2013b) The auditory brainstem is a barometer of rapid auditory learning. Neuroscience 243:104–114. doi:10.1016/j.neuroscience.2013.03.009

Slabu L, Escera C, Grimm S, Costa-Faidella J (2010) Early change detection in humans as revealed by auditory brainstem and middle-latency evoked potentials. Eur J Neurosci 32:859–865. doi:10.1111/j.1460-9568.2010.07324.x

Sloan TB (1998) Anesthetic effects on electrophysiologic recordings. J Clin Neurophysiol: Off Publ Am Electroencephalogr Soc 15(3):217–226

Song P, Wang N, Wang H, Xie Y, Jia J, Li H (2011) Pentobarbital anesthesia alters neural responses in the precedence effect. Neurosci Lett 498:72–77. doi:10.1016/j.neulet.2011.04.066

Stabler SE, Palmer AR, Winter IM (1996) Temporal and mean rate discharge patterns of single units in the dorsal cochlear nucleus of the anesthetized guinea pig. J Neurophysiol 76:1667–1688

Stiebler I, Ehret G (1985) Inferior colliculus of the house mouse. I. A quantitative study of tonotopic organization, frequency representation, and tone-threshold distribution. J Comp Neurol 238(1):65–76. doi:10.1002/cne.902380106

Ter-Mikaelian M, Sanes DH, Semple MN (2007) Transformation of temporal properties between auditory midbrain and cortex in the awake Mongolian gerbil. J Neurosci 27:6091–6102

Thomas JM, Morse C, Kishline L, O'Brien-Lambert A, Simonton A, Miller KE, Covey E (2012) Stimulus-specific adaptation in specialized neurons in the inferior colliculus of the big brown bat, *Eptesicus fuscus*. Hear Res 291:34–40

Tikhonravov D, Neuvonen T, Pertovaara A, Savioja K, Ruusuvirta T, Naatanen R, Carlson S (2008) Effects of an NMDA-receptor antagonist MK-801 on an MMN-like response recorded in anesthetized rats. Brain Res 1203:97–102. doi:10.1016/j.brainres.2008.02.006

Tollin DJ, Populin LC, Yin TC (2004) Neural correlates of the precedence effect in the inferior colliculus of behaving cats. J Neurophysiol 92:3286–3297

Ulanovsky N, Las L, Nelken I (2003) Processing of low-probability sounds by cortical neurons. Nat Neurosci 6:391–398

von der Behrens W, Bauerle P, Kossl M, Gaese BH (2009) Correlating stimulus-specific adaptation of cortical neurons and local field potentials in the awake rat. J Neurosci 29:13837–13849. doi:10.1523/JNEUROSCI.3475-09.2009

Winer JA, Saint Marie RL, Larue DT, Oliver DL (1996) GABAergic feedforward projections from the inferior colliculus to the medial geniculate body. Proc Natl Acad Sci USA 93(15):8005–8010

Willott JF, Parham K, Hunter KP (1988) Response properties of inferior colliculus neurons in young and very old CBA/J mice. Hear Res 37:1–14

Young ED, Brownell WE (1976) Responses to tones and noise of single cells in dorsal cochlear nucleus of unanesthetized cats. J Neurophysiol 39:282–300

Zhao L, Liu Y, Shen L, Feng L, Hong B (2011) Stimulus-specific adaptation and its dynamics in the inferior colliculus of rat. Neuroscience 181:163–174. doi:10.1016/j.neuroscience.2011.01.060

Zook JM, Winer JA, Pollak GD, Bodenhamer RD (1985) Topology of the central nucleus of the mustache bat's inferior colliculus: correlation of single unit properties and neuronal architecture. J Comp Neurol 231(4):530–546