
# Neuronal basis of auditory adaptation and temporal discrimination in the auditory cortex of the awake freely moving rat

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# *NEURONAL BASIS OF AUDITORY ADAPTATION AND TEMPORAL DISCRIMINATION IN THE AUDITORY CORTEX OF THE AWAKE FREELY MOVING RAT.*


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Explanation: This part of the thesis is written in Spanish, English and French in order to thank each person in the proper language.
Aclaración: Este apartado de tesis está escrito en español, inglés y francés para agradecer a cada persona en su idioma correspondiente.
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# INDEX

# 1. INTRODUCTION

## 1.1. Neuronal response adaptation in the auditory cortex

That adaptation occurs in the auditory system is a phenomenon that we all experience when we stop hearing background noises such as traffic that initially may have been perceived as disturbing. Adaptation to known stimuli increases sensitivity towards new ones, acting as a gain control mechanism and influencing stimuli perception. In the primary auditory cortex the responses of single units to sound stimulation are known to be affected by the preceding history of stimulation (Abolafia et al. 2010). In all, understanding how sensory cortices adapt to changing environments constitutes an important issue. The first study presented in the present thesis aimed at exploring the time course of auditory adaptation at the single unit level in the primary auditory cortex of the freely moving rat.

### 1.1.1. Timescale of adaptation: effect of the previous history of stimulation on the response amplitude of subsequent responses

Adaptation to auditory stimuli has been classically studied in different animal species (Anderson et al. 2006; Shechter and Depireux 2006; Gourevitch and Eggermont 2008; Wimmer et al. 2008; Hildebrandt et al. 2009; von der Behrens et al. 2009) under fast timescales (less than 400 ms) (Calford and Semple 1995; Brosch and Schreiner 1997; Sakai et al. 2009a) and the studies that have used very short interstimulus intervals often referred to adaptation as *forward inhibition or forward masking*. Typically, adaptation is maximal (Reale and Brugge 2000) for intervals approaching 50 ms, although it is still enhanced when short interstimulus intervals (ISIs) are used up to 400 ms.

Auditory adaptation has also been reported to be influenced by longer-lasting stimulus history (several seconds) (Malone et al. 2002; Ulanovsky et al. 2003; Ulanovsky et al. 2004; Werner-Reiss et al. 2006; Gourevitch and Eggermont 2008; Asari and Zador 2009). Long-term adaptation, ranging from minutes to weeks, has been also found (Gourevitch and Eggermont 2008) and it has been explained in terms of topographical changes in auditory cortex.

Acoustic environmental enrichment could also increase the response amplitude and synchronization of neurons to stimuli presented at slow rates, while the opposite occurs at faster rates (Percaccio et al. 2005). Moreover, (Delano et al. 2008) found that the silent period before the presentation of an auditory stimulus is a critical factor that affects stimulus-induced oscillations.

It has been suggested that neuronal response adaptation may scale with the duration of the ensemble of stimuli presented (Ulanovsky et al. 2004). Similarly, different timescales have been attributed to the firing rates of auditory cortex neurons (Nelken et al. 2003). Then, fast timescales (10 ms) would coexist with medium (100 ms) and slow (1 s) timescales in order to extract the relevant features of the auditory scene.

The influence of the interstimulus interval on subsequent responses has been shown to be also dependent on the frequency of the tones presented, what is called “specificity”. Therefore a certain stimulus having different frequency from the subsequent one would induce lesser adaptation in the later. If, on the contrary both stimuli have similar frequency the adaptation would be enhanced (Rauschecker 2005), and this phenomenon has been termed *stimulus specific adaptation*. Therefore, the degree of adaptation is dependent on the stimuli features. (Gourevitch and Eggermont 2008) showed that neuronal firing in the anesthetized cat tended to be more enhanced when a low density stimuli is being presented, while the opposite occurs for high density stimuli.

The ability of the auditory cortex to follow different stimuli repetition rates has been studied by (Lu et al. 2001; Wang 2007). Two different kinds of neuronal response were found. Stimulus synchronized responses showed always a spiking response to click trains, while non-synchronized ones showed no response immediately after each click stimulus. These two kinds of responses were suggested to encode temporal aspects of stimuli in two different ways. Then, synchronized responses would code stimuli by spike responses after each stimulus, therefore, coding slowly occurring events. On the contrary, non-synchronized responses would code stimulus repetition rate by means of spike timing, therefore, representing rapidly changing temporal intervals by their average firing rate.

An interesting study by (Werner-Reiss et al. 2006) recorded single units responses to broadband noise from the auditory cortex of the awake monkey to broadband noise. Monkeys were trained to maintain fixation to a visual stimulus while auditory stimuli was presented. Therefore, auditory stimuli were perceived under attentive brain state conditions. Under these conditions, interstimulus intervals of more than 2 seconds barely had any effect on subsequent responses, although some neurons showed adaptation lasting up to 5 seconds.

Similarly, (Anderson et al. 2006) focused on the study of the maximum repetition rate that auditory cortex can track. The authors recorded multiunit activity to click presentation (80 microsec; broadband noise) from the awake rat and they found that “slow” repetition rate (20-50 Hz) is encoded by responses synchronized with the stimulus (i.e. responses phase locked with stimuli). While fast repetition rates (>50 Hz) are encoded by transient increases in the firing rate (only in the first 50 ms to stimulus presentation) or by changes (increased or decreased firing rate) in the sustained firing.

The influence of the sound level of previous stimulus presentation has been scarcely tested (Bartlett and Wang 2005). The authors found in the awake marmoset

monkey that as the intensity of stimulus one (S1) increased the response to the second stimulus (S2) decreased its firing rate, although many units showed no change in response to S2 or even response facilitation. The authors presented a variety of stimuli like pure tones, band-pass-filtered noises, sinusoidally amplitude-modulated tones or noises, and sinusoidally frequency modulated tones. A similar finding was observed when the duration of S1 was augmented, such that the longer the S1 duration the further suppression of S2. Importantly, the timescale of the suppression or facilitation observed ranged between hundreds of milliseconds to more than one second. This results are in line with other studies in the anesthetized animal where the majority of units showed suppression to S2 as S1 increased in decibels or duration, although some units showed the opposite pattern (Brosch and Schreiner 1997, 2000).

The reduction of spontaneous activity right after stimulus offset – often called postadaptation – has not been thoroughly studied in auditory cortex. (DeWeese et al. 2003) recorded from cell-attached neurons in the auditory cortex of ketamine/medetomidine anesthetized rats. The authors observed a reduction of spontaneous and evoked activity for over 200 ms after stimulus presentation. (Anderson et al. 2006) also reported in the recorded multiunit of the awake rat an enhanced inhibition of around 60 to 100 ms following the first click presentation.

### 1.1.2. Spike timing and adaptation

The timing of neuronal firing has been also shown to be affected by the previous history of stimulation. The measure of response onset has been a matter of controversy given the high variability of the spiking responses, namely in the awake animal. (Ter-Mikaelian et al. 2007) recorded neuronal responses from the auditory cortex of the anesthetized and awake mongolian gerbil and they defined the response onset as the first bin (10ms) that contains the highest spikes number. The

authors compared the response variability or fidelity between auditory cortex and inferior colliculus (IC), being higher for the former than the later. Low temporal response onset variability (2 to 3 ms) has been widely reported in the auditory cortex, although temporal jitter of less than 1 ms was found (DeWeese et al. 2005).

Stimulus locked responses have been reported to be dependent on the stimuli presentation rate. Stimulus locking boundary could be of 30 ms interstimulus interval (Sakai et al. 2009a). Response latencies are also dependent on the firing properties of auditory neurons. (Chimoto et al. 2002) reported 3 kinds of cells with different sustained firing properties and response onset properties: a) tonic cells that maintained their firing along with stimulus duration and with an average response onset of 25.3 ms, b) phasic-tonic cells that tend to adapt to less than half of the maximum firing amplitude, and they showed a response latency of 19.8, and c) phasic cells that only responded transiently to the stimulus presentation and with an average response onset of 10.2. Therefore, response onset is shorter as the firing properties of the neuron become more transient. In this study the response onset was defined as the time interval from stimulus onset to the time the Perievent raster and histogram (PSTH) reached a value of 2 standard deviations above the mean.

Recordings in the anesthesized cat varied the click rate presentation from 1 to 32 clicks per second and found that the response latency increased with a decrease in response probability (Eggermont 1991).

### 1.1.3. Stimulus-specific adaptation

Stimulus-specific adaptation refers to the decreased response to the same sound when it is common, while an increased response occurs to rare sounds. Stimulus-specific adaptation has been described in the auditory cortex of the anesthetized animal (Brosch and Schreiner 1997; Ulanovsky et al. 2003; Ulanovsky et al. 2004; Pienkowski and Eggermont 2009; Szymanski et al. 2009b) but also in the

awake (Dahmen et al. 2008; von der Behrens et al. 2009). There the term *specific* is used in the sense that the cortex stops responding to repeated stimuli but remains responsive to rare, infrequent sounds. However, not all adaptation is specific to the preceding stimulus (Bartlett and Wang 2005). Adaptation has been also shown to be modulated by attention (Hudson et al. 2009). Moreover, that adaptation to auditory stimuli depends on the sensory context or stimuli ensemble has been suggested also by (Shechter and Depireux 2006). Similarly, sensory processing has been suggested to depend on the ongoing stimulus statistics (Diaz-Quesada and Maravall 2008; Dahmen et al. 2010). Finally, (Bartlett and Wang 2005) found that suppression of auditory response to S2 was stronger when its parameters were similar to the one of S1. i.e. when the carrier frequency, duration and sound level were similar in both stimuli.

### 1.1.4. Adaptation and anesthesia

It is known that anesthesia has an effect on cortical excitability. In this respect, the spectrotemporal dynamics of frequency response areas, which refers to the neuronal response amplitude along with stimuli duration, was found to be more variable in the anesthesized preparation (Qin et al. 2003). Pentobarbital and ketamine anesthesia also induces less precisely timed response onset and they occur more reliably than in the awake recordings of the Mongolian gerbil (Ter-Mikaelian et al. 2007).

(Wang 2007) suggests that non-synchronous responses to repetitive stimulation also carry information about auditory stimuli, and that this response pattern is affected in the ketamine/barbiturate anesthesized animals. Additionally, the synchronization boundary or the ability to follow repetitive stimulation has been suggested to be higher in the auditory cortex of the awake animal than in the anesthesized (Wang 2007). Similarly, (Wang 2007) compared the response patterns in the auditory cortex of the awake marmosets versus anesthesized cats. As opposed to

other studies, he found that neurons responded to wide-band and narrow-band clicks more weakly in the awake than in the anesthesized animals. Importantly, non-synchronized responses were not present in the anesthesized preparation.

The neuronal response patterns in the auditory cortex of the cat have been studied under halothane anesthesia (Moshitch et al. 2006). The frequency response area and the temporal response patterns resembled the ones obtained in previous studies in the awake animals. Moreover, halothane anesthesia affected to a lesser extent sustained responses than barbiturate or ketamine anesthesia (Moshitch et al. 2006).

In all, there is strong evidence that anesthesia affects neuronal activity. The influence of pentobarbital/chloral hydrate was quantified and the tuning properties of the awake versus anesthesized rat compared (Gaese and Ostwald 2001). The authors found that the frequency range of response areas was narrower in the anesthesia with respect to the awake animal. Similarly, the excitatory or inhibitory subregions of the frequency response area were shifted. Therefore, the authors found an overall enhancement of inhibition.

### 1.1.5. Cortical and subcortical origins of adaptation

Although adaptation was believed to be mostly cortical, it has been recently shown that auditory adaptation also exists in the inferior colliculus (Kvale and Schreiner 2004; Perez-Gonzalez et al. 2005) and in the auditory thalamus (Anderson et al. 2009; Malmierca et al. 2009). Some of the adaptation observed in the cortex may be transmitted from lower nuclei, and conversely, part of the adaptation observed in those areas may be of descending cortical origin (for a review see: (Robinson and McAlpine 2009). Since forward inhibition has been evidenced to be present in several structures along the ascending auditory pathway it has been suggested that cortical adaptation is generated by subcortical structures, although

intracortical mechanisms could also influence adaptation (Brosch and Schreiner 1997).

Alternatively, other authors have declined the implication of the auditory thalamus in stimulus specific auditory adaptation, given that no sensitivity to stimulus specific history was observed in the medial geniculate body (Ulanovsky et al. 2003; Ulanovsky et al. 2004). Therefore adaptation was believed to be mostly cortical where stimuli is encoded and remains in the memory for a long time period as observed in the so called phenomenon of stimulus specific adaptation. Similarly, the authors suggest that the degree of adaptation occurred to the same extent for nearby neurons located in the same cortical column.

In a stimulation protocol where the influence of a masker was tested on the probe, it was found that 50% of the response was recovered at 2 ms between stimuli in subcortical structures like the auditory nerve, anteroventral cochlear nucleus or superior olivary complex, while in the inferior colliculus the 50% recovery was found at 7 ms (Fitzpatrick et al. 1999). In the case of the primary auditory cortex, the 50% response recovery was found at 20 ms. Therefore, the authors found that adaptation was more enhanced in the primary auditory cortex than in subcortical structures. Indeed, the auditory cortex has been observed to be more sluggish than other subcortical structures (Nelken et al. 2003). Therefore, the ability of the auditory cortex to follow temporally fast occurring events is less than that of subcortical structures (Schnupp 2006). Similarly, (Wang 2007) suggested that stimulus selectivity increases along the auditory pathway, i.e. auditory nerve, cochlear nucleus, inferior colliculus, medial geniculate body and auditory cortex.

### 1.1.6. Mechanisms of adaptation

The fact that different adaptation patterns were found (Ulanovsky et al. 2003; Ulanovsky et al. 2004) makes it more difficult to pin down its underlying mechanisms. An additional problem to understand the basis of adaptation is that different laboratories use different experimental animals, anesthesia, protocols of stimulation, methods of analysis and even different conceptual frame and terminology (e.g. response suppression, forward masking, adaptation, forward suppression, response to repetitive stimuli).

A number of different mechanisms operating at the input of the neuron that could underlie cortical adaptation have been considered: synaptic depression (Wehr and Zador 2005; Rothman et al. 2009), decreased excitation, lateral inhibition (Shamma and Symmes 1985; Zhang et al. 2003; Qin and Sato 2004; Oswald et al. 2006), excitatory-inhibitory imbalance (De Ribaupierre et al. 1972b; Volkov and Galazjuk 1991; Ojima and Murakami 2002; Oswald et al. 2006; Sakai et al. 2009a) or increased inhibition (Metherate and Ashe 1994; Kilgard and Merzenich 1999; Zhang et al. 2003). Each of these mechanisms involves a network of interneuronal connections. However, the role of intrinsic properties have been addressed in the visual cortex (Sanchez-Vives et al. 2000a, b) or in the barrel cortex (Diaz-Quesada and Maravall 2008), while it was barely studied in other areas (Wang 1998). Moreover, the role of ionic currents on auditory adaptation has been largely ignored so far. One of the reasons is that the currents that usually underlie adaptation processes, namely $K^+$ currents, have not been characterized in the auditory cortex. Potassium currents have been thoroughly studied in other cortical areas, their activation is often activity-dependent and they can maintain neurons hyperpolarized ranging from tens of milliseconds to tens of seconds (Pennefather et al. 1985; Schwindt et al. 1988b; Sah 1996; Vergara et al. 1998; Bhattacharjee and Kaczmarek 2005). Potassium channels play a role in sensory adaptation in other sensory areas

(Schwindt et al. 1988a; Sanchez-Vives et al. 2000a; Diaz-Quesada and Maravall 2008; Kuznetsova et al. 2008). $Na^+$ dependent potassium channels also contribute to the slow afterhyperpolarization that follows repetitive firing (Bhattacharjee and Kaczmarek 2005). Depolarization and high frequency firing during sensory responses can induce an intracellular increase of ions such as $Ca^{2+}$ or $Na^+$ that activate ion-dependent $K^+$ channels and also membrane depolarization can directly activate voltage-dependent $K^+$ channels as it was proposed in visual system for contrast adaptation (Sanchez-Vives et al. 2000a, b). The activation of potassium currents hyperpolarizes the membrane potential and decreases neuronal responsiveness to subsequent inputs. This can happen even in the absence of spikes, *via* ions entering the cell through AMPA receptors (Nanou et al. 2008).

## 1.2. Spontaneous neuronal activity in the auditory cortex

Spontaneous activity in the auditory cortex of the awake animal has been rarely reported (Manunta and Edeline 1999; DeWeese and Zador 2006; Hromadka et al. 2008; Luczak et al. 2009; Sakata and Harris 2009; Harris et al. 2010) whereas most of the studies have reported spontaneous activity patterns under anesthesia (e.g. (Kilgard and Merzenich 1999; Valentine and Eggermont 2001; DeWeese et al. 2003; Turner et al. 2005; Eggermont 2006)). In this respect, barbiturates have been shown to reduce spontaneous activity in auditory cortex (Zurita et al. 1994).

Some studies have reported that in auditory cortex of the awake animal the spontaneous activity ranges between 0.1 and 41 Hz, the mean being 4.5 Hz (Manunta and Edeline 1999). Similarly, (Hromadka et al. 2008) reported, by means of whole-cell recordings in the head-restrained rat, firing rates as low as 0.01 Hz, with a population mean spontaneous activity of 4.9. The auditory cortex has also been reported to be dominated by slow fluctuations of <4 Hz (Harris et al. 2010). The spontaneous activity in the awake animal is of particular relevance in order to

disentangle the dynamics of the network activity where a balance of excitation and inhibition may play an important role (Wehr and Zador 2003; DeWeese and Zador 2006). Moreover, the particular pattern of spontaneous activity of a population of single units has been shown to be relevant on sensory coding given the sequential structure observed in evoked and spontaneous activity (Luczak et al. 2009; Sakata and Harris 2009; Harris et al. 2010).

## 1.3. Sustained neuronal firing in the auditory cortex of the passive listening and attentive animal

Some studies have shown the effects of sustained (>0.5s) auditory stimulation in different animal species like the awake cat (Mickey and Middlebrooks 2003), macaques (Malone et al. 2002) or marmosets (Barbour and Wang 2003). The responses to sustained stimulation have been also suggested to vary among regions within the auditory pathway, where cortical structures tend to respond weaker and more phasic than subcortical ones (Schnupp 2006). Similarly, neurons show higher sustained firing rate to preferred than to non-preferred stimuli and this specificity is more enhanced in cortical structures than in subcortical ones (Wang 2007). Therefore, the proportion of neurons showing sustained firing decreases along the ascending auditory pathway.

Previous studies have shown that responses to sustained pure tones in awake cats were classified into phasic, phasic-tonic and tonic firing (Chimoto et al. 2002; Qin et al. 2003). The authors compared the frequency response area for the three different kinds of firing patterns of auditory neurons and they concluded that neurons with a sustained firing, i.e. tonic, show best-frequency-firing narrower and more stable than phasic ones.

The set of stimuli presented to the awake animal could also influence the pattern of sustained activity. (Barbour and Wang 2003) presented random spectrum

stimuli and he observed that 89% of auditory neurons showed sustained spiking to this kind of stimuli. This kind of neurons show a non-synchronized firing that has been previously suggested to code for auditory stimuli by means of sustained firing rate instead of synchronous responses that code stimuli by means of phase locked responses (Lu et al. 2001).

Even though sustained stimulation and responses constitute an intrinsic feature of auditory cortex and correspond to naturalistic conditions, sustained auditory activity has been barely studied in auditory cortex of the behaving animal. (Recanzone 2000) used relatively long stimulus duration (200 ms) consisting on Gaussian noise and pure tones, and the author characterized the different firing patterns while the monkey performed an auditory spatial location task. The effect of the attentional demands on the neuronal responses was not measured in this study, though. In the study by (Okazaki et al. 2010) the neuronal substrate of sound duration discrimination was reported in the anesthesized guinea pig in the context of Mismatch Negativity neuronal phenomena (Okazaki et al. 2010). The authors alternated stimuli with different durations, showing that whenever a duration transition difference was greater than 75 ms there was mainly a response enhancement at the offset of stimulus. These results were interpreted in terms of the ability of auditory cortex neurons to discriminate sound duration changes.

## 1.4. Discrimination of temporal information in the auditory cortex

Perception of auditory stimuli is a robust phenomenon despite the highly variable temporal information that the auditory cortex receives. For that reason, a relevant aspect of auditory perception is the analysis of temporal information, although few studies have tackled the issue of how single units of auditory cortex code for temporal information (Bao et al. 2004; Polley et al. 2006; Fritz et al. 2007;

Lemus et al. 2009; Liu et al. 2010) of relevant stimuli during an auditory task (Selezneva et al. 2006).

The neuronal basis of attention and decision making has been largely studied in different cortical areas (Buffalo et al. 2010; Romo and Salinas 2003; Sugrue et al. 2005; Uchida et al. 2006; Gold and Shadlen 2007; Yang et al. 2008; Resulaj et al. 2009). Similarly, the neurophysiological correlates of auditory attention have been also addressed (Weinberger 2004; Blake et al. 2006; Polley et al. 2006; Hromadka and Zador 2007; Lemus et al. 2009; Otazu et al. 2009). Moreover, temporal processing of auditory cortex has been studied following a number of experimental manipulations. Therein, some studies have focused on interval production tasks in humans (Merchant et al. 2008), discrimination of stimuli repetition rate (Bao et al. 2004; Polley et al. 2006; Fritz et al. 2007; Lemus et al. 2009) or frequency categorization of tones (Ohl et al. 2001; Selezneva et al. 2006). However, few studies have shown the role of attention during timing tasks at the level of the single unit and in awake animals (Lemus et al. 2009; Liu et al. 2010). The later study focused on the discrimination of two different repetition rates while recordings from the auditory cortex of the awake monkey were obtained. The author suggests that auditory cortex codes for stimuli rates through their firing rate and stimulus locked responses during stimuli presentation and not during the working memory or decision making periods of the trial. Therefore, they attributed to auditory cortex a sensory role rather than a cognitive one. In the study by (Liu et al. 2010) cats were required to make a GO response whenever a deviant interval was presented with respect to the standard one. These authors found a correlation between behavioural performance and neuronal response, arguing that perception of auditory intervals can be explained by the neuronal response to the second sound. We aimed to disentangle how single units in auditory cortex code for temporal information in a decision-making task. To solve this problem we recorded the activity of single units with tetrodes in auditory cortex

of the behaving rat and we compared the information content, the variability, and the slow modulation of spontaneous activity between the attentive and passive states.

### 1.4.1. Information content of single units in auditory cortex

Understanding how neuronal responses encode sensory information remains a fundamental question in auditory processing. Information-theoretical analysis as Mutual Information (MI) has provided important insights into the information content of neuronal codes in auditory cortex (Gehr et al. 2000; Furukawa and Middlebrooks 2002; Nelken et al. 2005; Chechik et al. 2006; Nelken and Chechik 2007; Kayser et al. 2009). MI analysis has been classically used to measure the stimulus-response relationship in order to see whether neuronal activity is stimulus selective or not (Lu and Wang 2004; Chechik et al. 2006; Nelken and Chechik 2007). In our study, we performed MI analysis to quantify the information content of single unit activity during task performance in order to disentangle the neuronal codes responsible for temporal discrimination in a decision-making task. We first quantified the information content during stimuli presentation in a temporal auditory decision-making task. Then, we compared the information content during the attentive stage to the passive ones, and during the Stimulus 2 with respect to Stimulus 1.

### 1.4.2. Neuronal response variability during attention

A classical view has been that stimulus-driven mean response enhancement to the target tone with respect to the distractor is the best way to improve signal-to-noise ratio (Desimone and Duncan 1995). Alternatively, recent studies have emphasized the importance of reduced variability to encode and enhance the sensitivity to sensory stimuli (Churchland et al. 2010; Mitchell et al. 2007; Cohen and

Maunsell 2009; Mitchell et al. 2009). Decreased variability has been observed in evoked responses in visual cortex under anesthesia (Kara et al. 2000) and during attentive conditions (Mitchell et al. 2007; Cohen and Maunsell 2009; Mitchell et al. 2009), for a review see (Churchland et al. 2010). In this way, the signal-to-noise ratio is increased, yielding the basis for an improved encoding of the stimulus information. However, response variability in auditory cortex has only been studied under anesthesia (DeWeese et al. 2003; Curto et al. 2009). Therefore, it remains unknown whether neuronal response variability might be a neuronal code that contributes or accounts for the temporal discrimination in the auditory cortex of the awake animal. Here, we analyzed the reduction of trial-to-trial neuronal variability in order to compare the stimulus-driven and not stimulus-driven variability under different behavioural states. Even more, we report that trial-to-trial neuronal fluctuations are not only reduced during explicit stimulus presentation but are also reduced in the interval between the auditory stimuli, indicating that the whole system is prepared to discriminate the relevant temporal information.

### 1.4.3. Slow modulation of evoked and spontaneous activity during attention

The slow modulation of neuronal firing to evoked and spontaneous activity has barely been studied in the auditory cortex, while phasic firing or even few millisecond firing has been attributed an important role in sensory encoding (Yang et al. 2008; Kayser et al. 2009). On the other hand, (Selezneva et al. 2006) measured the influence of category discrimination of tone steps on the neuronal responses of the awake monkey. Sustained responses were observed to predict the behavioural outcome of decisions made by the monkey. Therefore, the authors attributed a cognitive role to the sustained responses observed in the primary and secondary auditory fields. In another study the same authors suggested that slow modulation of firing rate, or sustained firing, emerges as a consequence of a learning process where

two consecutive sensory or behavioural events are contingent on reinforcement (Brosch et al. 2010). Therefore slow modulation of firing could constitute an anticipating mechanism that associates events (stimulus-behaviour-reinforcer) that are relevant or adaptive to the environment.

# 2. METHODS

## 2.1. Tetrodes and Microdrives

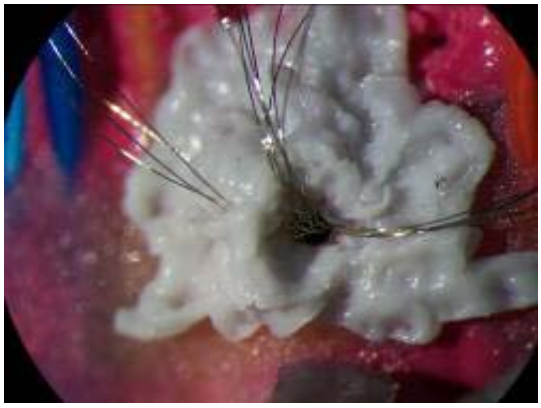
A tetrode was composed of four twisted strands (Fig. 1A). Each tetrode (Fig. 1B) was made from strands of HM-L-coated 90% platinum-10% iridium wire of 17 to 25 μm diameters (California Fine Wire, Grover Beach, CA). Four tetrodes were held by a cannula (Fig. 1C,D) that was attached to a microdrive supplied by Axona Ltd, St Albans, UK. This microdrive (Fig. 1E,F) allowed for dorsal to ventral movement of the tetrodes to search for new units. The tip of each electrode was burnt during less than a second in order to remove the isolation layer and, therefore, allow the connectivity of each electrode twisted to the microdrive´s cables. Then, each electrode was covered with liquid silver in order to further improve connectivity (Fig. 1G,H). After applying 9 V current in saline solution (Fig. 1I) in order to check for shorts among electrodes the top part of the cannula was closed with blutac$^{R}$ and then all the cables were covered with nail polish that acts as an isolator (Fig. 1J).

**A**


**B**

**C**


**D**



**E**

**F**

**G**

**H**

**I** **J**


**Figure 1. Tetrodes and microdrive making. A,B**. Each tetrode consisted of four strands twisted by means of a magnet. **C,D**. Each tetrode was placed in the cannula of the microdrive. Each tip was burnt in order to remove the isolation and improve connectivity when the electrodes were attached to each cable. **E-H**. Silver was placed in each cable tip in order to further improve connectivity with each electrode. **I**. 9 V current was applied to each microdrive channel in order to test for possible shorts. **J**. Cable tips were covered with nail polish in order to isolate and protect them.

## 2.2. Surgical Procedure

Recordings were obtained from Lister Hooded rats and Wistar (250-350 grs) chronically implanted with tetrodes in the primary auditory cortex. In the case of the temporal discrimination protocol, animals were trained during 21 days approx. and after a week of water and food ad libitum they were implanted a microdrive that held the tetrodes. To perform the surgery, anesthesia was induced using intraperitoneal injections of ketamine (60 mg/kg) and medetomidine (0.5 mg/kg). The animal was then mounted in a stereotaxic apparatus and the skull was exposed. A trephine was used to make a 3 mm diameter craniotomy with the centre located, from bregma, -5.3mm anterior-posterior, 6.6-7mm medium-lateral and 0.3mm dorso-ventral (Paxinos and Watson 1998). These coordinates were used in order to position the microdrive dorsally, which made it more stable than entering laterally right over auditory cortex. Body temperature was monitored through a rectal thermometer and maintained (36-38°) by means of an electric blanket. Heart rate

and blood oxygen levels were monitored. Reflexes were regularly checked during surgery to assure deep anesthesia. Other drugs were given during surgery and the recovery period in order to prevent infection, inflammation and for analgesia: antibiotics (enrofloxacin; 10mg/kg; subcutaneous (s.c.)) and topical application of neomycin and bacitracin in powder (Cicatrin®), analgesic (buprenorphine; 0.05mg/kg; s.c.), antiinflamatory (methylprednisolone; 10mg/kg; i.p.) and atropine (0.05mg/kg, s.c.) to prevent secretions during surgery. Rats were cared for and treated in accordance with the Spanish regulatory laws (Boletín Oficial del Estado 256; 25-10-1990) which comply with the European Union guidelines on protection of vertebrates used for experimentation (Strasbourg 3/18/1986).

Gold plating (Fig. 2A,B) prior to surgery allowed decreasing the electrode impedance to ca. 300 KΩ. Microdrives were attached to the skull with dental cement and 7 stainless steel screws. The auditory cortex was reached by vertical descent and the tetrodes were lowered 300 μm during the surgery (Fig. 3). A covert over the cannula protected the tetrodes and wax was placed around the craniotomy hole in order to protect the brain from dental cement. Dental cement covered the whole skull exposed in order to hold the microdrive. Vertical descent performed after surgery was of 50 μm per day until an auditory response was observed. All the recordings included in this study corresponded to auditory cortex (Doron et al. 2002). This estimation is based on the depth of the included recordings and on the anatomy. The auditory latencies were typically 10-20 ms, which are as well characteristic of auditory cortex (Ojima and Murakami 2002; Malmierca 2003; Nelken et al. 2003).

**A** **B**

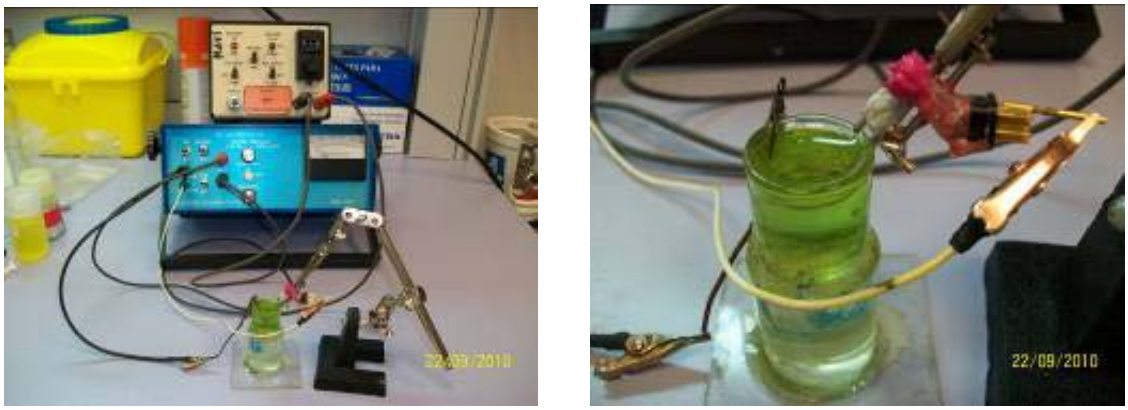



**Figure 2. Gold plating procedure. A,B.** Tetrodes impedance was first tested with the impedance tester. Tetrodes were placed in a gold solution. A ground and an electrode connection allowed for impedance testing. Impedance usually ranged between 1 and 2 MΩ. Then positive current was applied by means of the stimulus isolator during 1 second. Impedance was tested and current was repeatedly applied till 300 KΩ was obtained.

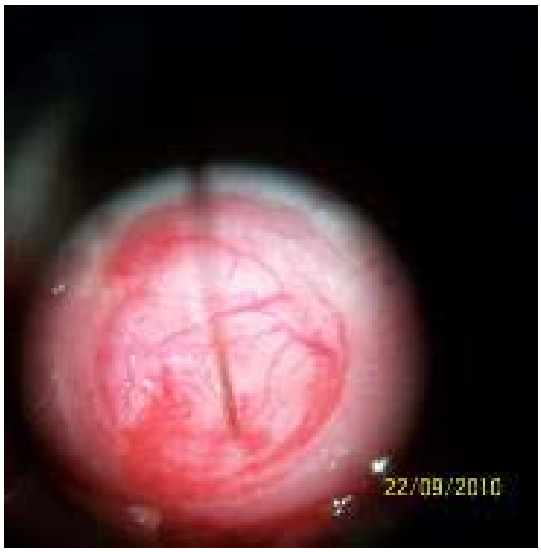



**Figure 3. Tetrodes implantation.** The animal was placed in a stereotaxic apparatus by means of ear bars and a nose holder. 7 stainless steel screws (one of them being the ground connected to the microdrive) together with dental cement allowed to hold the microdrive tightly. 4 tetrodes were vertically lowered while veins were avoided. **B**. Wax was applied all over the craniotomy hole in order to avoid contact with dental cement. Dental cement covered the skull exposed.

## 2.3. Experimental set up

The recordings were performed inside a box built in black acrylic (Fig. 4B) with a surface of 22 by 25.5 cm and the height of the walls was 35 cm. The box in which the recordings were performed was placed inside two wooden boxes placed one inside the other (Fig. 4A). Between each box two isolating foam rubbers (4 and 2 cm thick) were placed to soundproof for low and high frequencies. A wooden covert and equal soundproof foams closed the whole recording chamber and a hole permitted the entry of the recording wire (2mm thick) connected to the preamplifier. Water valves were placed outside the recording chamber. Animals poked their nose into 3 different sockets, 2 cm wide and separated by 3 cm each, and without covert in the top part of the socket to avoid hits with the microdrive. During the recordings the rat was freely moving within the limited space of the chamber. Recordings were obtained in darkness and the experiment was filmed with an infrared camera placed above the recording chamber.

**A** **B**

**Figure 4. Experimental setup. A**. 80x80 cm acoustic isolation wooden box allowed running the experiments in complete silence. Another 60x60 wooden box was placed inside and it was separated by 2 cm thick foams and 4 cm thick foam in order to isolated from high and low frequencies respectively. Acoustic resonance absortion foam covered the upper part of the outer box as well as the inner box. **B**. 22x25 acrylic box contained the nosepokes where the rat performed the attentive task while single unit recordings were performed. During passively listening recordings the nosepokes were occluded.

## 2.4. Electrophysiological recordings from awake freely moving rats

Animals lived in large cages of 28x42x30 cm (Charles River) in rich environment, under a 12 hr light/dark cycle, food ad libitum and water restricted during the training period. Before training and after a week of postoperative recovery period animals were habituated to the recording chamber. Prior to each recording session animals were connected with the headstage and earphones (Fig. 5). The electrode wires were AC-coupled to unity-gain buffer amplifiers. Lightweight hearing aid wires (2-3 m) connected these to a preamplifier (gain of 1000) and then to the filters and amplifiers of the recording system (Axona, St. Albans, UK) (Fig. 6). Signals were amplified (x15000-40000), high pass filtered (360 Hz) and acquired using software from Axona Ltd (St Albans, UK). Each channel was continuously monitored at a sampling rate of 48 kHz, and action potentials were stored as 50 points per channel (1 ms; 200 μsec prethreshold; 800 μsec postthreshold) whenever the signal from any of the prespecified recording channels exceeded a given threshold set by the experimenter for subsequent off-line spike sorting analysis. Before every experimental session, tetrodes were screened for neuronal activity. Once spikes could be well isolated from background noise the experimental protocol started.

**A** **B**

**Figure 5. Electrophysiological recording and sound stimulation. A.** Earphone holder is attached with dental cement to the animal´s head. Earphones are attached to holders by means of screws. **B.** Microdrive´s headstage is plugged and a clip is used in order to hold tightly the headstage. Earphones are taped to the wires that are protected by a semi-rigid plastic tube.

**Figure 6. Recording system.** Pre-amplifier (left) and analog-to-digital converter (right) allowed to electrophysiological recordings of single units and field potentials.

## 2.5. Presentation of Sound Stimuli

Protocols of stimulation were controlled through Matlab, a National Instrument card (BNC-2110) and a breakout box (FS 300 kHz). Sound triggers had μs precision. Sound stimuli were delivered through earphones (ER.6i Isolator, Etymotic Research Inc.) that were screwed in each recording session to the earphone holders

chronically attached to the skull of the animal with dental cement. The earphones allowed isolation from any sound unrelated with the protocol since they were adjusted inside the ear by means of silicone at their tip. Sound calibration was performed with a microphone (MM1, Beyerdynamic) placed 1 mm away from the earphone and a preamplifier (USB Dual Pre, Applied Research and Technology). The sound stimuli during the passive and attentive recording stages had a duration of 50 ms, intensity of 80 dBs SPL and pure tones of 5322 Hz with a 6 ms rise/fall cosine ramps, being therefore identical for both the first and second stimulus. Interstimuli interval (from the end of stimulus 1 to the onset of stimulus 2) varied depending on the experimental protocol and the same amount of trials (180-200) were presented on each recording. Similarly, the total number of correct trials in the attentive stage was the same as in the passive (180). The intertrial interval also had a similar duration in the attentive and passive stages (2-3 s). Tuning curve consisted of 9 different pure tones between 500 Hz and 8078 Hz presented 30 times during 20 ms each tone and 6 ms rise/fall cosine ramps. Four distinct intensities ranged between 50 and 80 dBs SPL (Sound Pressure Level) and interstimuli interval varied randomly between 1 and 1.5 s.

## 2.6. Behavioural protocols

The behavioural protocols presented in Fig. 19 and Fig. 23 consisted of 6 different recording stages with a total duration of 3 hours approx. Animals went through the whole session only once each day. A tuning curve (24 min approx) and a passive listening recording stage (17 min approx) were performed (Fig. 7A) before and after the attentive stage (40 min approx) (Fig. 7B). The final stage comprised a passive recording with reward (40 min approx) delivery after each stimuli/us was presented (Fig. 7C). The aim of the tuning curve recording stage was to compare offline the preferred frequency of the isolated units with respect to the stimuli/us

responses during the remaining recording stages. Additionally, the aim of the remaining passive stages was to compare the neuronal responses with respect to the attentive task. In the attentive task of Fig. 19 the animal was trained to poke its nose into de centre socket which immediately triggered the onset of one stimulus (500/900ms; 80dB;5322 Hz). The animal had to remain in the centre socket till the end of stimulus presentation. Animals had to discriminate the stimulus duration, 500 ms or 900 ms, which required a left or right poke, respectively, in order to get water reward. In the attentive task of Fig. 23 two identical stimuli (80dB, 5322 Hz, 50 ms duration) were triggered when the animal poked its nose in the central socket. Stimuli were separated either by 150 or 300 ms, which required left or right poke to obtain water reward, respectively. We also present just the behavioural performance of a different task (Fig. 24) where central poking of the animals triggered the presentation of either short (50, 100, 150, 200ms) or long (350, 400, 450, 500ms) interstimuli intervals (ISIs), which required left or right poking in order to obtain the reward, respectively. In the attentive task, false alarms (poking in the opposite side) or early withdrawals (withdrawal before stimuli termination) were punished with a 3 s time out and a white noise (WAV-file, 0.5 s, 80 dB SPL). During both passive recordings and both tuning curves the animal freely moved around the recording box with all the sockets occluded while listening to stimuli/us presentation. Finally, in the last passive recording stage, the left and right sockets were occluded while the animal repeatedly entered in the central one and listened to the same stimuli/us as in the attentive task, and after a 0.3 s delay a water drop was delivered.

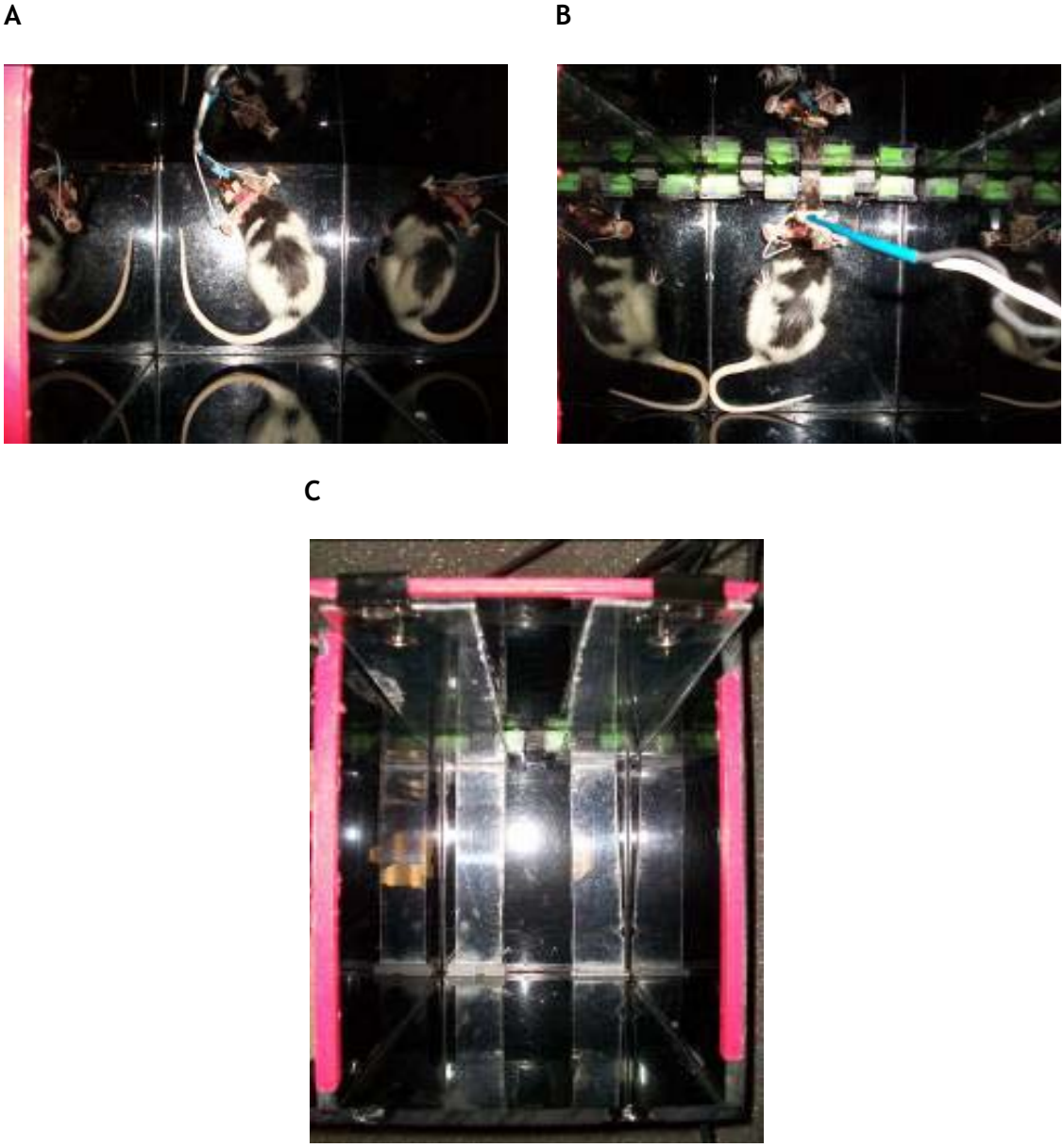



**Figure 7. Electrophysiological recordings in the experimental cage.** The electrophysiological recordings in the awake animal were performed under different conditions, i.e. with the nosepokes occluded and the animal freely moving (**A**), under attentive conditions working with the nosepokes (**B**) and with one or both lateral nosepokes occluded during the training period or passive recording session, respectively (**C**).

## 2.7. Data Analysis

Cluster cutting (isolating single units from the multiunit recording data) was performed using an Off-Line Spike Sorter (OFS, Plexon) (Fig. 8). Waveforms were first

sorted into units by using the valley-seeking algorithm (Koontz and Fukunaga 1972). Waveforms were considered as being generated from a single neuron when they occurred simultaneously in the four electrodes and it defined a discrete cluster in 3D principal component or peak to peak space that was distinct from clusters for other units using a MANOVA test. Single units exhibited a recognizable refractory period (>1 ms) in their ISI histograms and had a characteristic and distinct waveform shape and peak-to-peak amplitude when compared with other neuronal activity.

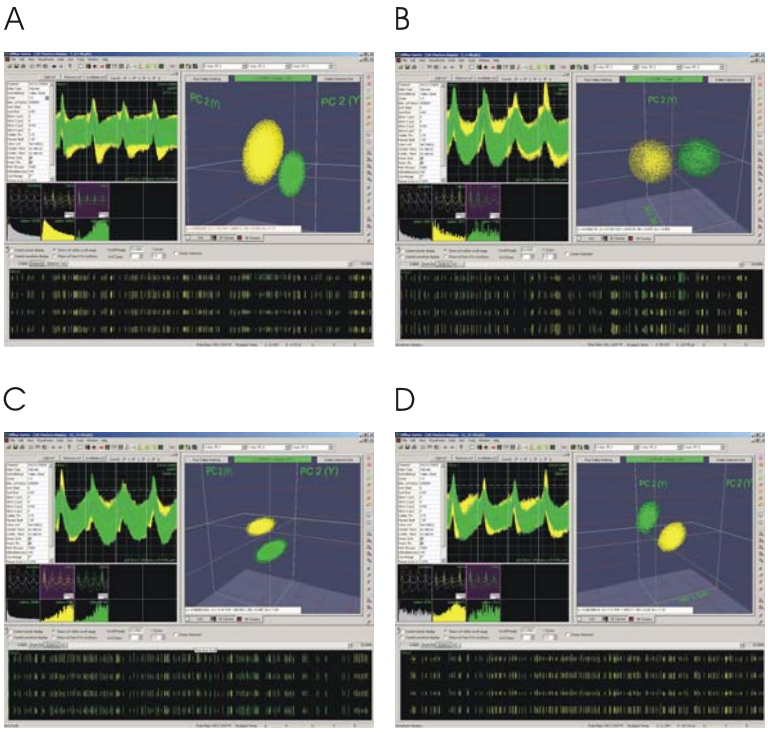



**Figure 8. Spike sorting. A-D.** Four pairs of neurons recorded within one tetrode in four different animals. **A.** Upper left. Spike waveform average recorded from each channel of the tetrode. Middle left. Interspike interval Histogram of each isolated neuron. Upper right. 3D view of the Principal Component analysis of two units. Bottom. Burst display of each channel of the tetrode. Same for **B**, **C** and **D**.

Analysis of peristimulus time histograms (PSTH) and rasters were performed using 10 ms bins to estimate accurately the responses to auditory events. Frequency response histograms were obtained averaging the spiking activity during the whole recording within each bin. The onset of each stimuli presentation was aligned to 0. Raster plot illustrates the spike activity of the aligned time windows of the whole

recording. PSTH were also performed to analyze the tuning curve before and after the attentive task and no changes in preferred frequency were observed.

Neuronal responses from the study presented in the epigraph 4.4. were classified into *onset*, *onset+offset*, *offset*, *non-responsive*, *pauser* and *unknown*. Neuronal response classification was performed by means of visual inspection and by means of two different quantitative analysis. Method 1 had a 72% overlap with the classification made by visual inspection. This method consisted of calculating the 99% confidence interval during spontaneous activity (200 ms before S1 presentation), S1 presentation and Interstimulus interval. Whether one bin crossed or not the confidence interval limits during and after stimulus presentation determined the response profile of each neuron. Method 2 had a 77% overlap with the classification made by visual inspection. The maximum peak value of the previous time windows was considered in order to classify the neuronal responses, where maximum peak value/s during and after stimulus presentation of more than twice the spontaneous activity were considered to be significant.

To estimate the information content carried by the firing rate of the neuron we performed Mutual information (MI) analysis (analysis done in collaboration with M. Martinez and G. Deco), which measures the strength of association between two variables, in our case "spike rate" and the "category of interstimuli interval". The MI was calculated as:

$$I(R;S) = \sum_{p\in P, s\in S} p(s,r)\cdot \log_2\left(\frac{p(s,r)}{p(s)p(r)}\right)$$

where p (s ), p (r ) are the marginal distributions and the p (s, r) the common distribution. The MI has a zero value if the two variables are independent. Calculation was based on spike counts in 50 ms time windows during each stimulus presentation. As MI estimate is subject to statistical errors we normalized over the

surrogates. A surrogate is the MI between the spike rate and category of interstimuli interval after the order of the samples has been randomly changed. The normalization consists in fitting the probability distribution of 1000 surrogates. That fitting has been used to test the statistical significance of the MI between rates and category.

Fano factor (FF) was computed (analysis done in collaboration with M. Martinez and G. Deco) as the ratio between the variance in the spike count across trials and the mean spike count. FF was calculated in 10 ms bins along with the trial duration (from -200ms to S2+750/600 ms). The statistical significance of the FF was assessed by comparing the minimum FF value during spontaneous activity (-200ms to 0) and FF from 0 to S2+750/600 ms. A neuron with significant FF would have three consecutive bins with FF lower than the minimum value obtained during spontaneous activity (whether short or long ISI). We obtained 11 significant neurons using this criterion, and we added 3 more neurons with a very phasic response in which the criterion was a single bin with FF lower than threshold. Bin match according to their firing rate was performed for <5% difference between the attentive and passive recordings of the same neuron and same bin location at each recording.

## 2.8. Histology

After performing all the electrophysiological recordings animals were perfused administering paraphormaldeide (4%) through a needle inserted in the heart of the animal. The brain was extracted and placed with glue in a saline solution of the vibratome. The brain slices were kept in interface chambers and then placed in microscope slides. After a day or two the slices were dried in microscope glass slides and then they followed a tinction process. Right afterwards they were covered with microscope cover glass using a mounting medium (Eukitt). Following the brain slices were pictured (Fig. 9).

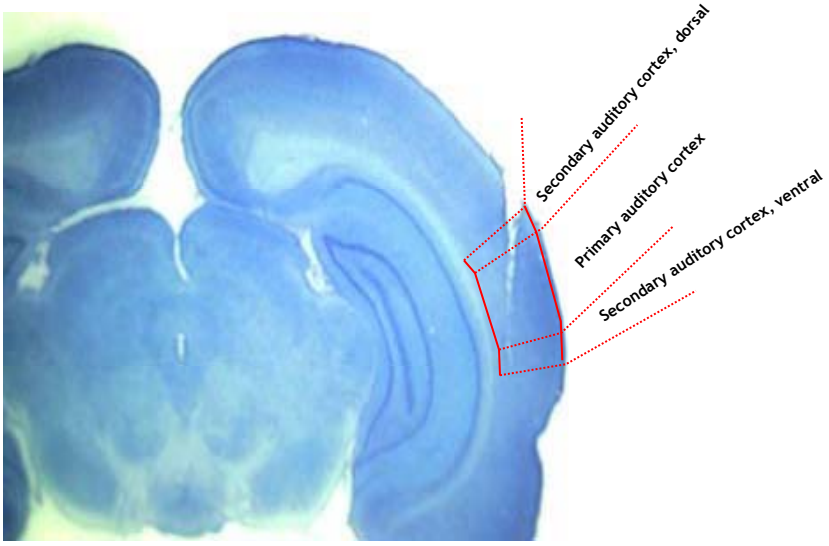



**Figure 9. Picture of a coronal brain slice after tinction.** Tetrodes socket can be seen in the auditory cortex at the right side of the picture.

# 3. OBJECTIVES

1. To determine the time course of auditory adaptation in single units of the auditory cortex from the freely moving animal.
   1.1. To determine how the interstimulus interval, duration and intensity of the previous history of stimulation influence the response amplitude and timing of subsequent responses.
   1.2. To quantify the postadaptation as a result of an adaptation phenomenon.
2. To explore the spontaneous activity patterns of single units in the auditory cortex of the awake animal.
3. To characterize the neuronal patterns to sustained auditory stimulation in the passive and in the attentive animal.
4. To study the neuronal basis of temporal discrimination in the auditory cortex of the attentive animal.
   4.1. To quantify the information content of single units responses during a temporal discrimination task.
   4.2. To analyze how the neuronal response variability is affected by attentional demands.
   4.3. To explore the slow modulation of evoked and spontaneous activity under attentional demands.

# 4. RESULTS

## 4.1. Timescale of auditory adaptation in the awake passive listening animal

In our first study, recordings from 76 isolated neurons were obtained in the primary auditory cortex of awake freely moving rats. Amongst these, detectable auditory responses were evoked in 54 neurons that are included in the study. Similar proportions of responsive neurons have been found by other authors (50% in (Hromadka et al. 2008).

Single units were identified by means of tetrode recording and subsequent cluster cutting from the freely moving rat. We were concerned to ascertain that the animal was awake during the whole protocol. Experiments were carried out in the dark, where rats have been reported to be awake during 54-66% of the time, around 5% in REM sleep, and the rest in non-REM sleep (Bertorelli et al. 1996; Stephenson et al. 2009). Those studies refer to a quiet environment and without sound stimulation, which was not the case here. Indeed, sound has been used before in protocols of sleep deprivation (Franken et al. 1995). Sound stimulation occurred in our study in random intervals between 8 and 15 s. In addition, we carried out analysis on the local field potential recordings obtained along with the single units. The analysis followed that described in (Gervasoni et al., 2004). In all analyzed cases (n=17) periods of low frequencies (see Fig. 1B in (Gervasoni et al. 2004) were rarely observed, while those of theta, normally associated to movement, predominated.

### 4.1.1. Interstimulus interval and adaptation

Auditory responses to pairs of 50 ms sounds (white noise) separated by interstimulus intervals spanning between 50 ms and 8s (from the end of stimulus 1

(S1) to beginning of stimulus 2 (S2)) were tested in a total of 30 neurons. After averaging the response to 50-100 trials of stimulation for each interval we observed that most neurons (83.3 %) showed some degree of adaptation in the form of a decreased response to the second identical sound, the adaptation being larger for shorter intervals. The remaining neurons showed either no difference between the two responses (13.3 %) or an increased response of the second response with respect to the first (3.4 %). Fig. 10 illustrates the peristimulus time histogram (PSTH) of the response (spike/s) to 50 trials (bottom) and raster plots (top) in each panel. The responses correspond to the intervals 100, 300, 500 and 2000 ms for one neuron (Fig. 10A-D) (see Fig. 1 in (Abolafia et al. 2010)). The response to the 2$^{nd}$ stimulus (R2) was in all cases smaller than the response to the 1$^{st}$ one (R1). The time course of adaptation is represented for this particular case (Fig. 10E). For a 50 ms interstimulus interval, the R2 was 0.35 the peak amplitude of R1. When the interval was increased to 1 s, R2 was on the average 0.80 of R1. In this neuron, a total recovery of the amplitude of R2 with respect to R1 was not completely achieved until an interval of 3 seconds had elapsed.

The average adaptation time course for 30 neurons is represented in Fig. 10F. In these cases, for a 1 s interval between the two auditory stimuli, R2 had approximately 0.80 the amplitude of R1, and was completely recovered for intervals between 2-5 s. We conclude that stimuli occurring up to 1 or even 5 seconds earlier have an influence on the amplitude of subsequent responses.

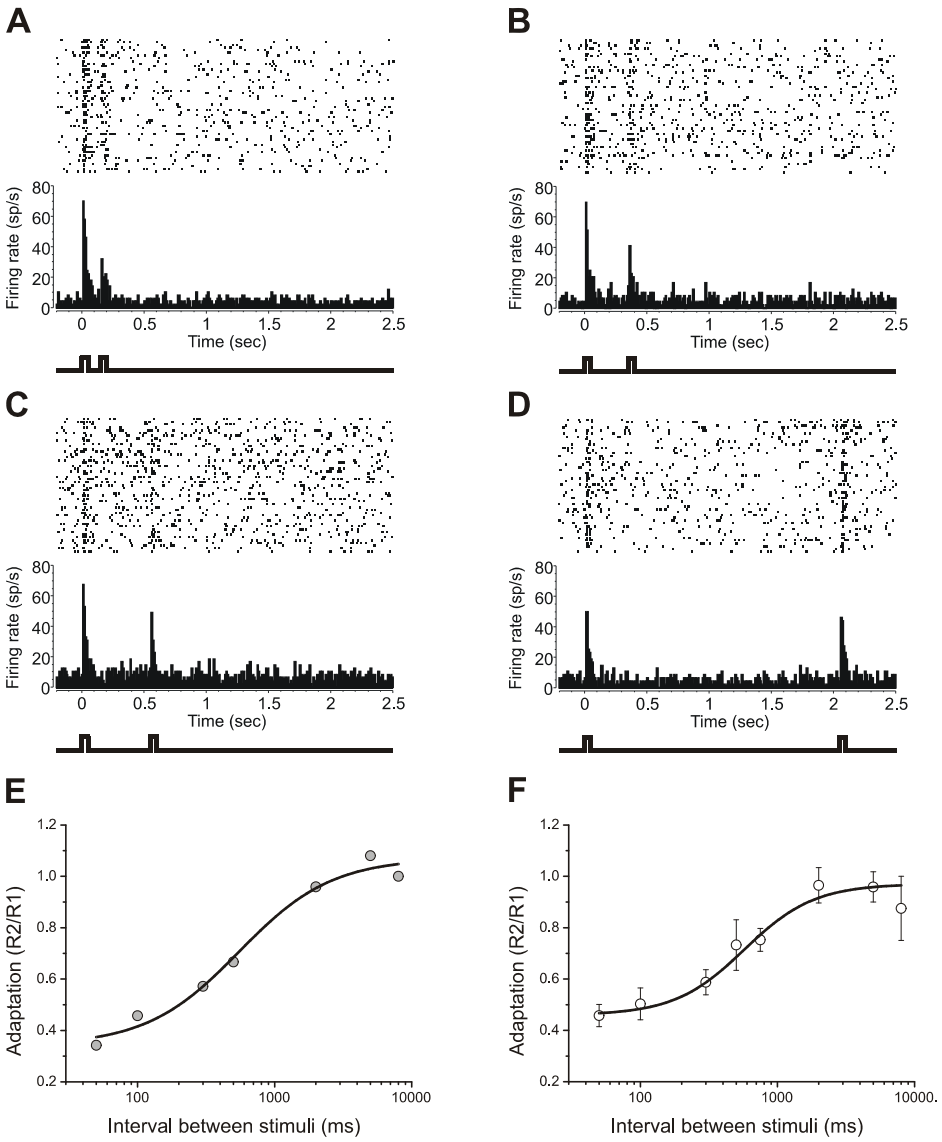



**Figure 10. Auditory adaptation in single neuron recordings from auditory cortex neurons in the awake freely moving rat. A-D.** Responses to two identical sounds (50 ms; 90 dB; white noise) separated by 100, 300, 500, 2000, ms intervals (A, B, C, D, respectively). Perievent raster and histogram (PSTHs) of the response (spike/s) to 50 trials (bottom) and raster plots (top) in each panel. **E.** Relative amplitudes of the peak responses to the second with respect to the first stimulus illustrating the time course of adaptation. The neuron is the same as in panels A-D. A sigmoid was fitted, and $R^2$ = 0.98. **F.** Plot of the relative amplitude of the response to the second sound with respect to the first one for different intervals for an average of 30 neurons. $R^2$ = 0.99. Error bars are s.e.m. Note that in average 2 s are needed for the second response to have an amplitude which is the same as the first one.

## 4.1.2. Duration of the first stimulus and adaptation

In 21 neurons recorded in auditory cortex of the awake rat the duration of the first auditory stimulus was randomly varied (50, 300, 500 and 700 ms) while the

duration of the second stimulus (50 ms) was maintained (Fig. 11A-C) (see Fig. 2 in (Abolafia et al. 2010)). The objective was to determine to what extent the adaptation of R2 was contingent on the duration of S1. This was tested for intervals of 300, 400, 500, 750 and 1500 ms between R1 and R2. In 62% of the observations we found an effect of S1 duration, the longer duration of the first stimulus further decreasing R2. In the remaining cases adaptation either remained the same (19%) or there was less adaptation than for shorter stimuli (19%).

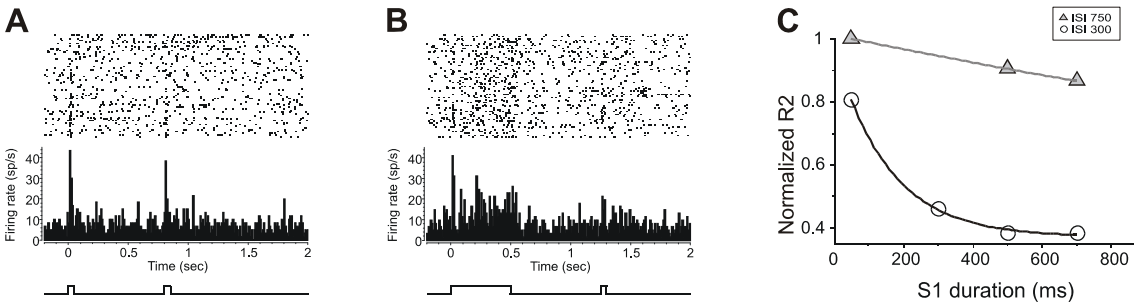



**Figure 11. Relative adaptation of the second auditory response with respect to the duration of the first one in auditory cortex of the awake rat. A.** Plot of the PSTH for one auditory cortex cell to 60 trials of two 50 ms white noise sounds (90 dB) presented at 750 ms interval**.** On top, raster plots. **B.** PSTH shows a decrease of the response to the second sound when the duration of the first is prolonged up to 500 ms**. C.** Normalized response to the second sound (R2; 50 ms) (R2 divided by the value of the maximum R2, which occurs when the duration of S1 equals that of S2) represented against the duration of the first one for two intervals (300 and 750 ms).

### 4.1.3. Intensity of the first stimulus and adaptation

The influence of the intensity of the first stimulus (70 dB to 103 dB) in the response to S2 was also explored in 21 neurons for various intervals (50, 100, 200, 300, 400, 500, 750, 1000, 1500 ms). The intensity of S1 was varied randomly. In 50% of neurons an influence of the intensity of S1 was observed on R2, such that higher intensity of S1 induced stronger adaptation (Fig. 12A-C) (see Fig. 2 in (Abolafia et al. 2010)). Of the remaining neurons, the adaptation of 40% remained constant independently from S1 intensity. Finally, the remaining 10% of neurons showed less adaptation for higher than for lower intensities of S1.

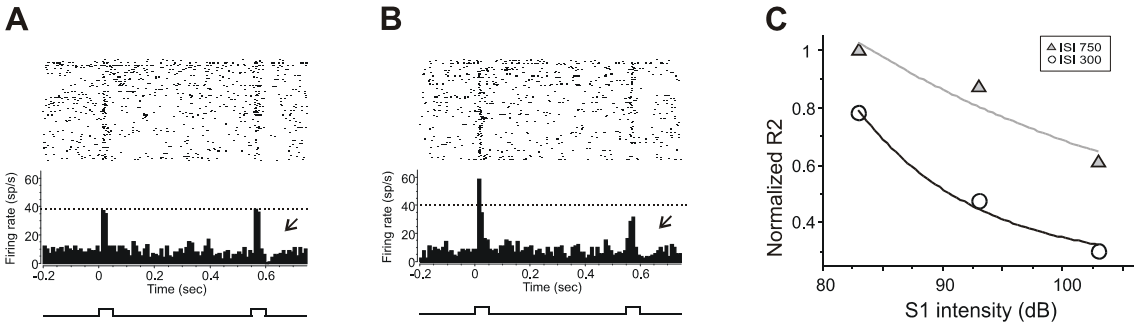



**Figure 12. Relative adaptation of the second auditory response with respect to the intensity of the first one in auditory cortex of the awake rat. A.** Plot of the PSTH for one auditory cortex cell to 100 trials of two 50 ms white noise sounds (90 dB) presented with 500 ms interval**. B.** PSTH of the responses to both stimuli when the intensity of the first stimulus has been increased to 100 dB. Note that the response R1 increases while R2 decreases. An arrow points to the post-adaptation following the second stimulus (D, E). **C.** Normalized response to the second sound (R2) (as in C) for different intensities of the first stimulus and for two different intervals (300 and 750 ms).

### 4.1.4. Latency of auditory responses and adaptation

As reported by others (Eggermont 1999), the occurrence of adaptation often not only affects the magnitude of responses but also their timing. Here the timing of responses was first quantified at the response's threshold. The mean firing frequency and the 95% of the confidence interval during the 300 ms preceding the stimulation were calculated. The response to multiple trials (80-100) was represented in a PSTH in 2 ms bins. After 10 ms of stimulus presentation, the first bin that crossed the 95% confidence interval was taken as the onset of the auditory response, and the exact time taken was the mid value of a 2 ms bin (Fig. 13) (see Fig. S3 in (Abolafia et al. 2010)).

Auditory responses following the shortest interstimulus intervals (<0.3 s) often did not cross the 95% confidence interval, and thus their latency could not be determined. In order to detect possible changes in latency with adaptation, interstimulus intervals were classified into short ISIs (0.05, 0.1, 0.3, 0.4, 0.5 s) and long ISIs (0.75, 2, 5, 8 s). Auditory responses following short ISIs had significantly longer delays (12±1 ms) than those following long ISIs (11±2 ms; n=26) ($p<0.0003$),

although the difference was small. It is possible that this difference was underestimated given that the most adapted responses (ISI <0.3 s) were not included.

Auditory responses to S2 following long duration S1 (500 ms) had also significantly longer response delays (mean: 12±2 ms; $p<0.003$) than those following shorter stimuli (50 ms, mean: 11±1 ms) (n=20). Latency was also significantly ($p<0.01$) longer for S2 stimuli following louder S1 stimuli (95dB) (S1=11.9ms, S2=13ms). Whereas for louder S1 (95 dB), the response's latency was significantly shorter (11±1 ms) than for 70 dB S1 (13±2 ms; $p<0.023$; n=21) as reported earlier (Polley et al. 2006). For details on statistical analysis of response delays see Table 1.

The difference in latency was consistent across individual cases, and for that reason it was statistically significant in spite of the small latency difference (around 1 ms). Even when the measure used here has been used by other authors, a decreased response amplitude may result in an artefactual increase of the latency when detected at the response's threshold (Bair et al. 2003). We therefore repeated the latency measures at the peak of the response and at 50% and 25% of the peak. In that case, no significant differences in the latency between the first and the second responses was detected (Table 1) (see Table 1 in (Abolafia et al. 2010)). To conclude, the consistent but small differences in latency detected at the threshold of the adapted responses were not robust enough to persist when measured at the 25, 50 or 100% of the response's peak amplitude.

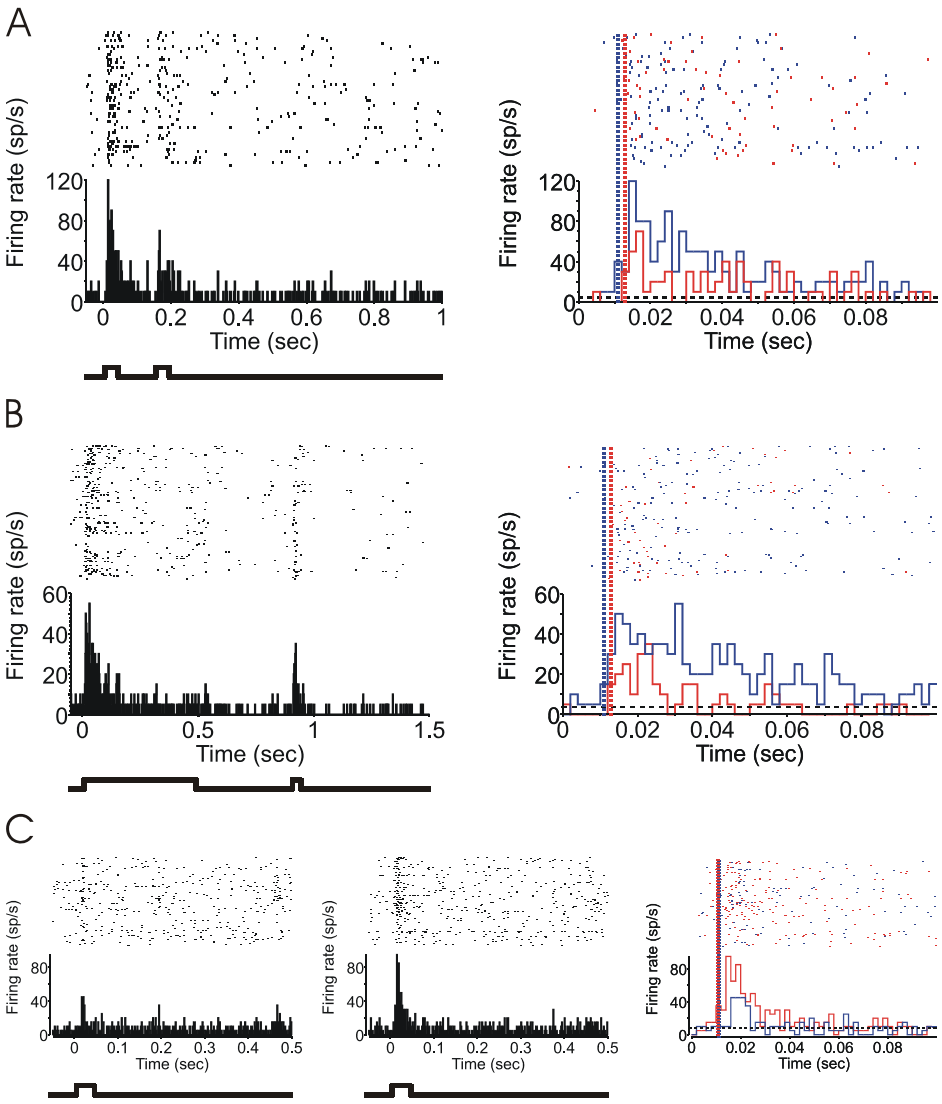



**Figure 13. Preceding stimulation modulates the timing of subsequent responses. A.** Left. PSTH of 2 ms bins shows the responses to two white noise stimuli of 50 ms duration and 90 dB, presented with an ISI of 100 ms. Right. Response onset occurs above confidence interval (horizontal dotted line) at 13 ms for the second sound (red dotted line) while the first response (blue dotted line) is delayed 11 ms. **B.** PSTH of 2 ms bins showing the response to 90 dB of white noise to 500 ms duration (first stimulus) and to 50 ms duration (second stimulus). The response lag crosses confidence interval (horizontal dotted line) of the second stimulus (red dotted line) at 13 ms, and the first (blue dotted line) at 11 ms. **C.** Left and Middle. PSTHs of 2 ms bins show the response to 50 ms duration of white noise stimulus; 70 dB and 95 dB are presented, respectively. Right. Response onset to S1 95 dB (red) occurs at the same time (11 ms) as S1 being 70 dB (blue), while peak response occurs at 15 and 17 ms for 95 and 70 dBs stimuli, respectively.

**Table 1**. Summary statistics of neuronal response delays in the awake rat.

| Protocol name | Mean delay S1 (ms)(short ISI) | Std. Deviation | Mean delay S2 (ms)(short ISI) | Std. Deviation | Wilcoxon signed ranks test Sig (2-tailed) |
|---|---|---|---|---|---|
| Varying Interstimulus Interval | 11.1 / 16.3 / 12.4 / 11.4 | 0.5 / 4.7 / 2.4 / 0.9 | 12 / 19.4 / 12.4 / 11.8 | 1.8 / 9.9 / 2.4 / 1.9 | .0003 / .2 / .9 / .3 |
| | **Mean delay S1 (ms)(long S1)** | **Std. Deviation** | **Mean delay S2 (ms)(long S1)** | **Std. Deviation** | **Wilcoxon signed ranks test Sig (2-tailed)** |
| Varying S1 Duration | 11.5 / 18.8 / 12.3 / 11.5 | 1.1 / 4.5 / 1.4 / 1.1 | 12.5 / 20.2 / 12.8 / 11.7 | 2.3 / 8.4 / 1.9 / 1.4 | .003 / .9 / .2 / .4 |
| | **Mean delay S1 95 dB (ms)** | **Std. Deviation** | **Mean delay S2 70 dB (ms)** | **Std. Deviation** | **Wilcoxon signed ranks test Sig (2-tailed)** |
| Varying S1 Intensity | 11.9 / 19.1 / 15.7 / 14.3 | 1.8 / 5.5 / 3.1 / 3 | 13 / 21.3 / 13.9 / 12.7 | 2.8 / 10 / 3.6 / 2.5 | .01 / .2 / .04 / .03 |

**Table 1: Summary statistics of latencies of cortical auditory responses in the awake rat.** Statistical parameters and its values are shown in rows, while the type of experimental manipulation is shown in the leftmost column. Each table cell represents the delay calculated by means of threshold at upper limit of confidence interval, at the peak response, at 50%, 25 % of peak response, respectively (1/2/3/4).

### 4.1.5. Post-adaptation following auditory responses

We refer to "post-adaptation" as the decay in the spontaneous activity firing following the end of an auditory response (Fig. 14A,B; see arrows) (see Fig. S4 in (Abolafia et al. 2010)). PSTHs (10 ms bins) were generated in order to quantify post-adaptation. A 95% confidence interval was determined, and the first bin following the auditory response that was below the 95% confidence interval was considered post-adaptation (Fig. 14). Post-adaptation was detected in 79% of cases following S2 and in 59% of cases following S1 (n=26 neurons).

We observed more post-adaptation following S2 when S1 was of longer (≥500 ms) duration (89 %) than with short (<500 ms) duration stimuli (70%). Finally, we found more post-adaptation after S2 when it was preceded by a short ISI (<0.3 s) (71%) than by a longer (>0.3 s) ISI (60 %).

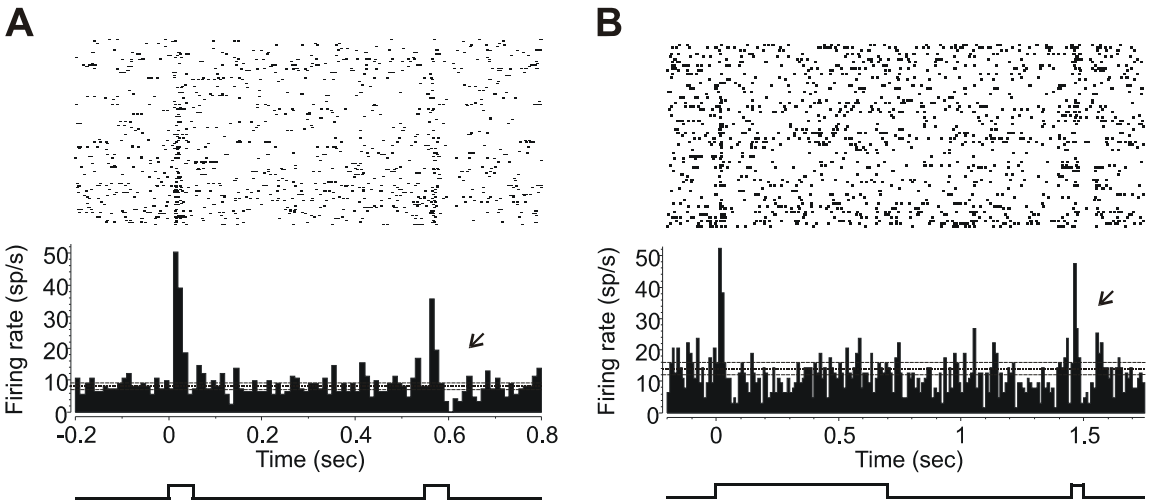



**Figure 14. Postadapting neurons**. **A.** Responses to two stimuli of 50 ms duration and 70 dB intensity. The interstimulus interval was of 500 ms. 123 trials were averaged. Note the prominent post-adaptation following the second stimulus. **B.** Responses to two stimuli of 700 ms (stimulus 1) and 50 ms (stimulus 2) respectively, both of 90 dB, separated by 750 ms interstimulus interval. 123 trials were averaged. In all panels the mean spontaneous response and the 95% confidence intervals are represented. In all figures, the raster plot is displayed on top of the PSTHs, where a spike is represented as a point.

### 4.1.6. Mechanisms of Neuronal Adaptation in Auditory Cortex in vitro

In order to illustrate to what extent the process of adaptation could be generated by activation of intrinsic membrane currents, it is shown Fig. 15 (see Fig. 3 and 7 in (Abolafia et al. 2010)) which illustrates the time course of adaptation for intracellularly recorded neurons (from R. Vergara and R. Reig). In order to evaluate the time course of adaptation of these neurons, a paradigm for neuronal stimulation was used that mimicked the one presented to the awake animal in the form of sounds. The paradigm used in vivo consisted of two sounds of 50 ms duration that were separated by different time intervals (ranging from 50 ms to 8 s), and it was replicated in vitro by means of current injection. Thus, pairs of square pulses of 50 ms duration separated by time intervals ranging from 50 ms to 5 s were injected. The intensity of the pulse was adjusted such that the first stimulus would induce a neuronal response of 3-5 action potentials, similarly to the response that a 50 ms sound stimulus evoked in the awake animal (Fig. 10). In addition, 6 of the recordings

were performed under local application of 100 μM CNQX, which did not affect adaptation or the subsequent afterhyperpolarization (AHP). Adaptation, defined as a decrease in the action potential frequency evoked by the second pulse with respect to the first pulse was detected in all cases (Fig. 15A,B) (n=36). The time course of adaptation was averaged for all neurons (Fig. 15I). For the intensities used, the response evoked by the second pulse was still diminished with respect to the first one for intervals of 1 second between stimuli, while for intervals of 2 s the response was almost totally recovered. Still, there is a large heterogeneity across neurons.

To explore the effect of ongoing activity on adaptation, spontaneous activity of one neuron in the awake animal was recorded for 20 min in the absence of auditory stimulation (Fig. 15C). A period of 10 s was selected, the average firing frequency during this period being 5.5 Hz. At the time of occurrence of each spike, a brief pulse of 2.5--4 ms was injected, adjusting the intensity such that it would evoke just one spike per pulse (2.4--3 nA) (Fig. 15D,E). This fake ‘‘spontaneous’’ firing also induced the activation of AHPs that would follow action potentials (Fig. 15F), and an average membrane potentials that was in average 3.01 ± 1.92 mV (n = 9) more hyperpolarized than that in silent neurons. The stimulation (background activity) was looping continuously over which the whole protocol of stimulation was carried out, and the intensity of S1 was adjusted such that the first pulse would evoke 3--5 action potentials (Fig. 15A,B,G). The degree and time course of adaptation were calculated for 9 neurons (Fig. 15H). The standard deviation of each of the points (SEM in the error bars) was larger than for the adaptation than in silent slices due to the noise added by the background activity. The adaptation curve and its time course in neurons with background activity were then represented along with the average one in the awake state and in the silent slice in Figure 15I. Interestingly, intrinsic adaptation evoked in neurons with ‘‘fake’’ background activity was larger than that in silent neurons and hence closer to that in the awake animal.

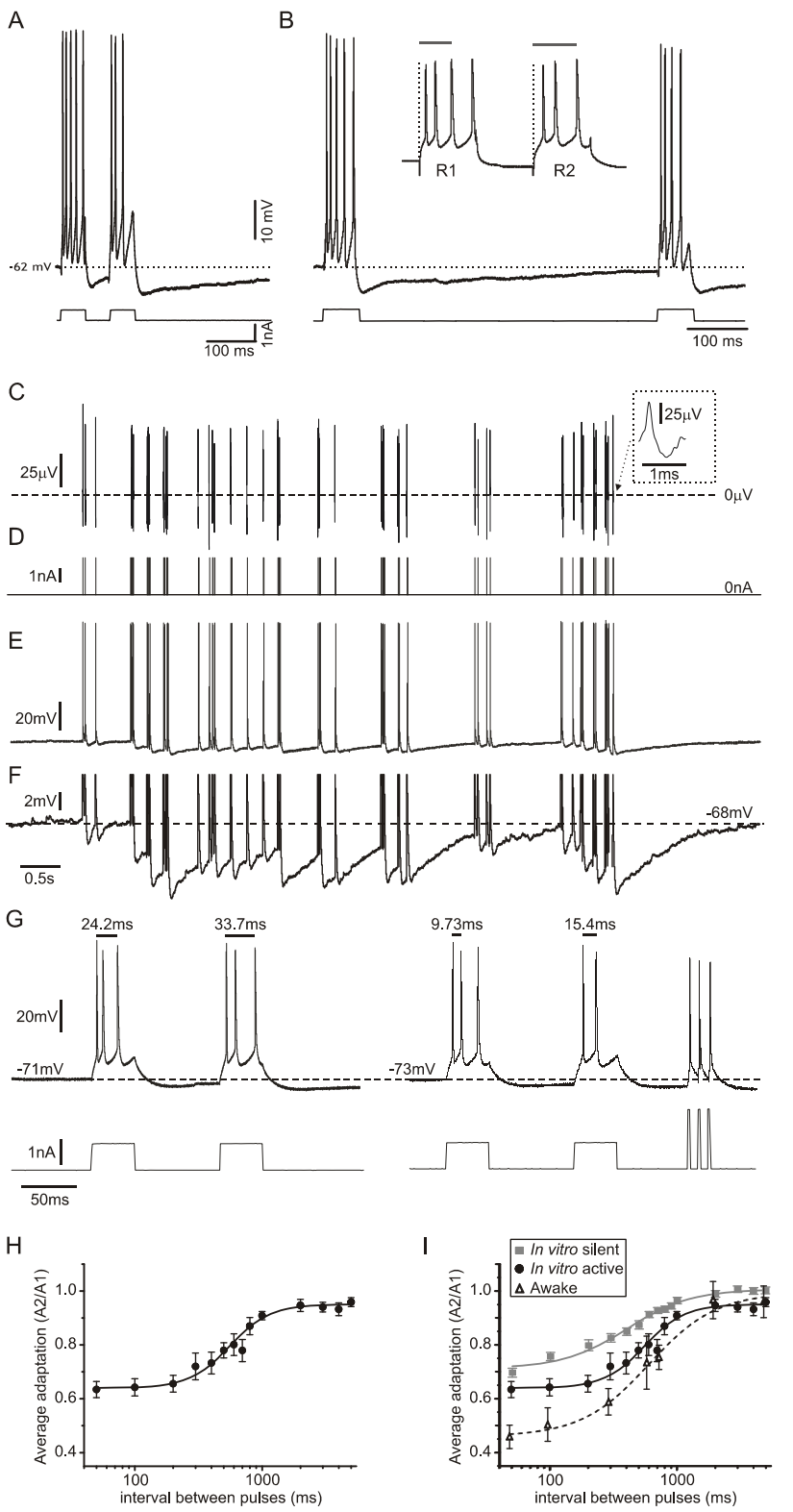

A
B
10 mV
-62 mV
1nA
100 ms
R1
R2
100 ms
C
25µV
25µV
1ms
0µV
D
1nA
0nA
E
20mV
F
2mV
-68mV
0.5s
G
24.2ms
33.7ms
9.73ms
15.4ms
20mV
-71mV
-73mV
1nA
50ms
H
Average adaptation (A2/A1)
1.0
0.8
0.6
0.4
100
1000
interval between pulses (ms)
I
In vitro silent
In vitro active
Awake
Average adaptation (A2/A1)
1.0
0.8
0.6
0.4
100
1000
interval between pulses (ms)

**Figure 15. Time course of adaptation in single units of auditory cortex in vitro. A**. Intracellular recordings illustrating the injection of two 50 ms pulses of 0.9 nA separated by a 50 ms interval. The intensity was adjusted to evoke 4-5 spikes with the first stimulus to mimic the sound stimulation *in vivo*. At the bottom trace, the current. **B.** Recording from the same neuron as in A, but now the interval is 500 ms. The inset illustrates how the spike rate computed as responses (R1, R2) was calculated. It was taken as the time for the same number of spikes to be fired in R1 and R2. The 3 spikes shown in the inset in figure 15B took, from time 0 (represented with a discontinuous line) 28.52 ms and 39.07 ms. **C.** Raw trace of single spikes recorded in the awake rat. The inset shows an expanded spike. **D**. Current square pulses (2.5--4 ms) following time pattern of the firing in C. The pulses we reinjected in silent neurons to mimic the firing in the awake animal. **E**. Intracellularly recorded neuron firing one action potential per injected pulse. **F**. Expanded trace of intracellular recording shown in (E) to illustrate membrane potential changes as well as the increases in AHP in response to spiking activity. The dash line marks the resting Vm while in silence. **G**. Two raw traces of the intracellular recording (top) to illustrate the neuronal response to two 50 ms square pulses (ISI 5 100 ms) (bottom). Left and right trace; neuron without and with ''fake'' spontaneous firing in response to short pulses, respectively. Note that the Vm is more hyperpolarized in the active neuron and its adaptation is larger. **H**. Time course of adaptation recovery average in neurons with ''fake'' spontaneous firing. A logistic function has been fitted ($R^2$ 5 0.91, N 5 9). **I**. Summary of the average adaptation time course in in vitro silent, in vitro active, and in awake preparations.

In order to understand the basis of the spike frequency adaptation in the auditory cortex cortical neurons in vitro, we explored the underlying mechanisms. An AHP followed the spike trains in all cases. The average AHP following a 50 ms pulse that evoked 4--5 action potentials (the average ''first pulse'' during the protocol) had a mean amplitude of 6.5 ± 0.4 mV when measured from a membrane potential of –60 ± 2 mV, and a mean duration of 1678.2 ± 107.1 ms (n = 36; x ± standard error of the mean [SEM]). The amplitude and duration of the AHP increased with the number of spikes up to a plateau. During the AHP, there was a decrease in the excitability of the neuron.

To explore the ionic basis of the AHP, two $K^+$ currents that for their time courses could be involved in the process were studied: apamin-sensitive $Ca2^+$-dependent $K^+$ current (Pennefather et al. 1985) and $Na^+$-dependent $K^+$ current (Schwindt et al. 1989). These two currents have been found to influence the time course of neuronal responses in the cat (Schwindt et al. 1988a).

The application of apamin (500 nM; local application) partially blocked the AHP following a 50 ms depolarizing pulse and spike train. The partial blockade was measured as the reduction of the AHP peak amplitude, which occurred in all cases (n

= 20) and consisted of an average decrease of the peak to 62% of the amplitude. Not only was the AHP partially blocked by apamin but the adaptation of the response to the second stimulus was also consistently reduced. For each of the 20 cells, the pairs of pulses separated by different intervals were given at the same membrane potential before and after the application of apamin. Adaptation was less in the presence of apamin, especially for those pulses separated by the shortest intervals (50 and 100 ms). This finding supports that apamin-sensitive $Ca2^{+}$- dependent $K^{+}$ current underlies at least the earlier adaptation ( <200 ms) following a 50 ms spike discharge.

Apamin did not block the slower part of the AHP. We next explored to what extent $Na^{+}$-dependent $K^{+}$ current, a current of a slower time course was playing a role on spike frequency adaptation in auditory cortex. To this end, [$Na^{+}$] was reduced from 152 to 26--42 mM by replacing NaCl with choline chloride in the bath. To prevent the action of choline on muscarinic receptors, we include the muscarinic antagonist scopolamine (10 lM) in the bath. Scopolamine per se does not have a direct effect on the AHP (Uchimura et al. 1990; Sanchez-Vives et al. 2000a), and this is in agreement with what we observed here. In these conditions, the size and duration of the AHP were significantly reduced (n = 11). Indeed, when [$Na^{+}$] was decreased following apamin application, the AHP often was totally blocked (n = 7). Spike frequency adaptation was again tested (n=7) with the same protocol as above while in apamin plus low [$Na^{+}$]. In low [$Na^{+}$], action potentials were still generated, although their amplitude was reduced and their duration increased. Lowering [$Na^{+}$] further blocked the adaptation already reduced by apamin. Still, a certain level of spike frequency adaptation remained, probably partly the result of the remaining $Na^{+}$-dependent $K^{+}$ current given that [$Na^{+}$] was not totally eliminated from the bath. Reduction of sodium has a similar effect to replacement with lithium (Franceschetti et al. 2003) and has been used before to study $Na^{+}$-dependent $K^{+}$ currents (Sanchez-

Vives et al. 2000a). Still, the caveat of reducing the size of action potentials is that it may affect the activation of other channels.

One of the differences that exist between the awake animal and the slice is that neurons in the slices were silent, while in the awake animal there was ongoing activity. Ongoing firing may have an influence on the intrinsic adaptation itself, setting neurons in a different adaptive state. To explore the effect of ongoing activity on adaptation, spontaneous activity of one neuron in the awake animal was recorded for 20 min in the absence of auditory stimulation. A period of 10 s was selected, the average firing frequency during this period being 5.5 Hz. At the time of occurrence of each spike, a brief pulse of 2.5--4 ms was injected, adjusting the intensity such that it would evoke just one spike per pulse (2.4--3 nA). This fake ‘‘spontaneous’’ firing also induced the activation of AHPs that would follow action potentials, and an average membrane potentials that was in average 3.01 ± 1.92 mV (n = 9) more hyperpolarized than that in silent neurons. The stimulation (background activity) was looping continuously over which the whole protocol of stimulation was carried out. The same protocols of adaptation used above were repeated, following what was described earlier for silent slices. The intensity of S1 was adjusted such that the first pulse would evoke 3--5 action potentials. The degree and time course of adaptation were calculated for 9 neurons. Interestingly, intrinsic adaptation evoked in neurons with ‘‘fake’’ background activity was larger than that in silent neurons and hence closer to that in the awake animal.

## 4.2. Spontaneous activity in the auditory cortex of the awake animal

In our recordings of the awake animal described in the epigraph 4.1., we also measured the mean spontaneous activity. We found that the mean spontaneous activity was 5.07± 3.25 Hz (mean ± s.d.), ranging between 0.6 and 16 Hz. The distribution of values is illustrated in Fig. 16A (see Fig. S2 in (Abolafia et al. 2010)).

Mean and standard deviation were similar to reported data from cell-attached recordings in head-fixed animals (Hromadka et al. 2008). The spontaneous frequency was calculated by averaging the firing rate value of each 10 ms bin during a 300 ms time window preceding auditory stimuli (between 50 and 100 trials, 15 to 30 s intertrial interval). The peak frequency (2 ms bins) of the subsequently evoked auditory responses (50 ms, 80-90 dB) was also measured. A significant positive relationship was found between the spontaneous frequency and the maximum frequency evoked by the stimuli (Fig. 16B-E).

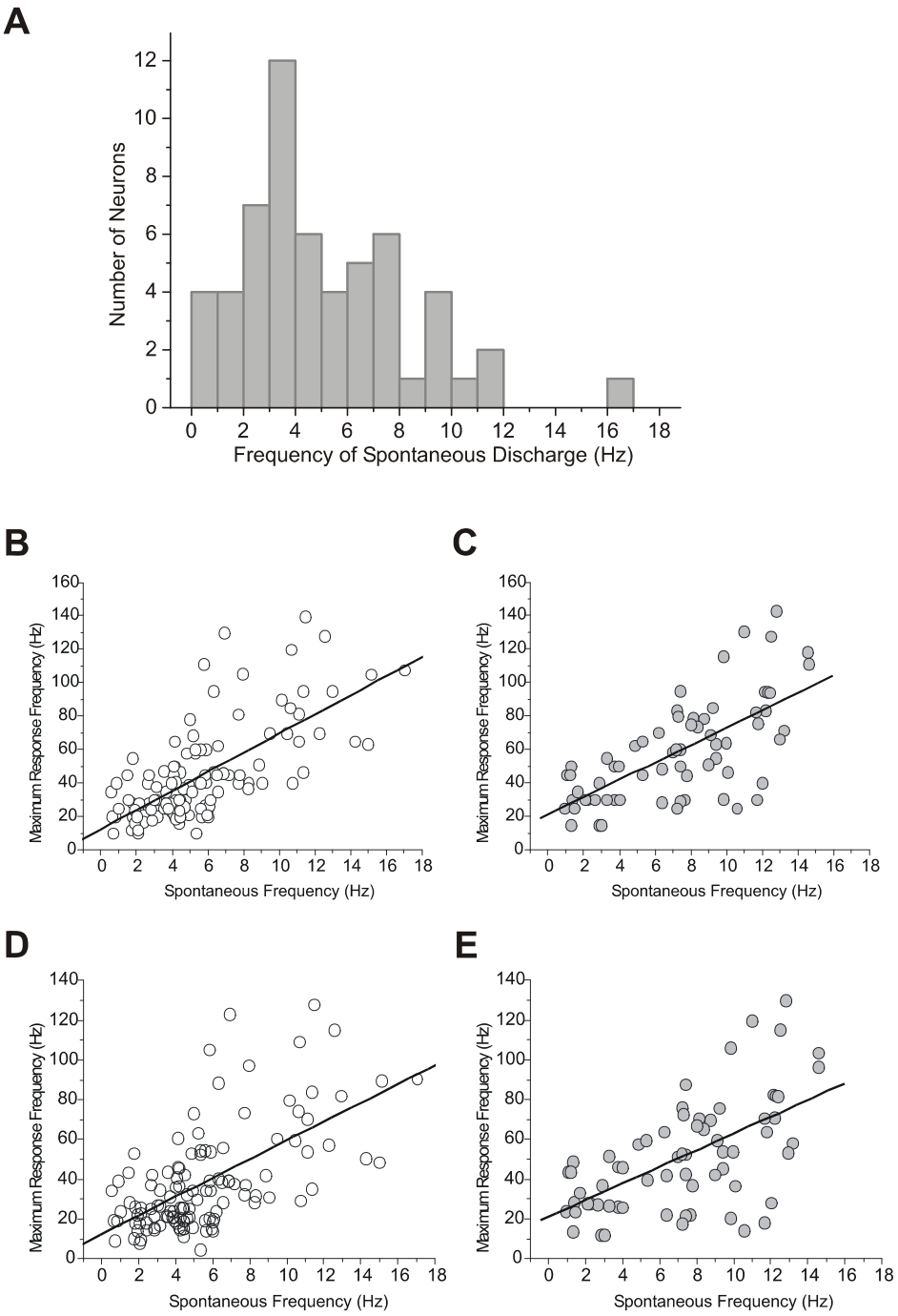



**Figure 16. Spontaneous activity in auditory cortex cortical units from the free moving animal. A**. Distribution of mean spontaneous activity in 54 neurons. Mean spontaneous activity was obtained from 50 to 100 windows of 300 ms. 5.07± 3.25Hz (mean ± s.d.) ranged between 0.6 and 16Hz. **B and C.** Relation between spontaneous activity of different neurons and their averaged maximum frequency evoked by a sound (n=43). Between 2 and 6 points per unit were obtained from different recordings. Each value corresponds to the average between 50-100 time windows of 300 ms. B and C correspond to measurements from different types of protocols in the same sample. Fitted line values were: slope of 5.7, R=0.69, n=122, p<0.0001 in B. Slope of 5.2, R=0.67, n=66, p<0.0001 in C. **D and E**. Same representation as in B and C, but here the spontaneous activity has been substracted from the maximum response. Fitted line values were: slope of 4.7, R=0.61, n=122, p<0.0001 in D. Slope of 4.19, R=0.59, n=66, p<0.0001 in E.

Additionally, we aimed at exploring the variability of interspike intervals of simultaneously recorded single units. With that purpose we recorded the spontaneous activity during longer time periods (30 to 45 min) from 13 neurons from the auditory cortex of 3 awake animals by means of tetrodes. We isolated 3 groups of 5, 4 and 4 simultaneously recorded neurons. In Fig. 17 it is shown one of the recorded groups where different neurons show very dissimilar patterns of interspike intervals, which vary in their length from 0.001 s to 53.976 s. Despite these differences may be attributed to the cell class, we found heterogeneity in less dissimilar simultaneously recorded neurons. Therefore, heterogeneity of firing of simultaneously recorded neurons was found as in (Rothschild et al. 2010). Finally, we explored whether there was a temporal structure in the firing that was common to simultaneously recorded neurons, as the one reported in (Luczak et al. 2009; Sakata and Harris 2009; Harris et al. 2010) which resembles oscillatory activity in the form of Up and Down states. Autocorrelograms performed to all neurons showed no pattern or rhythmic activity (Figs. not shown), although further recordings and analysis of single units and EEG activity in the awake and asleep animal could provide additional information on firing patterns.

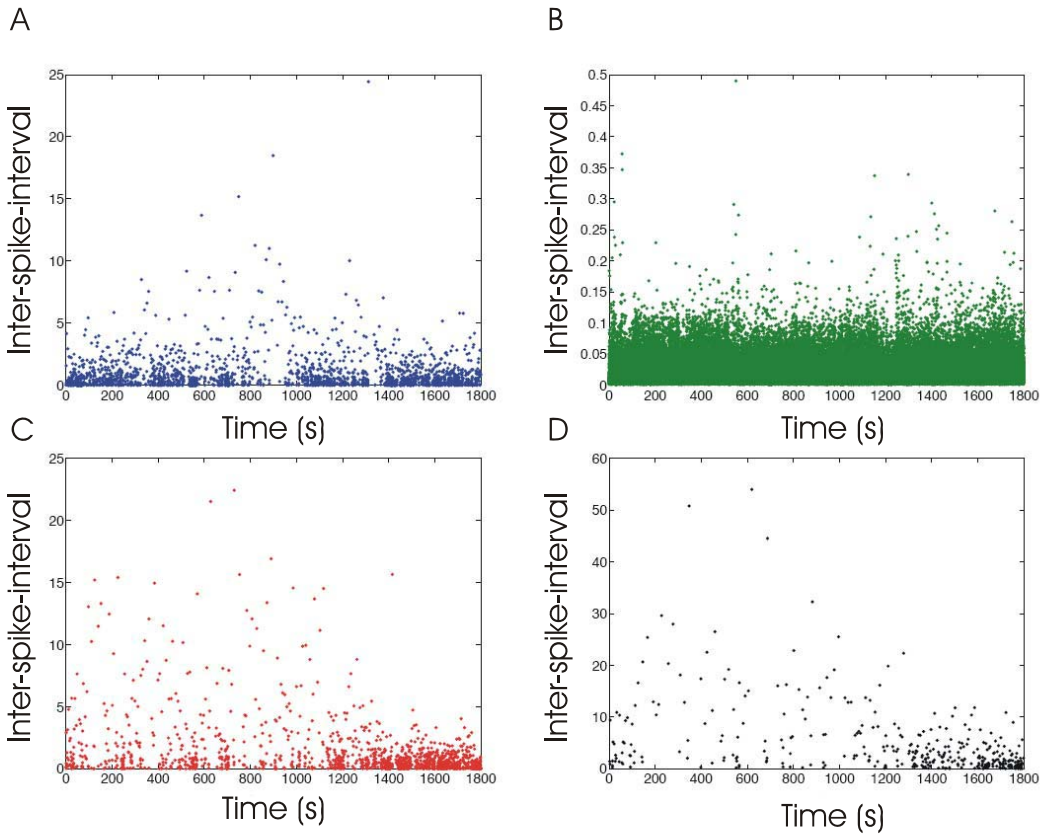



**Figure 17. Distribution of interspike intervals along the recording session. A-D.** Four adjacent isolated single units show different interspike interval patterns. Note that each neuron has a different range in the y axis. Neurons A,C,D show longer interspike intervals than neuron in B. Average values of interspike intervals are: 0.713, 0.023, 1.115 & 4.744 s for neurons A,B,C & D, respectively. (Analysis performed by B. Sancristobal).

## 4.3. Response patterns to sustained auditory stimulation in the passive listening and attentive animal

To determine the time course of auditory adaptation during long pulses and its recovery in the cortex of the awake passive listening rat, 28 isolated single cells were selected given their significant auditory response. The stimulation consisted on 200, 300, 400, 500, 700 and 1000 ms pulses of white noise evoking an auditory response.

Fig. 18A-B shows spike frequency adaptation during long auditory responses. In order to study adaptation during the stimulus, longer stimuli (200, 300, 400, 500, 700 and 1000 ms) were presented to awake rats. Stimuli of different durations were presented randomly, and each resulting PSTH was the result of averaging 80-100

trials (Fig. 18A,B). We observed a large heterogeneity on the response patterns across neurons and we divided the responses in “tonic” and “phasic”. In order to classify between phasic and tonic responses, the first 50 ms and the last 150 ms of the response (10 ms bins) were analyzed (Recanzone 2000; Chimoto et al. 2002; Qin et al. 2003). If both, the first and last response components had a mean frequency above the 95% confidence interval (C.I.) with respect to the mean rate, the neuron was considered tonic. If only the first 50 ms had a response above the C.I. the neuron was considered phasic. According to this criteria, 78.57 % of the neurons were phasic (n=22) and 21.42 % of the neurons were tonic (n=6), independently of the pulse duration.

The average decay of the firing between the first 50 ms and the last 150 ms was of 35% in tonic neurons and to 68% in phasic neurons. Therefore, the response of tonic neurons did as well show adaptation during long pulses. All tonic neurons and some phasic showed some degree of oscillatory behaviour during responses (Fig. 18B). By oscillatory behaviour we mean that following the first peak of the response there is a decay and between 1 and 3 subsequent peaks.

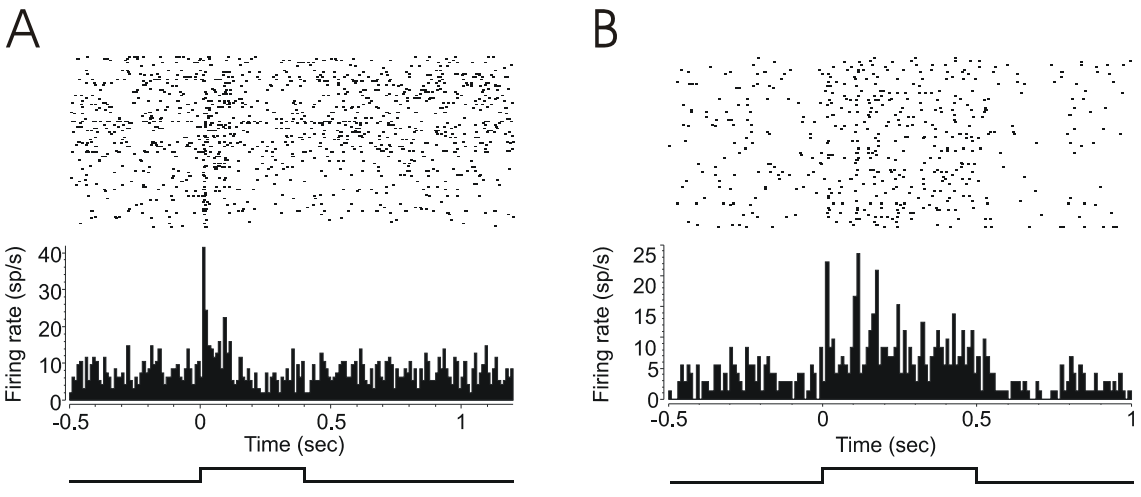



**Figure 18. Adaptation during the stimulus. A.** Neuron with phasic response. PSTH corresponding to the response to an auditory stimulus of 400 ms and 90 dB. 94 trials have been averaged. Note how the response decays after the first 100 ms. **B.** Neuron with tonic response. PSTH corresponding to the response to an auditory stimulus of 500 ms duration and 90 dB. It corresponds to the average response of 72 trials.

Neuronal response patterns of auditory cortex to long-lasting stimuli (>200 ms) during an attentive task have barely been studied (Recanzone 2000). We additionally recorded from 13 single units by means of chronically implanted tetrodes in the auditory cortex in order to characterize the neuronal response patterns in the awake animal during a long-duration-stimulus discrimination task. The experimental protocol consisted of a sequence (Fig. 19A) of recording stages with a total duration of 3.5 hours. A tuning curve was recorded in the freely moving animal at the beginning of the recording sequence and right after the attentive task. Two passive recording stages, “initial passive” and “passive post” were recorded also before and after the attentive task, respectively. In the attentive task (Fig. 19B) the animal had to enter in the central socket which triggered the presentation of a single stimulus with a duration of 500 ms or 900 ms, that required left or right poking, respectively, in order to obtain a reward. The “passive+reward” recording stage (40 min. approx.) was performed as a final step in the recording sequence. The aim of these recordings sequence was to compare the neuronal response of a single unit recorded during the attentive task *versus* the initial passive, passive post and passive+reward, having all of them the same amount of trials (200 trials each side), stimuli (80dB; 5.3 kHz) and intertrial interval (2 to 3 s). Animal performance during the recordings is shown in Fig. 19C,D. Recordings were considered valid only in case the percentage of correct reactions was above 70% (in this animal from day 4 in the trialwise randomized learning stage). Animal learning performance from 2 other rats in the same task showed a similar behavioural pattern (Figs. not shown).

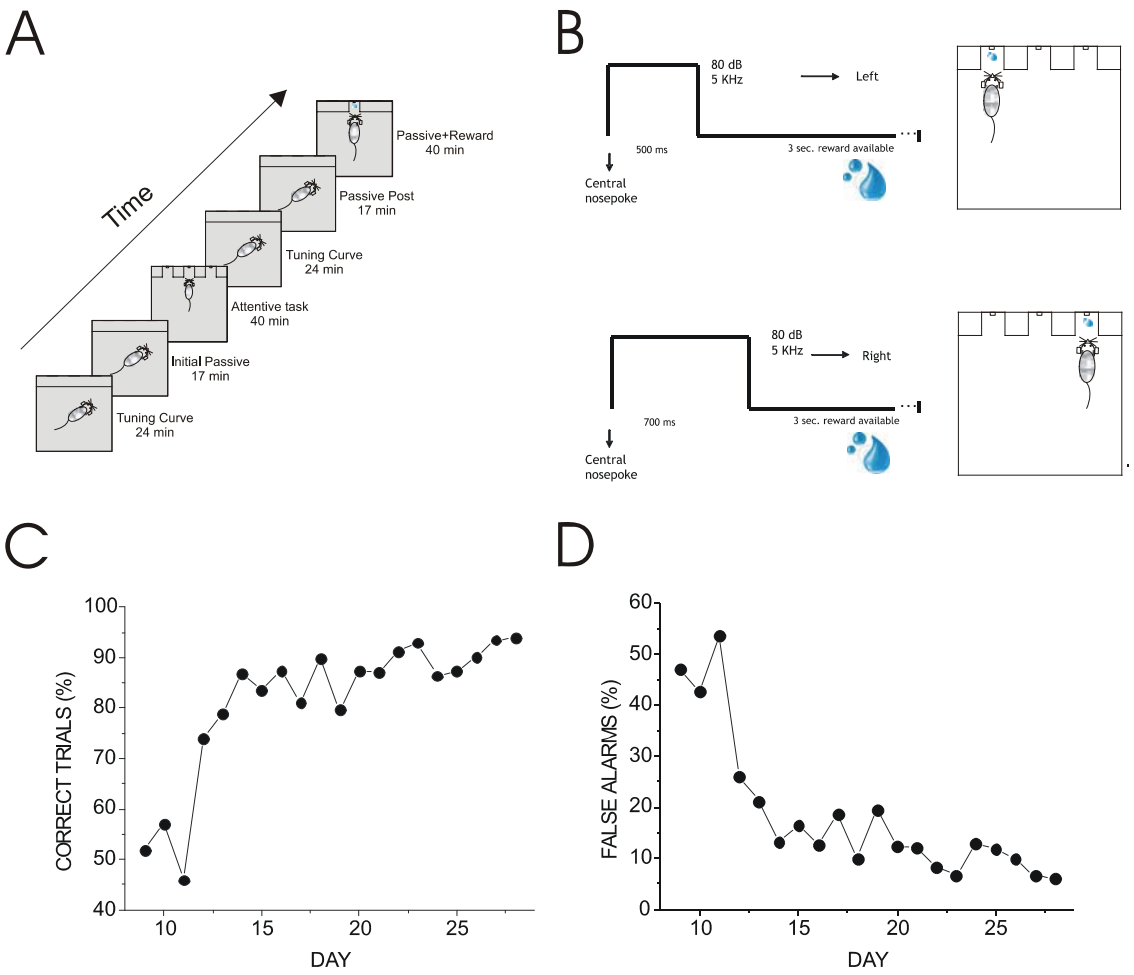



**Figure 19. Discrimination of long duration stimuli. A.** Sequence of recording stages in chronological order. Passive listening of auditory stimuli occurred in the tuning curve and the passive recording stages that were run before and after the attentive task. A passive recording with reward after each stimulus presentation is also performed as a final recording stage. The auditory stimuli (500ms or 900 ms; 80dB; 5322Hz), intertrial interval (2-3 s) and trials repetitions (200) were the same in each recording stage but the tuning curve. The total duration of the recording protocol was 3.5 hours. **B.** In the attentive task the rat enters in the central socket and a 500 ms or 900 ms stimulus duration is presented (80dB; 5322Hz) through earphones. 500 ms or 900 ms stimulus duration indicates left or right reward delivery, respectively. **C.** Animal 1 performance (correct trials (%)) along training days. **D.** Error trials (false alarms (%)) along training days. The animal was implanted and then trained. Learning performance is shown in C and D from day 4, where the stimuli durations are randomized trialwise. Previous training sessions included "shaping" and blockwise stimulus presentation.

Hereby, we report 3 different response patterns while the animal discriminated the duration of stimuli. First, we found that some neurons (n=4) showed an abrupt decrease in the firing rate at the onset and offset of stimulus presentation with respect to spontaneous activity. This enhanced inhibition at the onset and offset of stimulus presentation is absent in the passive state of the animal

where a response peak can be observed at the onset and offset of stimulus presentation (Fig. 20). Other neurons (n=3) showed only an onset - offset response component to stimulus presentation in the attentive task with respect to the passive state where any response can be observed to the stimulus presentation (Fig. 21). Finally, we also found other neurons (n=3) with an enhanced postadaptation after the peak response followed by a rebound of excitatory activity, while in the passive state there was no firing to the stimulus presentation (Fig. 22). Additionally, we observed that these response patterns show between the onset and offset of the stimulus whether an increase (Fig. 20;Fig. 22) or a decrease (Fig. 21) of firing rate. Altogether, these results show different kinds of activity patterns that may help to discriminate stimulus duration in the behaving animal. Importantly, the key stimulus components in our long-lasting-stimulus discrimination task, being the onset and offset, are clearly coded by means of an enhanced excitation or inhibition.

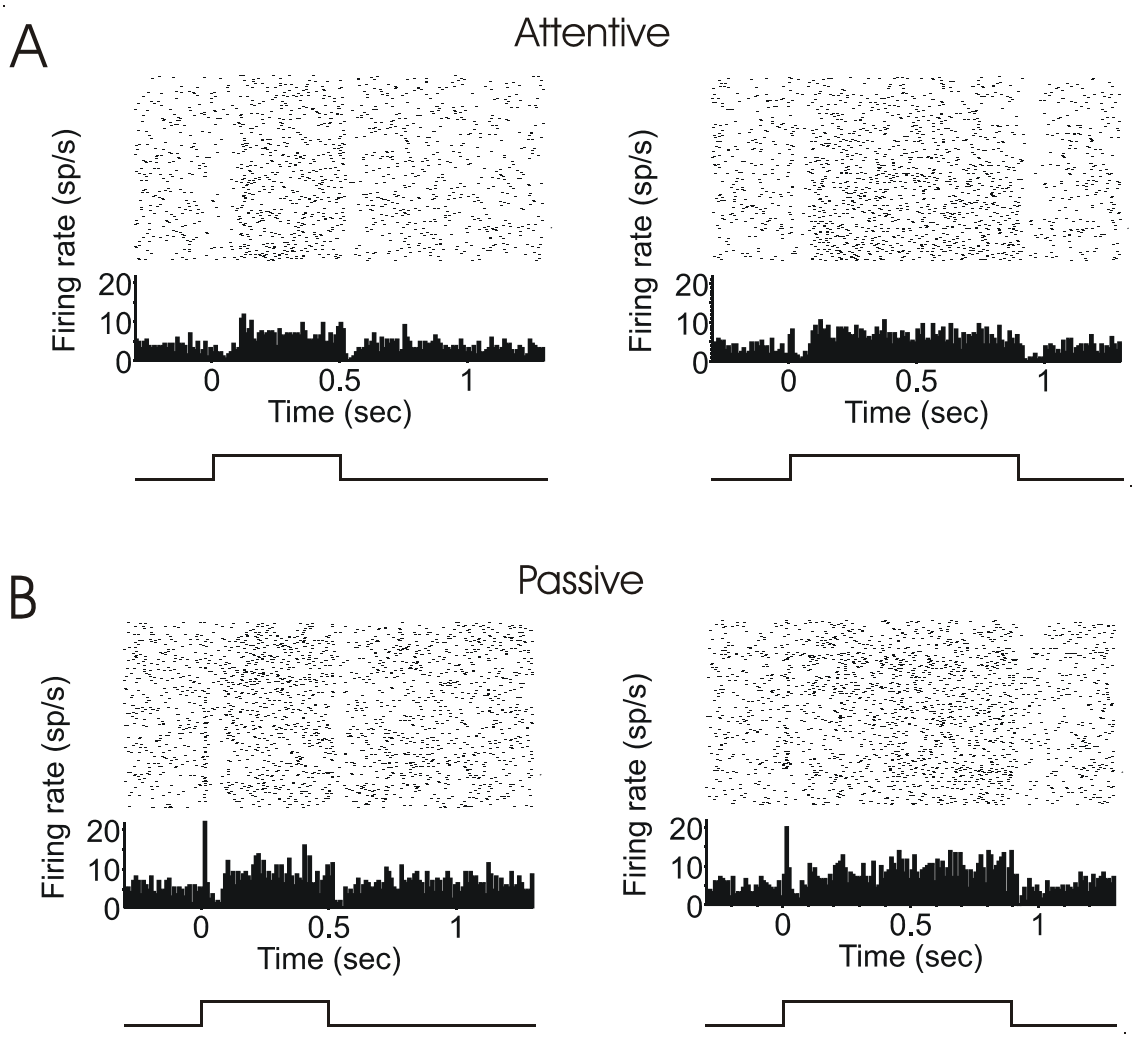



**Figure 20. Enhancement of inhibition at the onset and offset of stimulus presentation. A.** PSTH of the response of a single neuron to 500 ms (left) and 900 ms (right) stimulus presentation in the attentive task. Responses to approximately 200 stimuli repetitions show an inhibition of activity at the onset and the offset of stimulus presentation with respect to the passive state (**B**). Between the onset and offset there is an enhanced activity with respect to the spontaneous in the attentive condition.

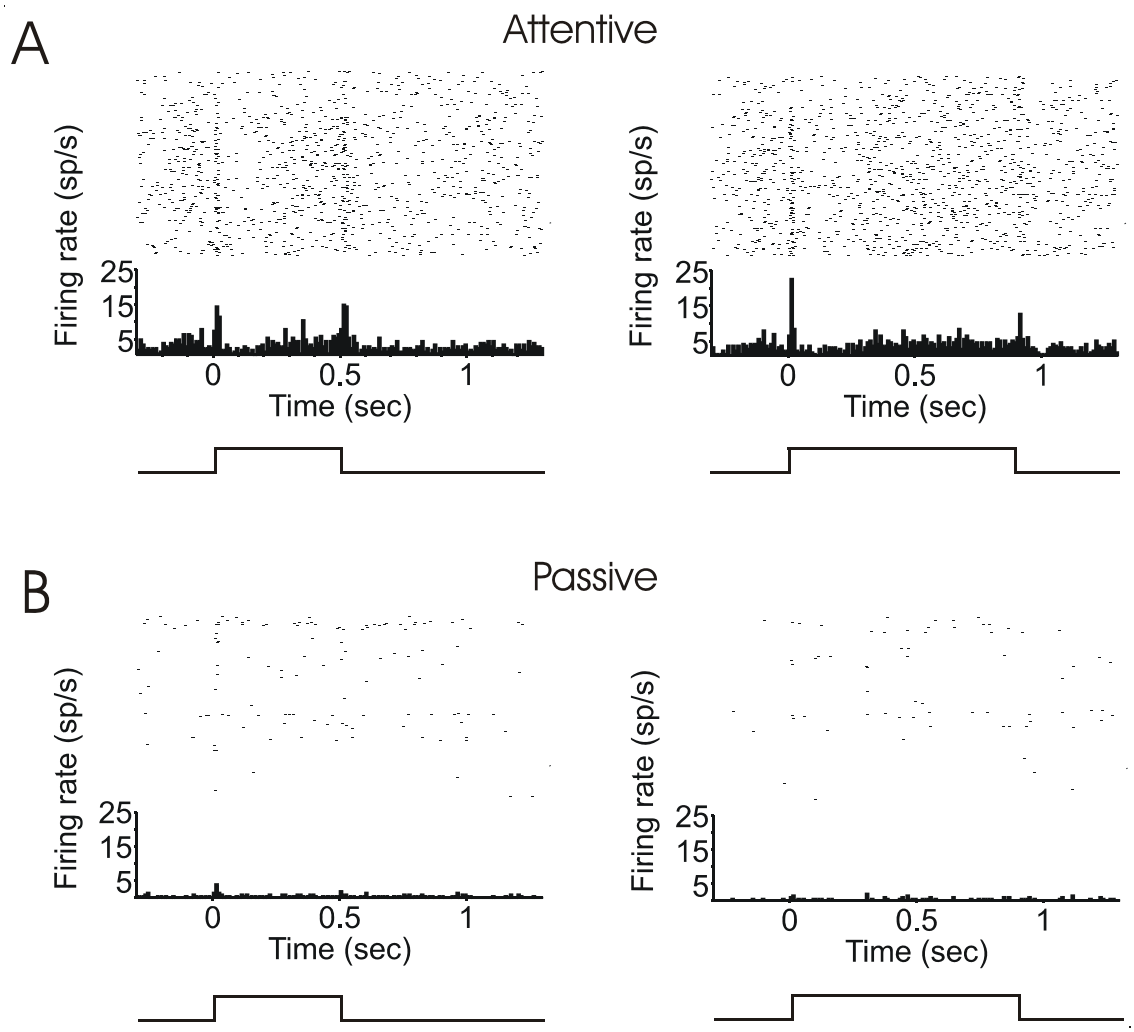



**Figure 21. Onset - offset response pattern to long duration stimuli. A.** PSTH of the response of a single neuron to 500 ms (left) and 900 ms (right) stimulus presentation in the attentive task. Responses to approximately 200 stimuli repetitions show enhanced onset and offset responses. No response to the stimulus presentation is seen in the passive state (**B**).

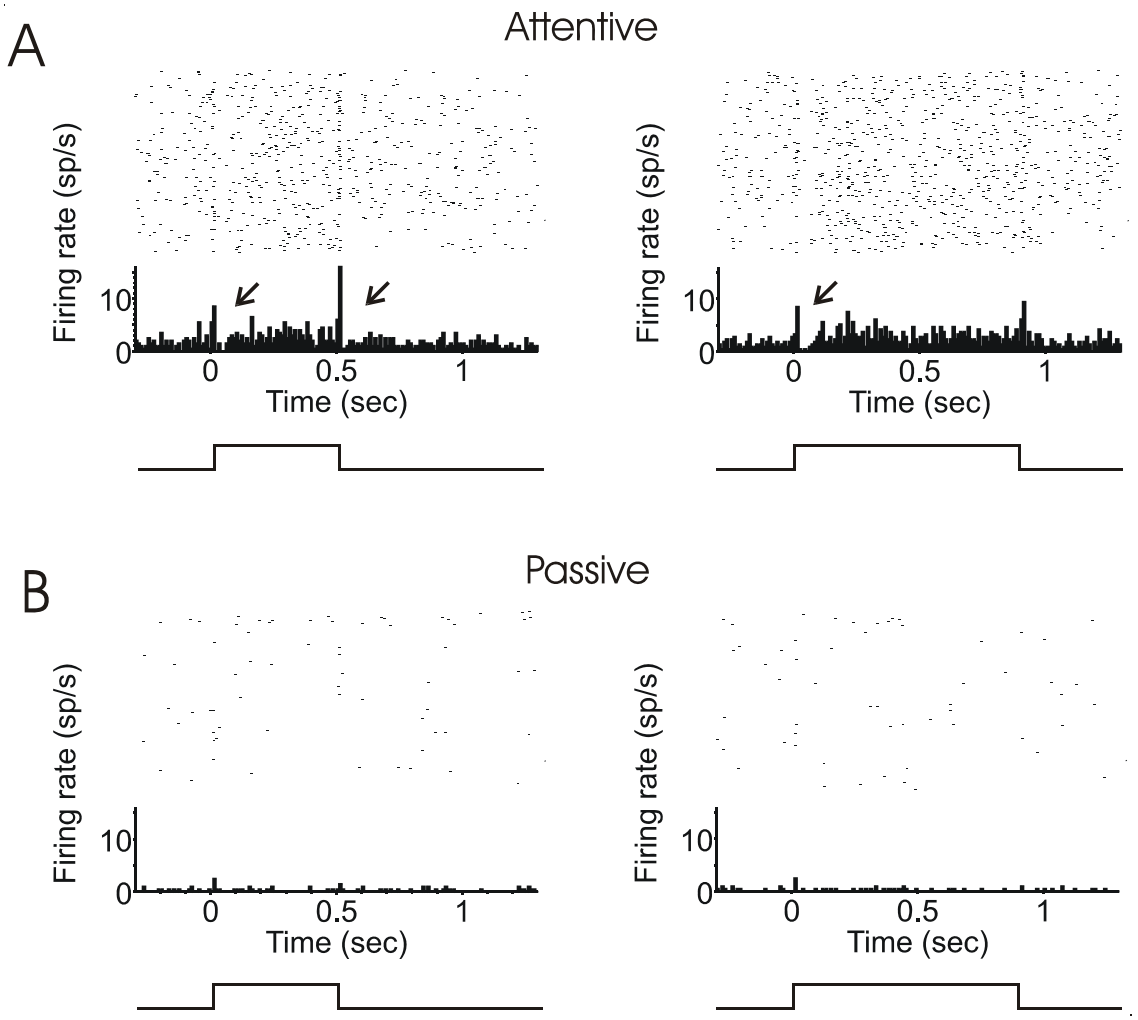



**Figure 22. Onset - offset response pattern followed by postadaptation and excitatory rebound of activity. A.** PSTH of the response of a single neuron to 500 ms (left) and 900 ms (right) stimulus presentation in the attentive task. Responses to approximately 200 stimuli repetitions show an onset and offset response pattern followed by postadaptation (indicated by arrows) and a rebound of excitatory activity. No response to the stimulus presentation is seen in the passive state (**B**).

## 4.4. Neuronal codes for temporal discrimination in the auditory cortex of the attentive animal

Further, we performed recordings in the auditory cortex of two awake freely moving rats chronically implanted with tetrodes. We isolated 86 single units which were classified according to a similar method and categorization (Recanzone 2000) into *onset* (25.5%), *onset+offset* (12.7%), *offset* (2.3%), *non-responsive* (43.0%), *pauser* (12.8%), and *unknown* (3.4%). A similar percentage of non responsive neurons

was observed by (Hromadka et al. 2008) by means of cell-attached recordings. The experimental procedure consisted of a sequence (Fig. 23A) of recording stages with a total duration of 3h. A tuning curve was recorded in the freely moving animal at the beginning of the recording sequence and right after the attentive task. Two passive recording stages, “initial passive” and “passive post” were recorded also before and after the attentive task, respectively. In the attentive task the animal had to enter in the central socket which triggered the presentation of two identical stimuli separated by an ISI of 150 or 300 ms. Rats had to categorize the two different ISIs, 150 and 300 ms, which required left or right poking, respectively, in order to obtain a reward. The “passive+reward” recording stage (40 min. approx.) was performed as a final step in the recording sequence. The aim of these recordings sequence was to compare the neuronal response of a single unit recorded during the attentive task *versus* the initial passive, passive post and passive+reward, having all of them the same amount of trials (180 trials each side), stimuli (50ms; 80dB; 5.3 kHz), ISI (150 and 300 ms) and intertrial interval (2 to 3 s).

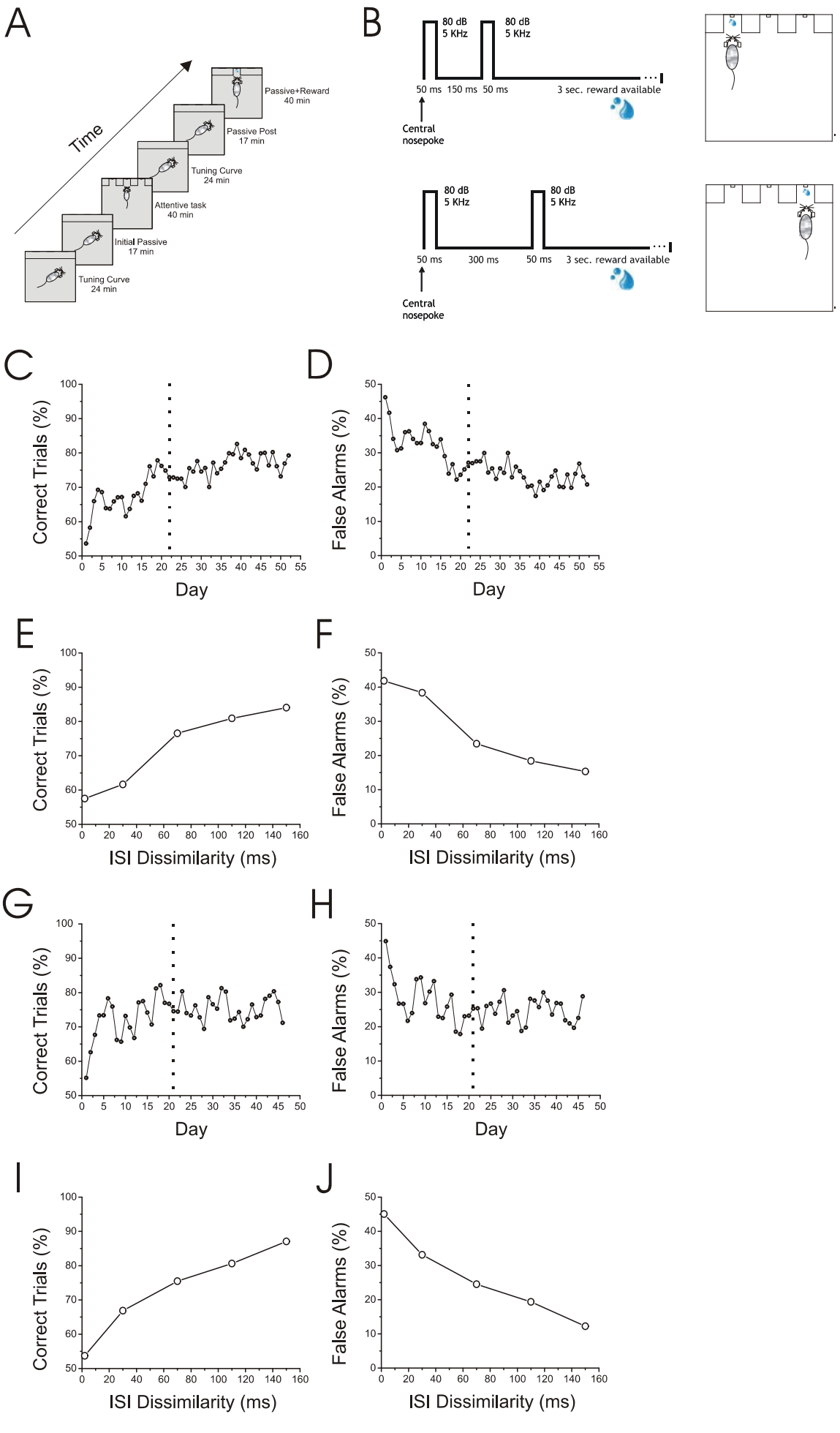

A
Time
Passive+Reward
40 min
Passive Post
17 min
Tuning Curve
24 min
Attentive task
40 min
Initial Passive
17 min
Tuning Curve
24 min
B
80 dB
5 KHz
80 dB
5 KHz
50 ms 150 ms 50 ms
3 sec. reward available
Central
nosepoke
80 dB
5 KHz
80 dB
5 KHz
50 ms
300 ms
50 ms
3 sec. reward available
Central
nosepoke
C
Correct Trials (%)
Day
D
False Alarms (%)
Day
E
Correct Trials (%)
ISI Dissimilarity (ms)
F
False Alarms (%)
ISI Dissimilarity (ms)
G
Correct Trials (%)
Day
H
False Alarms (%)
Day
I
Correct Trials (%)
ISI Dissimilarity (ms)
J
False Alarms (%)
ISI Dissimilarity (ms)

**Figure 23. Behavioural protocol and performance. A.** Sequence of recording stages in chronological order. Passive listening of auditory stimuli occurred in the tuning curve and the passive recording stages that were run before and after the attentive task. A passive recording with reward after each pair of stimuli presentation is also performed as a final recording stage. The auditory stimuli (50ms; 80dB; 5322Hz), interstimuli interval (150 and 300 ms), intertrial interval (2-3 s) and trials repetitions (180) were the same in each recording stage but the tuning curve. The total duration of the recording protocol was 3 hours approx. **B.** In the attentive task the rat enters in the central socket and two identical stimuli (50ms; 80dB; 5322Hz) are presented through earphones. 150 or 300 ms interstimuli interval indicates left or right reward delivery, respectively. **C.** Animal 1 performance (correct trials (%)) along training days. **D.** Error trials (false alarms (%)) along training days. In C and D: dashed line indicates beginning of recorded sessions. **E.** Psychometric curve shows improved performance within one session as the difference between both ISI (ms) increases. **F.** Same session shows increased difficulty to categorize both ISIs as they become closer. **G-J.** Same as C-F but for animal 2. Animal 1 and 2 were trained before tetrodes implantation. Learning performance in animals 1 and 2 shown in C,D;G,F corresponds to short and long ISIs randomized trialwise.

In order to further substantiate and validate our behavioural task shown in Fig. 23, we trained successfully 2 additional rats in the protocol shown in Fig. 24. In the later protocol the rats were presented two different groups of ISIs whenever they entered in the central socket. Therefore short (50, 100, 150, 200ms) ISIs indicated left response while long ones (350, 400, 450, 500ms) indicated right response in order to obtain water reward. Hence, this task allows us to confirm that rats are able to discriminate and categorize temporal information of auditory stimuli, an aspect that has been largely neglected so far. Moreover, the validation of the current behavioural protocol would allow us to further substantiate how the significance of MI or response variability varies according to the difficultness of discrimination of ISIs categories.

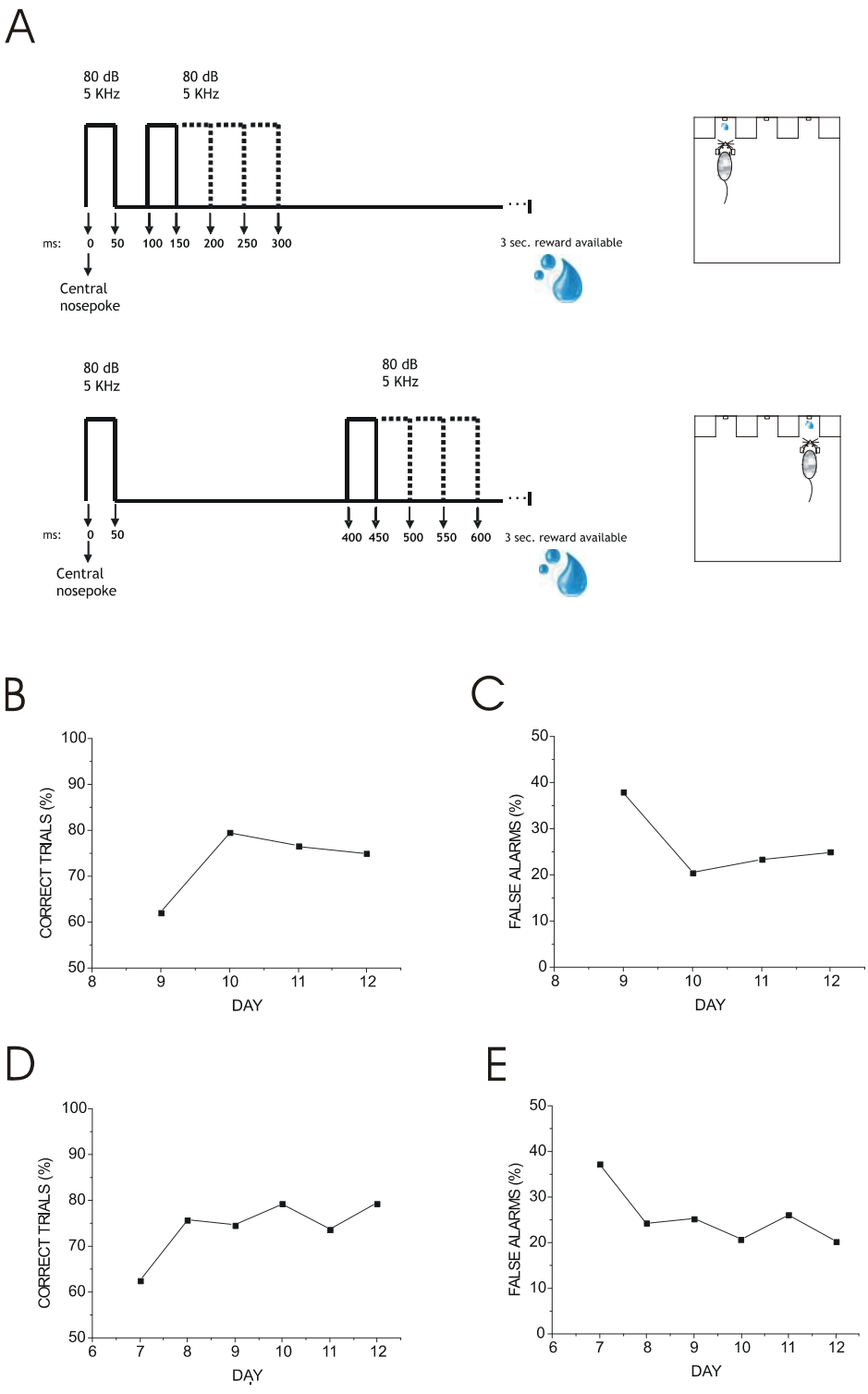



**Figure 24. Behavioural protocol and performance. A.** The rat enters in the central socket and two identical stimuli (50ms; 80dB; 5322Hz) are presented through earphones. Short (50, 100, 150, 200ms) and long (350, 400, 450, 500ms) interstimuli intervals indicate left or right reward delivery, respectively. **B.** Animal 1 performance (correct trials (%)) along training days. **C.** Error trials (false alarms (%)) along training days. **D-E.** Same as B-C but for animal 2. Learning performance in animals 1 and 2 are shown whenever short or long ISIs were randomized trialwise. Previous training sessions included "shaping" and blockwise stimuli presentation.

### 4.4.1. Information content is higher during attention than in passive brain states

Mutual Information (MI) measures the strength of the relationship between two variables and it allows quantifying how much information is shared between them. Therefore, MI analysis provides an accurate measure of how much knowing one variable we can reduce the uncertainty of the other. Mutual Information (MI) analysis has been previously used to estimate the information content present in spike trains of the auditory system in anesthesized animals (Lu and Wang 2004; Nelken et al. 2005; Kayser et al. 2009). Here we performed MI analysis to find out whether single units in auditory cortex of the awake animal code for the information related to the temporal category of stimuli. MI between the variable "spike count" during stimuli presentation and the variable "Interstimuli interval (ISI) category" (150 or 300 ms) was calculated. Hence, if the variable "spike count" provides information on the variable "ISI category" then we could say that there is mutual information between them, and the MI value would be high.

In our time-discrimination task the animal had to decide on whether two identical stimuli were separated by 150 or 300 ms. In that task, the key stimulus that determines if the ISI category is "short" or "long" (150 or 300 ms) is the second one. Hence, we compared the MI significance in the response to stimulus 1 (S1) *versus* that to stimulus 2 (S2). The raster plots and PSTHs corresponding to ISIs of 150 ms and 300 ms in a neuron are illustrated in Fig. 25A. The response to the second stimulus was typically decreased as a result of auditory adaptation processes (Abolafia et al. 2010). In the case illustrated in Fig. 25, MI was significantly higher during the response to S2 than to S1 (Fig. 25B) both in the attentive (S2:0.99; S1:0.06) and in the initial passive stages (S2:0.82; S1:0.19). This was not the case though during the passive-post stage, where the significance of MI was zero both for the first and the second stimulus (S2:0; S1:0).

MI was calculated in 21 selected cases (out of 86) that showed highly significant firing rate to the stimulus onset. We found that 13 cases showed significant MI to one or both stimuli in the attentive task while 8 did not. Figure 25C shows the average of 13 neurons with significant MI to one or both stimuli. It is shown that in the attentive task, the MI is significant to S2 (mean: 0.95) but not to S1 (mean:0.69). However, there is no MI significance in the initial passive (S1:0.31; S2:0.42) and passive-post (S1:0.36; S2:0.36). More precisely, in the attentive task, we found that in 6 cases S2 was significant while not S1. Another 3 neurons showed significant MI to S1 but not to S2. These results are suggestive that the second stimulus tends to carry more information than the first one. Finally, 4 neurons showed significant MI both to S1 and S2.

In all, MI shows higher significance to stimuli under attention than in the passive state of the animal, both in the initial passive and passive-post, indicating that information content is augmented under attentional demands where stimuli must be precisely encoded (see also Figure 26).

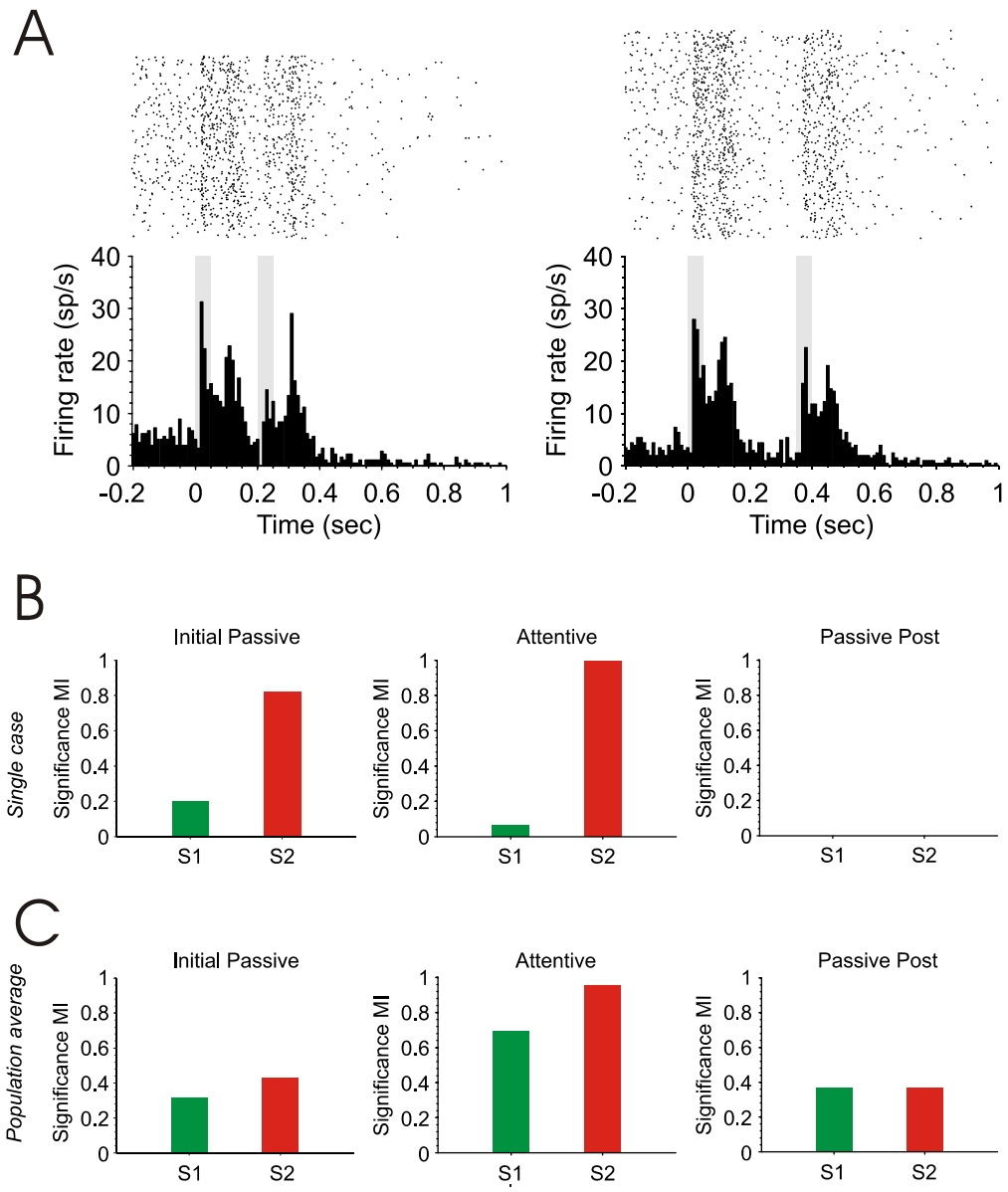



**Figure 25. Information content is higher during the relevant stimuli (stimulus 2 (S2)) as compared to the non-relevant (stimulus 1 (S1)). A.** Perievent raster and histogram of an example neuron shows the frequency response histogram (bottom) using 10 ms bins and the spikes raster plot (top) to 180 trials approx. A response peak can be observed during and after each identical stimuli (50ms;80dB;5322Hz) for 150 ms ISI (left) and 300 ms ISI (right). Grey bars indicate stimuli presentation. **B.** In the attentive task there is enhanced significance of Mutual Information (MI) in S2 compared to S1 and that pattern is consistent with the initial passive recording. No significance in either stimuli is present in the passive post stage. **C.** The same pattern as in B is shown for the average of 13 neurons with significant MI.

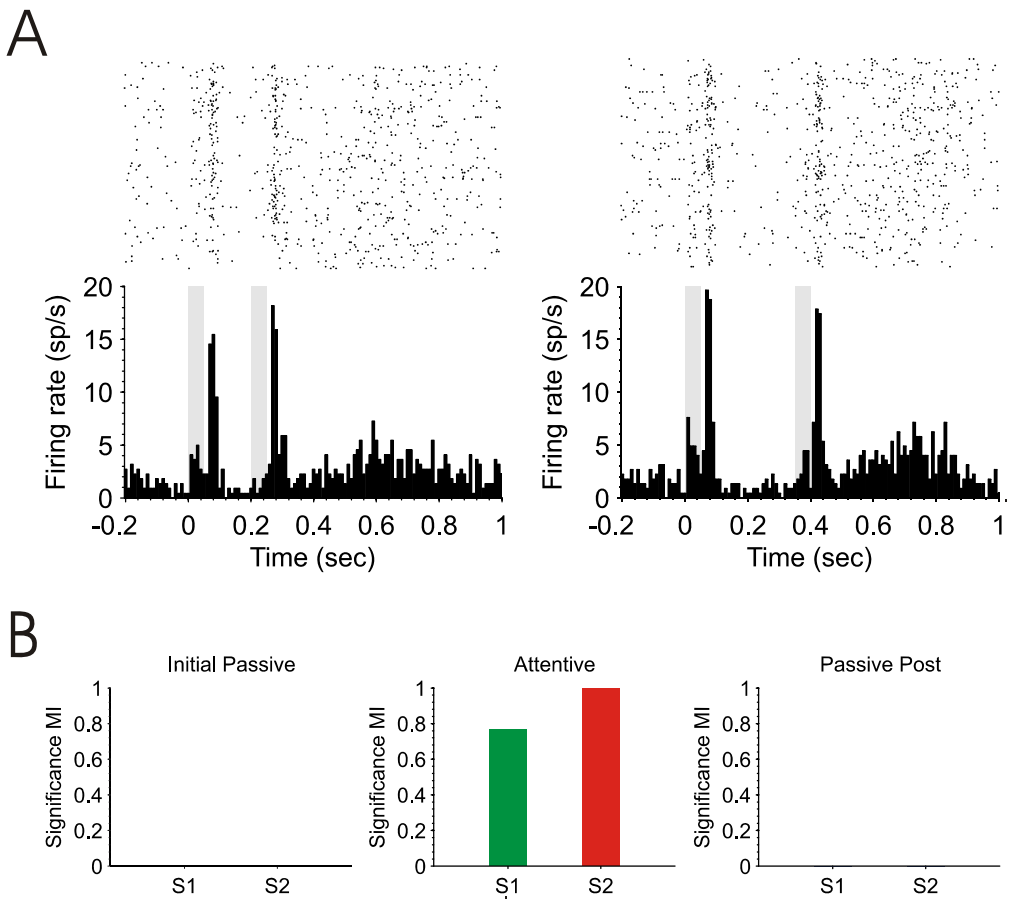



**Figure 26. Enhanced significance of Mutual Information during S2 in the attentive task. A.** PSTH (180 trials) of a single neuron shows a small onset response amplitude and enhanced offset response to two identical stimuli (50ms; 80dB; 5322Hz) separated by 150 ms (left) or 300ms (right). Grey bars indicate stimuli presentation. **B.** Higher MI significance to S2 (1) than in S1 (0.76) is shown in the attentive task. No significant MI is present in neither of the passive stages, having zero value in all of them.

Since S2 constitutes the most relevant stimulus in order to discriminate the ISI category, we compared MI to S2 response between the attentive condition and the non-attentive recording stages, i.e. “initial passive” and “passive-post”. Two different response profiles, one corresponding to a tonic neuron and one with an “onset-offset” pattern are illustrated in Fig. 27A and B, respectively. Irrespective to the neuronal type, MI was significantly higher in the response to S2 in the attentive than in the passive tasks. This was the case in the tonic firing neuron, where MI during attention to S2 was 0.87 while it was lower during passive responses (“initial passive”:0.2; “passive-post”:0.41) (Fig. 27C, left).

A significant increase in the MI to S2 was found in another neuron (Fig. 27B) which shows an “onset-offset” response pattern. Here the MI in the response to S2 was 0.98 during the attentive task while it was lower during the passive ones (initial passive:0.51; passive-post:0.13) (Fig. 27C, middle). Similarly, 13 (out of 21 neurons with significant firing rate) showed a significantly higher average MI value to S2 (0.95) than in the initial passive (0.42) or passive post (0.36) (Figure 27C, right). These results suggest that the attentive state of the animal is having an effect on the information content carried by stimulus-induced spike trains (see also Fig. 28).

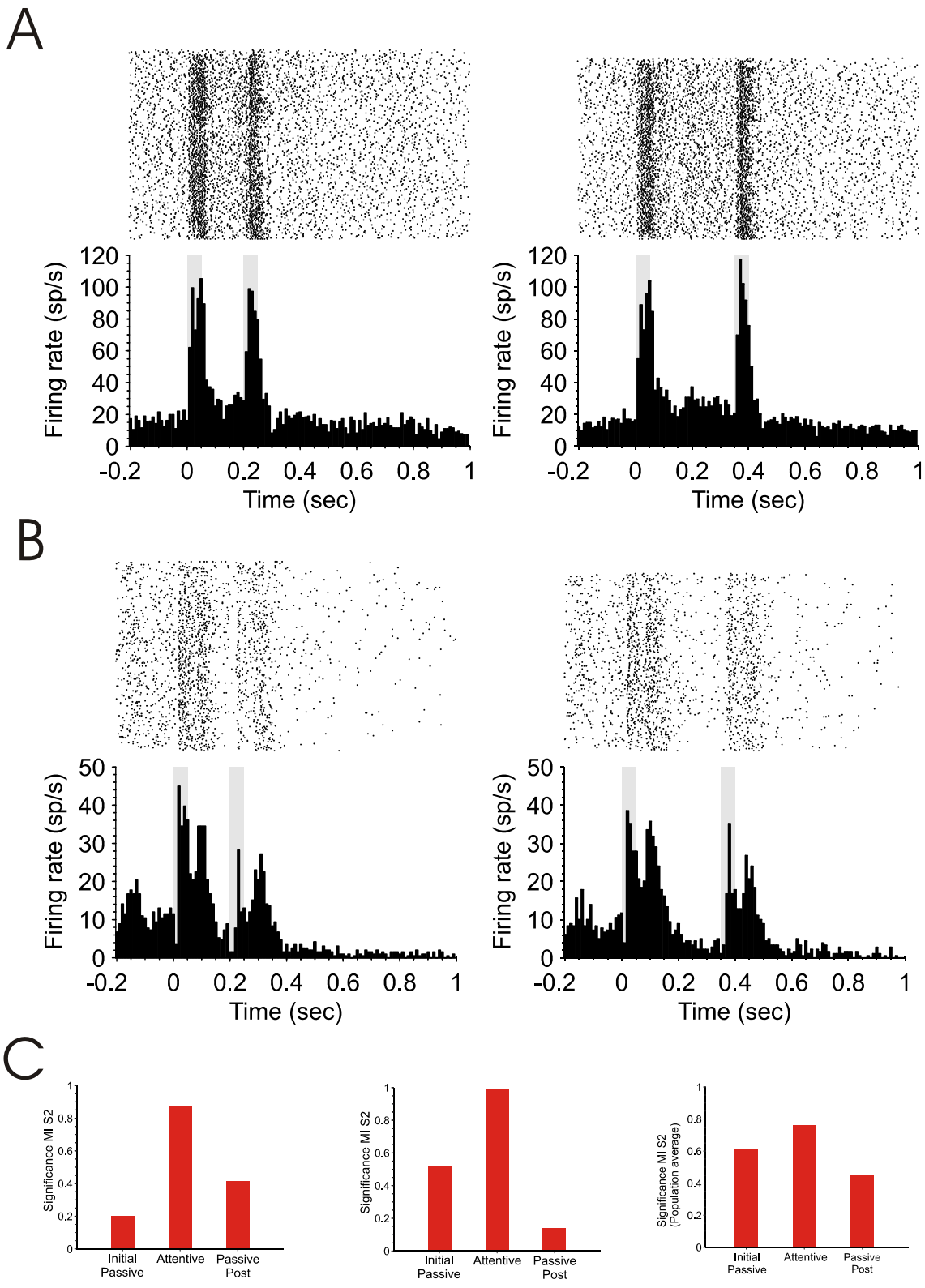



**Figure 27. Information content during S2 is higher during the attentive task than in the passive recording stages. A.** PSTH (180 trials) of a single neuron showing sustained responses along with the stimuli duration (grey bars) to 150 (left) and 300 (right) ms ISI and two identical stimuli (50ms;80dB;5322Hz). **B.** PSTH (180 trials) shows the response of a single neuron to the onset and offset of two identical auditory stimuli for 150 (left) and 300 (right) ms ISI. **C.** Significance of MI to S2 is increased during the attentive task as compared to the initial passive and passive post stages for neuron in A (left), in B (middle) and for the average of 13 neurons with significant MI (right).

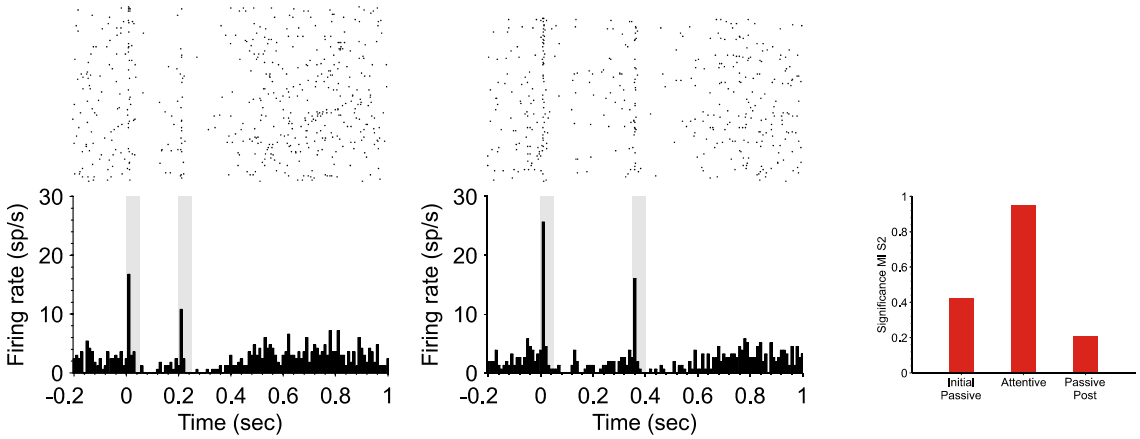



**Figure 28. Enhanced significance of Mutual Information during the attentive task with respect to the initial and passive post recording stages.** PSTH (180 trials) of a single neuron showing onset responses to 150 (left) and 300 ms (middle) ISI and two identical stimuli (50ms; 80dB; 5322Hz). Grey bars indicate stimuli presentation. Right. MI significance to S2 is increased during the attentive task (0.94) as compared to the initial passive (0.42) and passive post (0.20) stages.

We also compared the MI between the attentive task and the passive+reward recording. An example is illustrated in Figure 29, where a PSTH illustrates the response pattern of another neuron with an onset-offset response. There, higher MI to S2 (0.88) response during the attentive task than in the passive+reward recording stage (0.60) was measured. This same pattern was detected in 13 neurons (out of 21 with significant firing rate) previously reported as with significant MI to S2 response (attentive:0.95; passive+reward:0.58). This comparison strongly suggests that attention increases the information content carried by neurons in response to relevant stimuli.

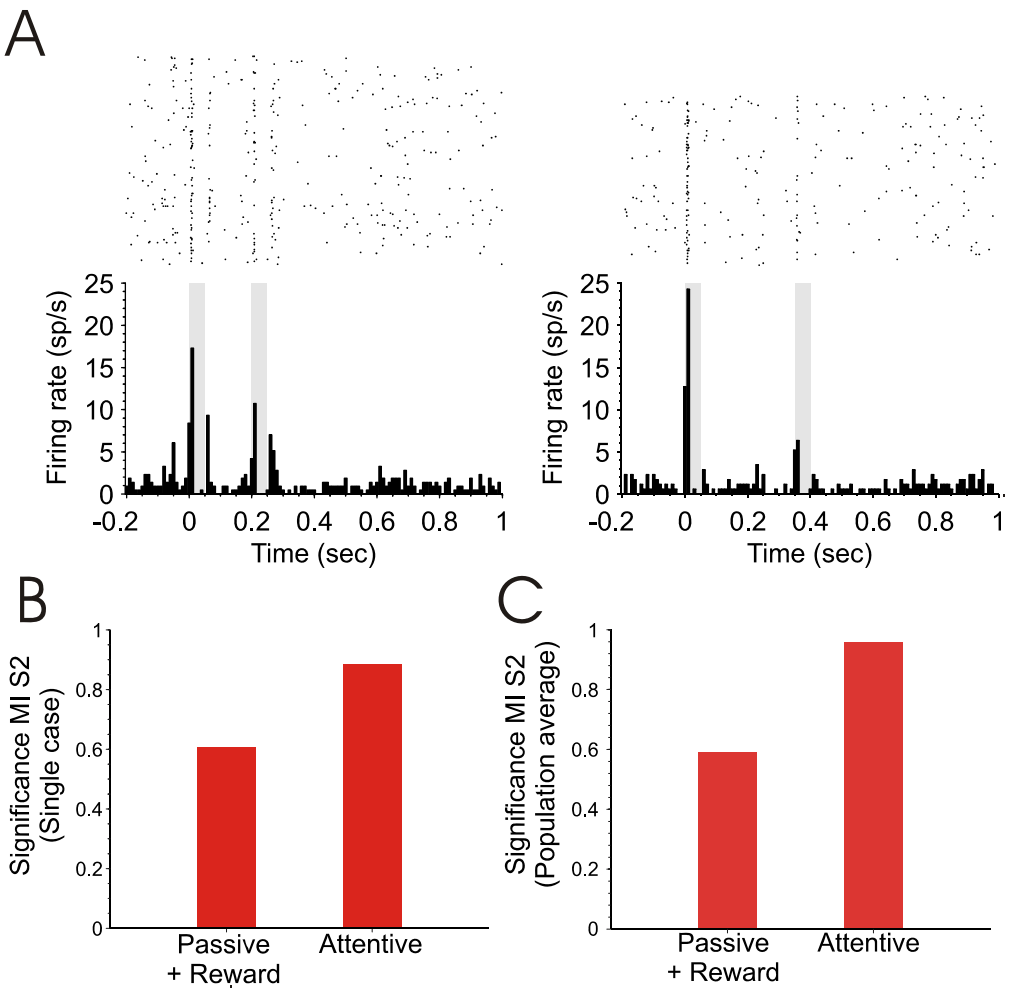



**Figure 29. Information content is higher during the attentive task than in the passive+reward recording stage. A.** Onset and offset neuronal response pattern of a single neuron is shown in the PSTH (180 trials) for two identical stimuli (50ms; 80dB; 5322Hz) separated by 150 (left) and 300 ms (right). **B.** Significance of MI in S2 during the passive+reward recording and during the attentive stage for the same neuron. **C.** MI average of 13 significant neurons shows enhanced MI during the attentive task as compared to passive+reward stage.

We also studied whether offset responses would carry information during their response after S2 termination. Seven neurons showed offset responses to auditory stimulation, 4 out of which showed additionally onset responses (e.g. Fig. 27B). MI during the offset response component was calculated after S2 termination in those neurons that were classified as “onset-offset” or “offset” (n=7). We analyzed the MI during a time window of the same duration as the one used to calculate the MI during stimuli presentation (50 ms). For that purpose, we selected 25 ms before and after the response peak of the offset response of S2. We found that MI was also significant

during the offset response (mean:0.84). This result suggests that the offset neuronal response after S2 termination not only carries information, but that it carries the same amount of information as during S2 presentation about the temporal category of the ISI.

Finally, we analyzed the distribution of reaction times (RT) (Fig. 30) and tested whether there was a relationship between MI of the responses and RT. RT distribution is plotted between 0.2 and 0.8 s since RT of these values comprised 96.6% of the trials. The probability of significantly high MI was higher for RT between 0.5 and 0.7 s for animal 1 and between 0.3 and 0.5 s for animal 2. Even though each animal has a different RT peak and distribution (animal 1 was slower than animal 2), the significance of MI is clustered around a group of RTs. This result suggests that the group of RT with more significant MI is the most optimal for animal´s performance in order to discriminate temporal information. This relationship between neuronal and behavioural data provides support for the information content in neuronal responses previously shown.

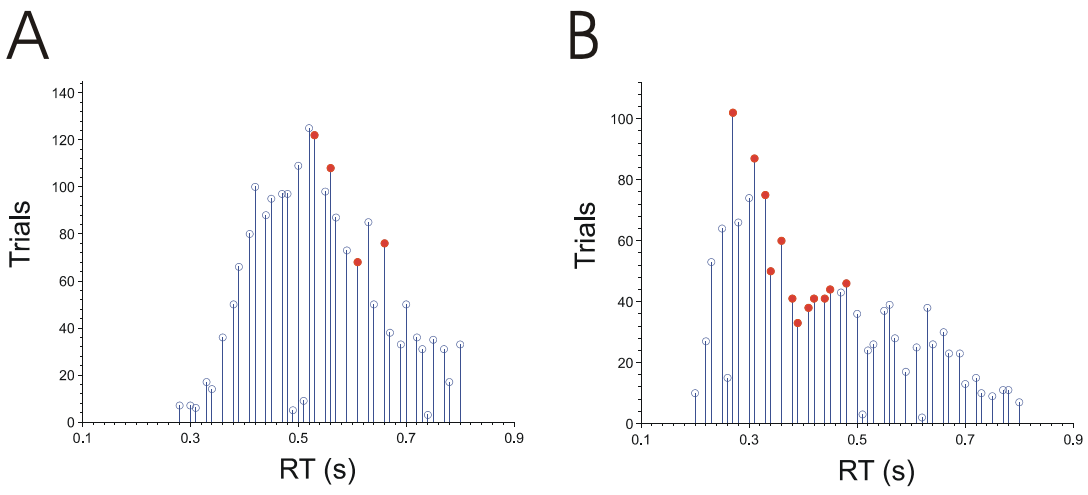



**Figure 30. Reaction time correlates with MI. A.** Animal 1 (A) and 2 (B) distribution of reaction times (RT) of all recorded attentive tasks. The significance of Mutual Information for each value of RT was calculated. Red dots indicate that MI is significant for RT ranging between 0.5 and 0.7 (animal 1) and between 0.3 and 0.5s (animal 2).

### 4.4.2. Attention during an interval discrimination task induces lower firing variability in auditory cortex

Enhanced responses to the relevant stimuli have been observed during auditory attentive tasks (Blake et al. 2002; Fritz et al. 2005; Blake et al. 2006; Atiani et al. 2009) as well as decreased ones under sustained attention (Otazu et al. 2009) or even both (Beitel et al. 2003). In this study we explored whether attention alters neuronal response variability in the auditory cortex of the behaving animal. We calculated the Fano Factor (FF) in order to test how neuronal variability changes as a function of the behavioural state of the animal.

Figure 31 shows, for two single units (A,B and C,D), the FF variation along with the trial duration for short (A,C) and long (B,D) ISI and for the attentive state (red) *versus* passive states (blue). A reduction in the FF during the attentive task can be observed during stimulus presentation for short (Fig. 31A) (mean: 0,475) and long (Fig. 31B) (mean: 0,466) ISI with respect to the passive ones in short (mean: 0,696) and long (mean: 0,690) ISI. Moreover, we also found during the ISI a reduced variability in the attentive task for short (mean:0,727) and long (mean: 0,743) ISIs with respect to the passive state (mean: 0,829; mean: 0,874). A similar trend is illustrated in other neuron which shows in the attentive state a reduction in FF during the response onset (Fig. 31C: mean:0,870; Fig. 31D: mean: 0,857) with respect to the passive state (mean: 0,954; mean: 0,960.). The same pattern was also found at the offset response component (see Fig. 31C,D). Importantly, we also found that during the spontaneous activity period (-0.2-0) there was a decreased variability in the attentive (Fig. 31A: mean: 0,839; Fig. 31B:mean: 0,866) with respect to the passive state of the animal (Fig. 31A: mean:0,927; Fig. 31B: mean: 0,934). In all, the reduction of variability during evoked and spontaneous activity (-0.2-0) was also observed in other 12 neurons out of 14 in which it was calculated. Attention also decreased variability in spontaneous activity during interstimulus intervals (n=8). Moreover, the neurons that showed significant information content mostly

overlapped with those neurons that showed a reduced variability and sustained modulation of firing patterns (4.4.3).

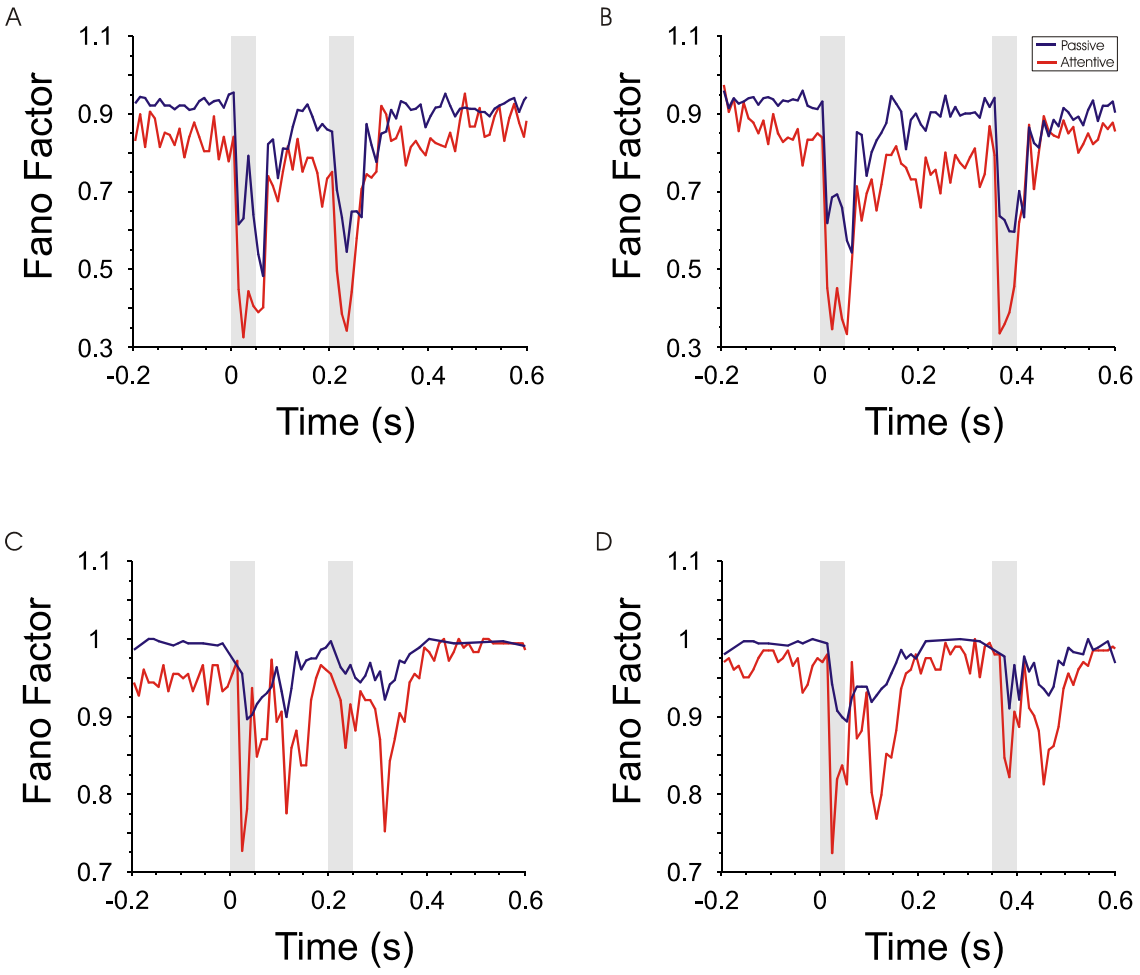



**Figure 31. Response variability is reduced under attentive states. A,B.** Fano Factor of an example neuron is shown for short (A) and long (B) ISI and for the attended (red) versus passive stages (blue). Grey bars indicate the presentation of S1 and S2. A reduction in the FF during the attentive task can be observed during stimulus presentation for short (A;mean: 0,475) and long (B;mean: 0,466) ISI with respect to the passive ones in short (mean: 0,696) and long (mean: 0,69) ISI. During the ISI there is a reduced variability in the attentive task for short (mean:0,727) and long (mean: 0,743) ISIs with respect to the passive state (mean: 0,829; mean: 0,874). During the spontaneous activity period (-0.2-0) there was a decreased variability in the attentive (A;mean: 0,839; B;mean: 0,866) with respect to the passive state of the animal (A;mean:0,927; B;mean: 0,934). **C,D.** Same as in A,B for another example neuron with an onset-offset response pattern. In the attentive state there is a reduction in FF during the onset (C;mean:0,870; D; mean: 0,857) and offset (C;mean: 0,897; D;mean: 0,935) with respect to the passive state in the onset (mean: 0,954; mean: 0,960.) and offset (mean: 0,961; mean: 0,960) response of that neuron.

It has been shown by other authors that FF is not contingent on the firing rate (Churchland et al. 2010; McAdams and Maunsell 1999; Kara et al. 2000; Mitchell et al.

2007). We also tested this, and for that we matched bins with very similar firing rate (<5% difference) between the attentive and passive recording stages. Then we plotted (Fig. 32) for each matched bin the FF value in the passive versus the attentive state for each neuron with significant FF (n=14). It is shown in Fig. 32 that most of the values remain above the bisecting line, which indicates that FF values are bigger in the passive state than in the attentive. We also found that the mean of positive values of the difference between FF-passive and FF-attentive was 0.0346 while the mean of negative values was 0.0042. This result shows that the Fano Factor is 8.2 times bigger in the passive recordings with respect to the attentive task, and that a decrease in FF during attention is not a mere artefact of an increase in firing rate.

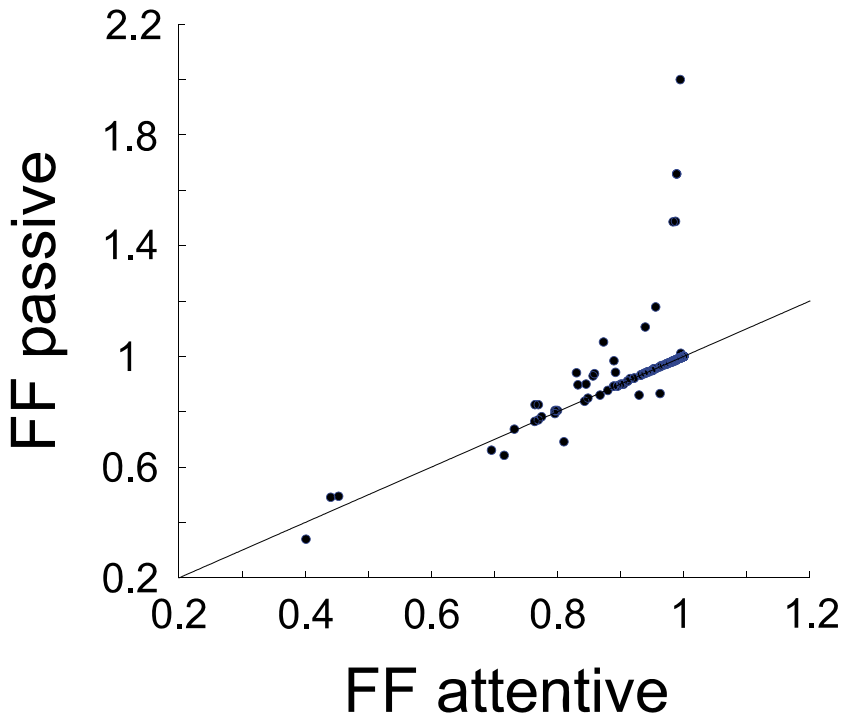



**Figure 32 – Fano Factor is not dependent on the firing rate.** The Fano Factor value of the passive recordings (Y axis) is plotted against the Fano Factor value of the attentive ones (X axis). The Fano Factor value was calculated only for each pair of bins with equal spike count (<5%) between the passive and attentive state. Only neurons with significant Fano Factor were included (n=14). Most of the values remain above the bisecting line. The mean of positive values of the difference between FF passive and FF attentive is 0.0346 while the mean of negative values is 0.0042.

### 4.4.3. Slow modulation of evoked and spontaneous activity varies with the ISI category and the attentional state of the animal

Slow modulation of firing rate in the auditory cortex of the behaving monkey has been previously shown to be related with the processing of stimuli, motor decision or even reward aspects of the task (Selezneva et al. 2006; Brosch et al. 2010). PSTH analysis of single neuron discharges allowed us to observe slow modulations of firing rate. This is illustrated in Fig. 33A, which shows the responses in the attentive task for short (left) and long (right) ISIs. The response to S1 is absent while following it there is an increase in firing rate during the spontaneous activity until the presentation of S2 which is followed by inhibition and a rebound of excitatory activity. Moreover, we compared the firing pattern between the attentive task and the passive recording stages. It is shown in the initial passive stage (Fig. 33B) an absence of response along the trial duration. The same pattern was also present in 14 neurons. Therefore, 17.4% of the neurons showed whether an increase or higher spontaneous sustained activity during the ISI that terminated when S2 began. A similar pattern is shown in Fig. 34.

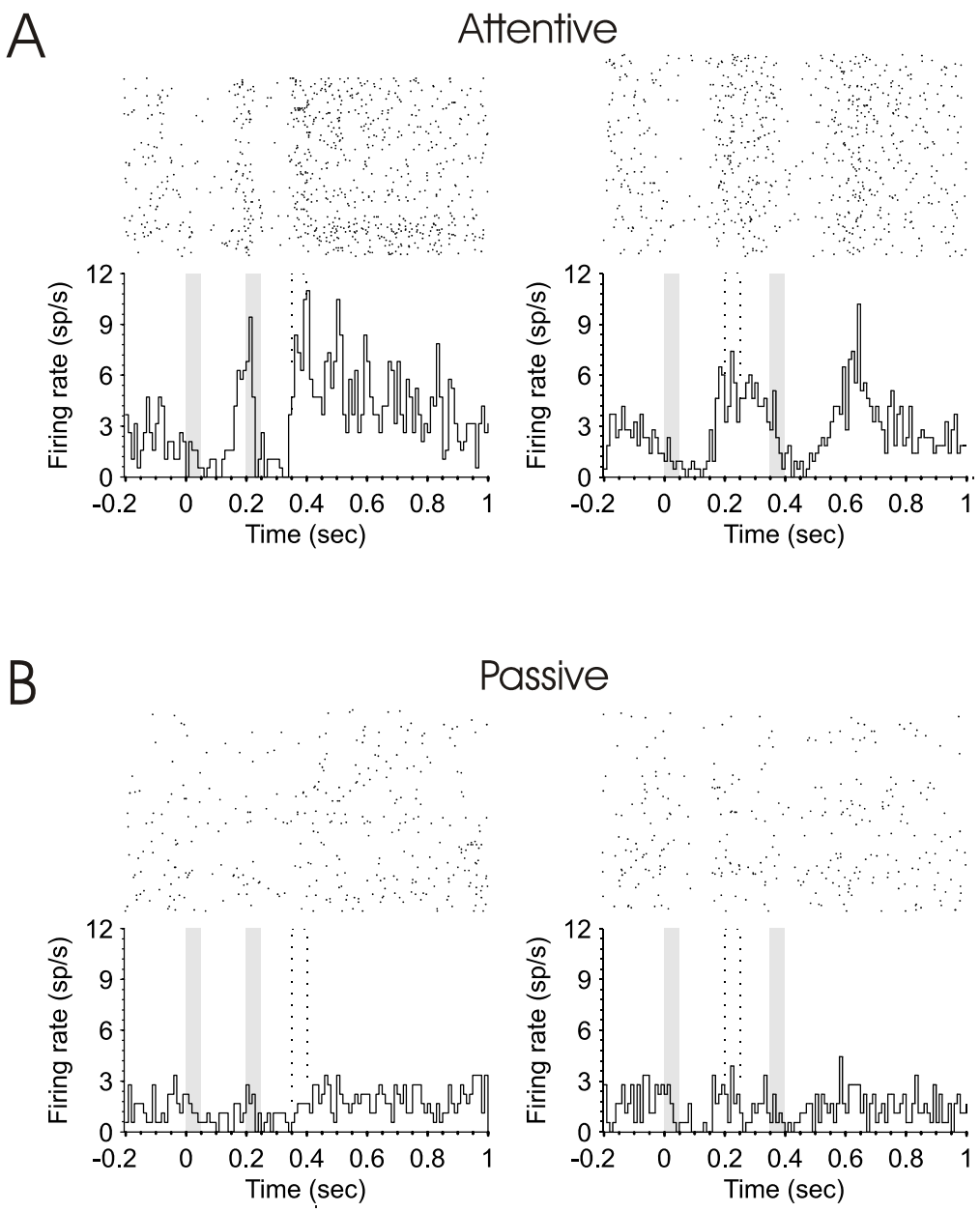



**Figure 33. Sustained firing of single units in auditory cortex code for temporal category information. A.** PSTH (180 trials) of a single neuron during the attentive task shows the response pattern to two identical stimuli (50ms; 80dB; 5322Hz) separated by 150 ms (left) and 300 ms (right). Grey bars indicate stimuli presentation and dotted bars indicate the location of S2 if the opposite ISI would have occurred. PSTHs show no response during S1 but a progressive increase of firing rate until S2 presentation. An abrupt response decrease is observed after S2 presentation. **B.** The same neuron in the initial passive stage shows an absence of response under the same stimuli conditions.

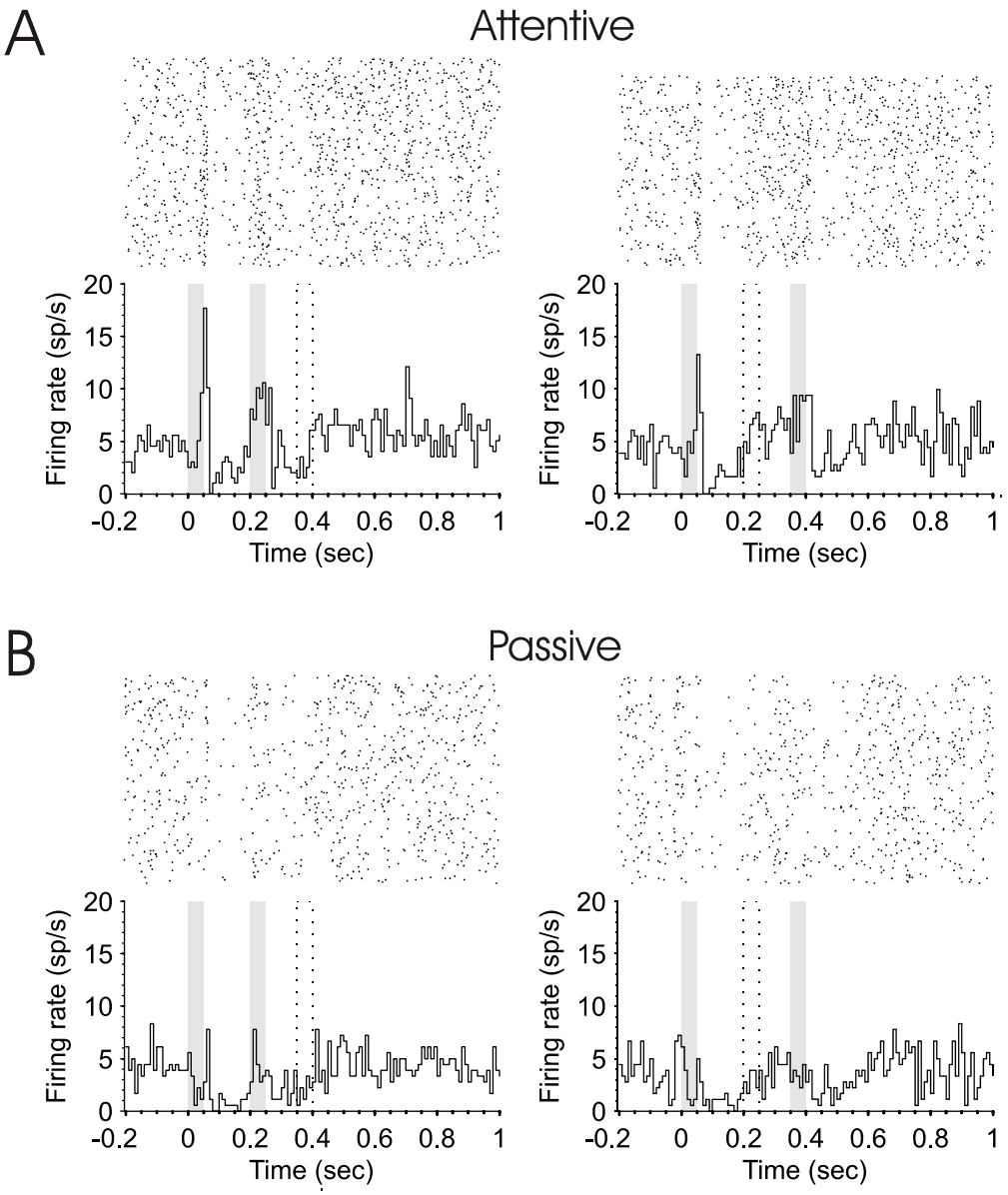



**Figure 34. Sustained firing of single units in auditory cortex code for temporal category information. A.** PSTH (180 trials) of a single neuron during the attentive task shows the response pattern to two identical stimuli (50ms; 80dB; 5322Hz) separated by 150 ms (left) and 300 ms (right). PSTH shows an offset response to S1 and onset response during S2. **B.** No identifiable response pattern can be observed during the initial passive recording stage under the same stimuli conditions.

Additionally, we illustrate (Fig. 35) another type of slow modulation in firing pattern at the offset of stimuli presentation. During the attentive state, we found 5 neurons (5.8%) that showed an enhanced sustained offset response. However, this was not the case in the passive state. Hence, this sustained post-stimulus response duration may help to track the timing between stimuli presentations.

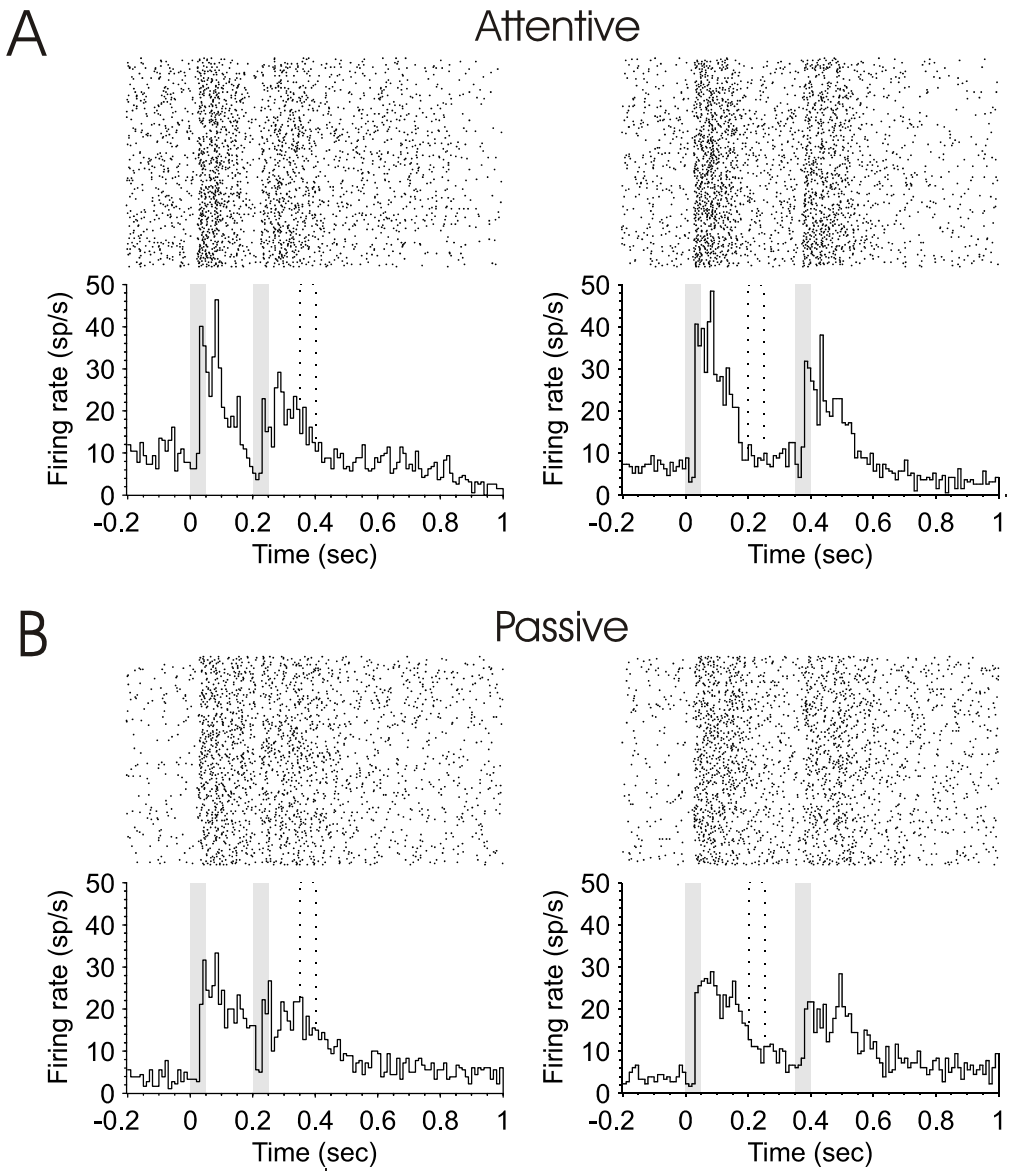



**Figure 35. Sustained firing of single units in auditory cortex code for temporal category information. A.** PSTH of a single neuron during the attentive task shows the response pattern to 180 trials of two identical stimuli (50ms; 80dB; 5322Hz) separated by 150 ms (left) and 300 ms (right). PSTHs shows an enhanced sustained offset response. **B.** The same neuron with equal stimuli conditions in the initial passive stage shows less firing rate after stimulus presentation.

Interestingly, some neurons (n=4) showed an increased firing to evoked activity (Fig. 36) and an average firing rate during interstimulus interval (long ISI:29.8 Hz) significantly higher than that preceding the first stimulus (long ISI:-0.2 to 0 s; 16.8 Hz). This is noticeable both in the PSTH and in the raster plots. The same

was the cases for the short intervals, where the firing rate during the ISI was 28 Hz versus the 15 Hz of spontaneous activity preceding stimulation. Furthermore, this increased firing rate during the ISI only occurred during attention and not during passive recording periods, where there is a generalized decrease of excitability during both the evoked and spontaneous activity. This is an indication that attention modulates not only evoked activity but also the spontaneous.

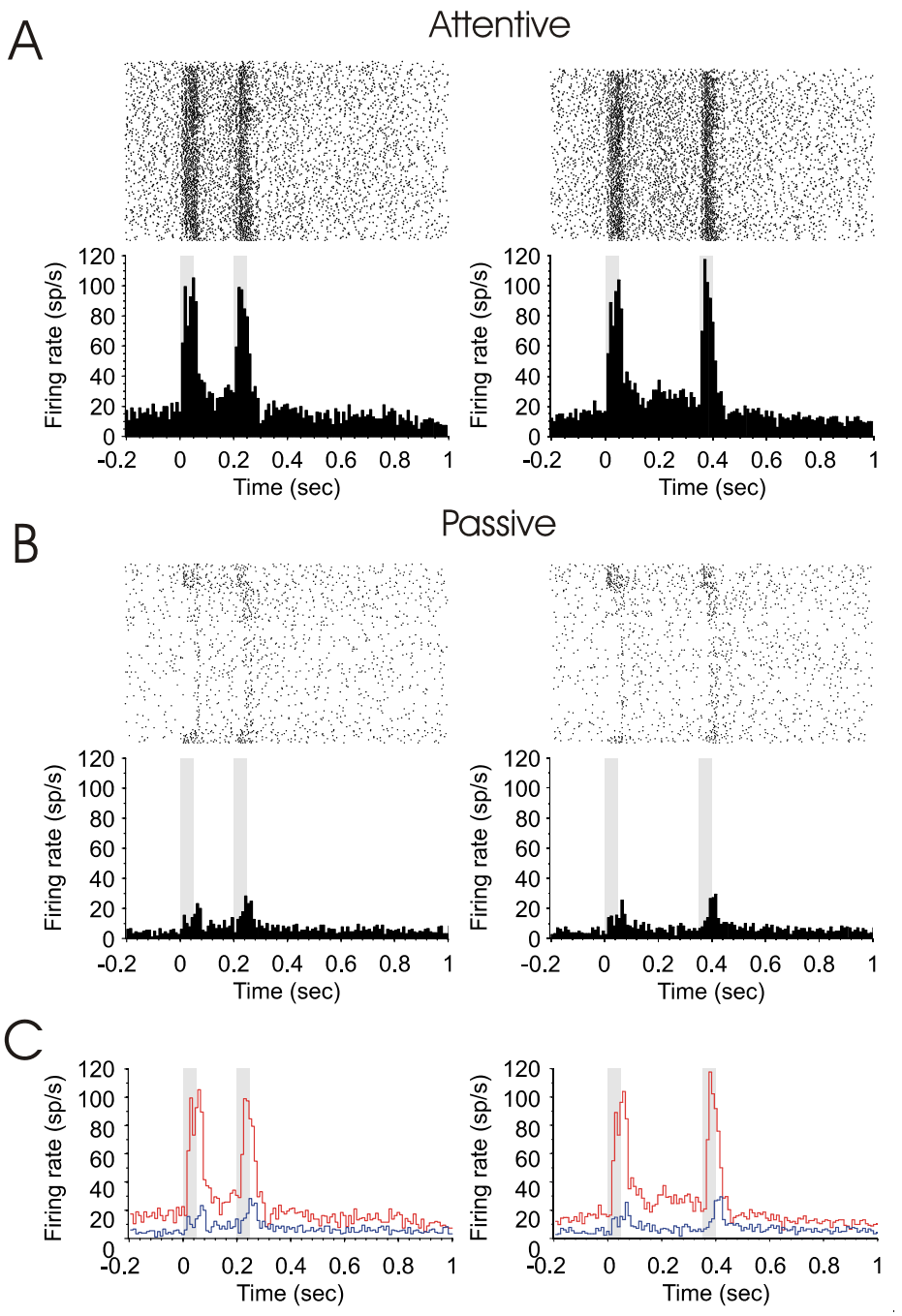



**Figure 36. Enhancement of spontaneous and evoked activity during attention. A.** (same neuron as in Fig. 27A) PSTH (180 trials) of a single neuron during the attentive task shows the response pattern to two identical stimuli (50ms; 80dB; 5322Hz) separated by 150 ms (left) and 300 ms (right). PSTH shows a tonic response and offset response to S1 and S2. **B.** Evoked activity in the passive listening animal is significantly decreased as well as the spontaneous before, between and after stimuli presentation. **C.** Overlaid histograms from A and B are plotted in order to show the difference in their evoked and spontaneous activity. Average spontaneous activity (-0.2 to 0) in attentive (A) is 15.07Hz and 16.79Hz for short and long ISI, respectively, while in passive (B) average spontaneous activity is 4.68Hz and 4.49Hz, respectively. The average spontaneous activity during the ISI in the attentive task (A) is 28HZ and 29.8Hz for short and long ISI, respectively, while in the passive (B) the average is 10.88Hz and 8.12Hz, respectively.

Finally, we report (Fig. 37) a firing pattern that we found in 3 neurons where a significant enhancement of spontaneous activity occurred previous to S1 presentation (-0.2 to 0). In that case, the spontaneous activity was around 20 times higher in the attentive task (9.52 and 12.34 for short and long ISI, respectively) than in the passive (0.44 and 0.58 for short and long ISI, respectively). This increased spontaneous excitability did not occur though in the passive state (Fig. 37B). Interestingly, we also found 2 other neurons that showed a specific decrease in the attentive task previous to S1 presentation (data not shown). Therefore, it is possible that phasic increased or decreased excitability would serve to improve sensory encoding of relevant stimuli under attentional demands.

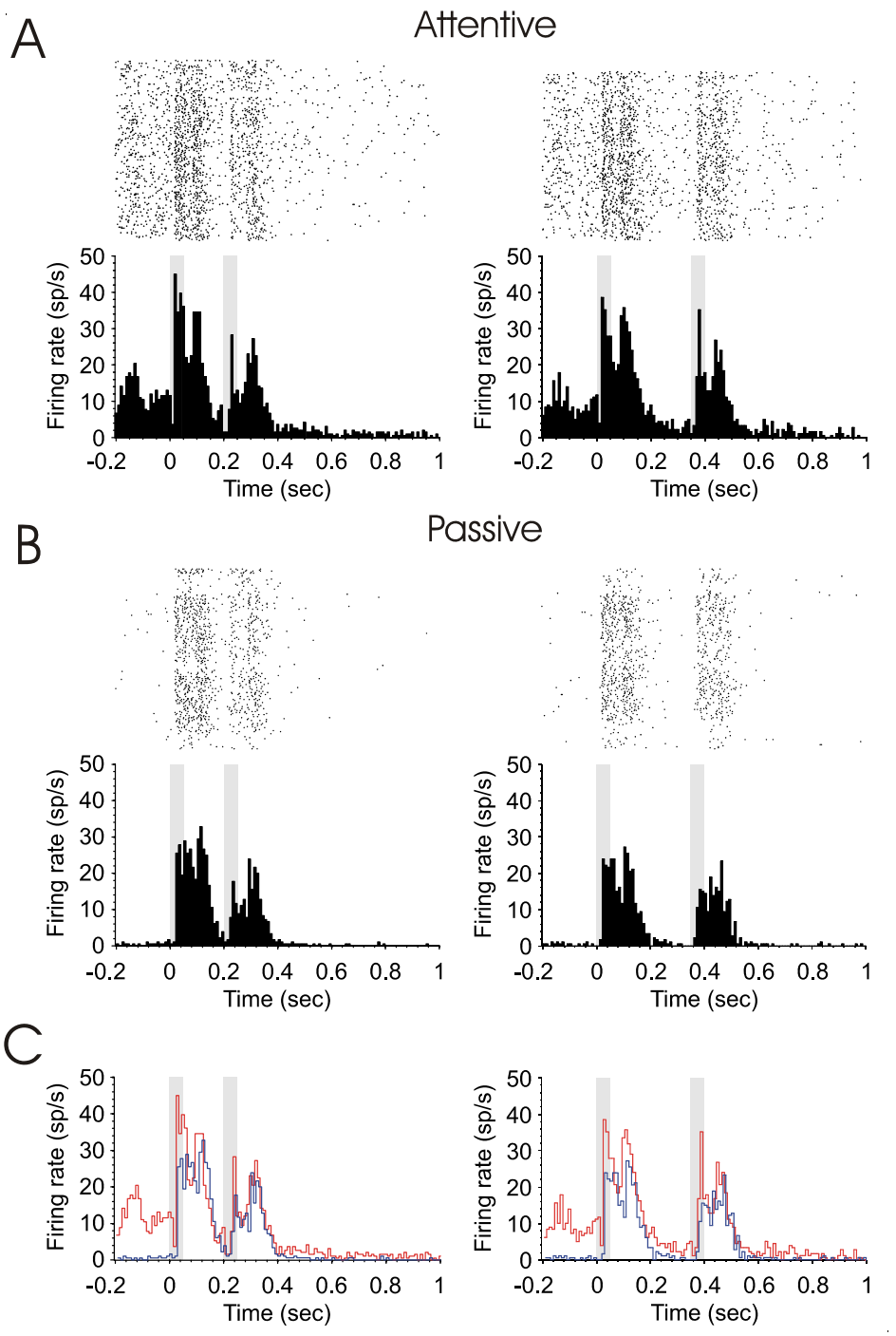



**Figure 37. Enhancement of spontaneous activity during attention. A.** (same neuron as in Fig. 27B) PSTH (180 trials) of a single neuron during the attentive task shows the response pattern to two identical stimuli (50ms; 80dB; 5322Hz) separated by 150 ms (left) and 300 ms (right). PSTH shows a tonic response and a enhanced offset response to S1 and S2. **B.** Spontaneous activity before stimulus presentation (-0.2 to 0) is significantly decreased in the passive with respect to the attentive state of the animal. **C.** Overlaid histograms from A and B are plotted in order to show the difference in their spontaneous activity. Average spontaneous activity (-0.2 to 0) in attentive (A) is 9.52Hz and 12.34Hz for short and long ISI, respectively, while in passive (B) average spontaneous activity is 0.44Hz and 0.58Hz, respectively.

# 5. DISCUSSION

## 5.1. Timescale of auditory adaptation in the awake passive listening animal

The results from epigraph 4.1., revealed auditory adaptation using pairs of auditory stimuli in single neurons in the awake freely moving rats. Heterogeneity across cells with respect to the degree of adaptation and its time course was found, the time courses ranging from hundreds of milliseconds to several seconds. The motivation to characterize auditory adaptation in the awake rat was to obtain data on adaptation in a wide timescale and in freely moving animals, given that most of the previous studies were done in anesthetized preparations and shorter timescales.

### 5.1.1. Auditory adaptation in the anesthetized and the awake preparations

Studies in anesthetized animals have been relevant for our current understanding of auditory adaptation. However, anesthesia induces changes in neuronal excitability, facilitates oscillatory activity, modifies spontaneous activity, or increases neuronal frequency tuning sharpness (Gaese and Ostwald 2001; Rojas et al. 2006; Koranda et al. 2008; Simon et al. 2010). The effects vary with different anesthesias, affecting phenomena such as sensory adaptation (Wehr and Zador 2005; Moshitch et al. 2006; Rennaker et al. 2007). Wehr and Zador (2005) showed that pentobarbital anesthesia in the rat prolonged the very slow component of adaptation (several hundreds of ms in that case), while ketamine has been reported to reduce the maximum rate of responses to repeated clicks (Rennaker et al. 2007). Cortical auditory adaptation in anesthetized cats has been described for different anesthesias and protocols displaying a variety of time courses including 50-1600 ms (Hocherman

and Gilat 1981), or up to 400 ms (Calford and Semple 1995; Brosch and Schreiner 1997), and even reaching tens of seconds (Ulanovsky et al. 2004; Pienkowski and Eggermont 2009). In all, awake animal preparations bypass the problems associated with anesthesia.

### 5.1.2. The influence of the previous history of stimulation on subsequent responses

We have found that the impact of a 50 ms sound on subsequent responses to an identical stimulus persists for several seconds in the awake rat. This effect was normally expressed through attenuation of the response to the second stimulus. Larger responses induced by the first stimulus due to longer or more intense stimuli provoke a larger attenuation of R2. Similarly, previous studies found adaptation in the responses to the second sound in a two-sound sequence for intervals up to 1–2 s in the anesthetized cat (Hocherman and Gilat 1981; Calford and Semple 1995; Brosch and Schreiner 1997; Reale and Brugge 2000) or up to 300 ms in the awake cat (Fitzpatrick et al. 1999). Neuronal adaptation in the passive listening primate was found to last no more than 2.2 s (Bartlett and Wang 2005), while in the primate performing a spatial location task has been reported to last up to 5 s (Werner-Reiss et al. 2006). This increased duration of adaptation in the attentive animal with respect to the passive seems counterintuitive and it could be explained as a result of the different set of stimuli used on each study. Interestingly, other studies (Bartlett and Wang 2005) found that S2 was not affected by the length of the interstimulus interval. The authors argue that S2 response amplitude is regulated namely by the context, i.e. the previous features of stimuli presentation like frequency of stimuli and the duration or intensity of S1.

The fact that cortical adaptation also exists in the awake restrained rat was also observed by (Anderson et al. 2006). Using a different conceptual framework, different stimulation paradigms (broadband 80μs clicks) and a different recording

technique (multiunit recordings), Anderson et al (2006) focused on the detection of the maximum stimulus rate that auditory cortex responses can track (synchronization boundary). In spite of these differences some of the reported findings in Anderson et al. are in agreement with those presented here, specifically the decrease in mean response rate and duration with the stimulation rate, as well as the description of post-adaptation. On the other hand, opposite to what we found, the authors observed that the time-to-peak response decreased with an increase in the presentation rate, although the response onset measurement probably differs from ours. Additionally, the intertrial interval in their experiments is of 1 second while ours varies between 10 to 15 seconds, although stimuli duration is shorter in the study of Anderson. Similarly, (Kilgard and Merzenich 1999) recorded from different sites and stimuli repetition rate was presented in order to check for the temporal response properties of neurons. The obtained optimal response rate was quite variable, varying from 5 stimuli per second to 15.

Previous experiments have shown the pattern of adaptation by means of pure tones in the anesthetized animals (Brosch and Schreiner 1997, 2000; Ulanovsky et al. 2004; Pienkowski and Eggermont 2009; von der Behrens et al. 2009). In the awake rats, adaptation has been observed following stimulation with pure tones, although only short term adaptation was studied. The issue of how the time course of adaptation to pure tones is in the awake animal is an interesting issue that can be the subject of future studies.

Our results show that 40% of the neurons showed no change in adaptation when the intensity of S1 increased or even showed less adaptation for higher than for lower intensities of S1 (10%). Auditory cortical neurons have been shown to be even more suppressed under low decibels stimuli than under high ones in the anesthesized rat (Scholl et al. 2008). These results resemble the well described phenomena of Mismatch Negativity, previously described (Naatanen and Alho 1995; Escera et al.

1998; Escera et al. 2001; Armstrong et al. 2002; Escera et al. 2002; Naatanen et al. 2004; Tubau et al. 2007; Dominguez-Borras et al. 2009) in humans by means of Electroencephalogram recordings, and it is defined as the difference between the Event Related Potential for the deviant stimulus and the standard one. This phenomena has been interpreted as a preatentional state since it shows an increased readiness to respond. Therefore, our results could be interpreted in terms of an increased readiness of the neuron to respond to intensity transients (Kilgard and Merzenich 1999) and therefore an increase in the probability of stimulus perception. In this line, some authors (DeWeese and Zador 1998) have suggested that adaptation requires an estimate of the present statistics of the stimuli being presented with respect to the recent past, therefore, abrupt increases in variance would be more readily detectable than decreases. A similar interpretation has been suggested in the case of barrel cortex (Diaz-Quesada and Maravall 2008).

Another interesting study that recorded in the auditory cortex of the pentobarbital anesthetized cats showed that approximately 90% of the neurons responded more strongly to a tone presented in a sequence than to a single one (Brosch and Schreiner 2000). Our results and some other studies (Bartlett and Wang 2005) have shown that a percentage of neurons show no change or even stronger responses as a result of a decrease in ISI duration or increase in previous stimulus intensity or duration. Therefore it is possible that adaptation and facilitation occur, and even in the same neuron depending on the previous history of stimulation. Presynaptic facilitation could be an important mechanism, in which cellular signals evoked by a first stimulus augment the signals evoked by consecutive ones. From the functional implications point of view, the response enhancement of stimuli within a sequence contributes to a robust cortical representation of the temporal structure of sound sequences and auditory streams that are quite natural in our environment such as speech or animal vocalizations. It is important to notice, though, that SOA

constitutes an important variable that could be enhancing stimulus detection. The lack of a systematic relationship between the increase of sound level of S1 and its effect on S2 has been interpreted as a suppressed synaptic input to the recorded cell, presynaptic depression of neurotransmitter release, shift in the balance of excitation and inhibition or even the interplay of different mechanisms at a time (Bartlett and Wang 2005).

### 5.1.3. Cellular and network mechanisms of cortical auditory adaptation

Different mechanisms that could underlie cortical auditory adaptation have been proposed. Additionally, given the existence of different cell classes (Nowak et al. 2003), adaptation has been suggested to be influenced by the cell type (Hildebrandt et al. 2009). Therefore, the heterogeneity of neuronal classes recorded in the awake animal could also influence the differences with respect to in vitro, though. Hence, further studies should elucidate the different adaptation patterns and timescales of each cell class.

Adaptation occurring at lower levels, in the inferior colliculus (Malmierca et al. 2009) or the auditory thalamus (Anderson et al. 2009; Antunes et al. 2009), could contribute to what is observed in the cortex. Synaptic mechanisms such as synaptic depression at the cortical as well as the thalamus level could also influence the neuronal excitability to repeated stimuli presentation (Castro-Alamancos 2002; Katz et al. 2006; Oswald et al. 2006). Still, adaptation appears to be larger in cortical layers that do not receive subcortical input (Szymanski et al. 2009a), suggesting that part of it is being generated through the cortical circuitry.

Synaptic mechanisms have been attributed an important role in auditory cortex. In this respect, synaptic depression, both thalamocortical and intracortical, has been proposed as a suitable stimulus-specific mechanism since it can be associated to specific inputs to the neuron (Ulanovsky et al. 2004; Percaccio et al.

2005) and it can provide a variety of time scales (Varela et al. 1997; Carandini et al. 2002). Still, synaptic depression in active networks is less than in silent networks (Boudreau and Ferster 2005), showing an inverse relationship with the occurrence of ongoing activity in the cortical network (Reig et al. 2006). Although presynaptic inhibition has been attributed a major role in the so called auditory adaptation phenomena of forward inhibition (Brosch and Schreiner 1997), it does not account for the slow component of auditory adaptation, since previous studies have reported adaptation lasting up to several seconds(Ulanovsky et al. 2003; Ulanovsky et al. 2004). Alternatively, some authors have suggested the importance of both, mechanisms operating at the output of the neuron (intrinsic properties) and at the input (synaptic properties) (Eggermont 1999; Kilgard and Merzenich 1999).

Inhibition could also play a role in auditory adaptation, a mechanism that would provide stimulus specificity (Zhang et al. 2003), as proposed by (Eytan et al. 2003) based on *ex vivo* networks. Balanced inhibition might increase temporal precision of auditory stimuli, by inhibiting responses within a stimuli sequence (Wehr and Zador 2003). Intracellular recordings in the cortex of the anesthetized rat revealed that inhibition plays a role in the adaptation during the first 50-100 ms after the stimulus (Wehr and Zador 2005). Evoked activity recorded by means of whole-cell (Hromadka et al. 2008) have shown that the shortest interspike interval was of less than 10 ms, which potentially would discard the possible influence of intrinsic mechanisms as in favour of circuit mechanisms (DeWeese et al. 2003) playing a role in adaptation, where the neuron can receive input from others nearby. However this does not explain the adaptation of slower time course spanning from 100 ms to tens of seconds, for which $GABA_B$ receptors proposed by some authors (Buonomano and Merzenich 1998) but were found not to be involved by others (Wehr and Zador 2005). Afterhyperpolarizations following auditory responses were described already in the first intracellular recordings from this region (De

Ribaupierre et al. 1972a), being mostly attributed to IPSPs (De Ribaupierre et al. 1972a; Tan et al. 2004; Wehr and Zador 2005). We suggest that at least part of these AHPs may be due to the activation of potassium currents.

### 5.1.4. Intrinsic mechanisms and cortical adaptation

Most of the studies dealing with adaptation or forward masking/suppression have ruled out intrinsic mechanisms as a possible underpinning mechanism in the auditory system. However, hyperpolarizing membrane currents have been found to play an important role in sensory adaptation in other sensory systems (Sanchez-Vives et al. 2000a, b; Diaz-Quesada and Maravall 2008; Kuznetsova et al. 2008). The lack of dependence on the input is the reason why ionic currents have been largely ruled out as a suitable player in auditory adaptation, which is stimulus-frequency specific (Ulanovsky et al. 2003; Ulanovsky et al. 2004; Wehr and Zador 2005).

So, how could $K^+$ currents support frequency-specific adaptation? First, not all auditory adaptation is stimulus-specific (Bartlett and Wang 2005). Second, in auditory cortex different frequencies follow a tonotopic map (Doron et al. 2002) and usually neurons in the same electrode track share the same preferred stimulation frequency (Read et al. 2002). Adaptation is very similar in each particular cortical column, with no differentiation according to layers (Ulanovsky et al. 2004 but see Szymanski et al. 2009) but across columns. In a highly interconnected network such as a cortical column, the activation of hyperpolarizing currents in even a small percentage of neurons reverberates in the local circuits, inducing modulation of activity in the whole circuit (Compte et al. 2003). Given the predominance of vertical *versus* horizontal connectivity, adaptation would not necessarily propagate to adjacent areas of different preferred frequency. In this way intrinsically mediated adaptation could also become stimulus-specific.

A role for potassium currents in adaptation is compatible with the participation of other mechanisms with which intrinsic properties would interact. Hence, the combination of spike frequency adaptation with synaptic depression in the network allows the computation of the rate of change of the stimulus, and even stimulus anticipation (Puccini et al. 2007), and a higher detectability of unexpected stimulus (Puccini et al. 2006), a property described in the auditory cortex (Ulanovsky et al. 2003).

A similar time course of adaptation was found in comparison between in vitro and in awake responses but the attenuation of the responses was larger in the awake animal. One possible factor contributing to this difference is the heterogeneity of neuronal classes recorded in the awake animal while in vitro only regular spiking neurons were included. Intrinsically bursting neurons recorded in vitro were not included given the difficulty to measure adaptation in a way comparable with that in regular spiking neurons. In any event, cortical intrinsically bursting neurons in rat sensorimotor cortex have been described to have as well sodium-dependent potassium currents contributing to their AHPs (Franceschetti et al. 2003). Ongoing firing in the awake animal is probably responsible at least in part for the lesser adaptation observed in vitro than in the awake animal. Adaptation in vitro increased when neurons were not silent, but awake-like background firing was artificially induced. Still, adaptation in vitro remained less than in the awake animal. These findings suggest that intrinsic properties could underlie at least part of the sound adaptation existing in awake animals for intervals between 50 ms and 2 s, while leaving room for additional mechanisms. Other adaptation features found in the awake state were also observed in slices. These were the dependence of response (R2) on the duration and intensity of the first stimulus and the increased delay of adapted responses.

Potassium currents have been thoroughly studied in vitro, however, their role in the awake, functioning brain is not well known. Studies of ionic currents require the use of techniques that are not fully viable in the awake freely moving animal, where only relatively short intracellular recordings are possible (Lee et al. 2006). The study of ionic channels in vitro is based on the idea that those currents must exist in the brain tissue in situ. However, it is difficult to extrapolate the knowledge obtained from in vitro, or even from the anesthetized preparations, to the awake and functioning brain. Here, we have concentrated on the activation of potassium currents by spikes. But it should be taken into account that in vivo, in addition to the changes in conductance and membrane potential induced by potassium channels activation, there are conductance and membrane potential changes due to excitatory and inhibitory inputs to neurons. The last ones were not mimicked in our in vitro simulations of in vivo discharge patterns.

The ionic currents that we describe here in A1 slices must exist in the brain of the awake rat, given that both species and brain area were the same. Furthermore, the slices studied here were obtained from adult animals and recorded at 34-35 Cº. Ongoing spontaneous activity was generally absent in our slices while we report an average of 5 Hz firing rate in awake animals. Ongoing activity in the awake may induce $K^+$ currents activation even in the absence of stimulation, thus neurons could have a basal “preadaptation”. We show that even if a neuron in vitro is tonically firing at a similar rate to spontaneous activity in vivo an AHP and decreased excitability follow a spike discharge. And indeed, we found that neurons with spontaneous activity display larger adaptation than silent ones. Still, ionic currents may be up or down regulated in the awake brain. On the one hand, norepinephrine blocks both $Ca2^+$ and $Na^+$-dependent $K^+$ currents (Foehring et al. 1989), while acetylcholine does the same on the $Na^+$-dependent $K^+$ current (Schwindt et al. 1989) hence they neuromodulate depending on the brain state. $K^+$ currents in vivo could on

the other hand be amplified in the network by reverberation in cortical circuits (Lorente de Nó 1949). In an active interconnected network, the decreased firing induced by $K^+$ currents is transmitted to connected neurons as decreased synaptic activity. The observation that $Na^+$ entering the cells through AMPA receptors is enough to activate $Ca2^+$ and $Na^+$-dependent $K^+$ current (Nanou et al. 2008) would contribute to the even further amplification of its effect at the network level.

## 5.2. Spontaneous activity in the auditory cortex of the awake animal

Previously, we have shown that spontaneous activity can be altered as a result of adaptation (postadaptation) but also it depends on the brain state of the animal where changes in firing rate and reduced variability during spontaneous activity could enhance stimuli perception. With respect to the functionality of spontaneous activity in auditory cortex, some authors suggested that the spontaneous neuronal firing gates temporally locked responses to auditory stimuli, being, therefore, a mechanism for rapid and reversible modulation of cortical processing (Loebel et al. 2007). Moreover, spontaneous activity has been suggested to follow a sequential structure that is similar to the evoked by auditory stimulation (Luczak et al. 2009; Sakata and Harris 2009; Harris et al. 2010). Similarly, spontaneous activity in the awake state could have a structure that resembles the Up and Down states reported in the anesthetized animal or in slices (Sanchez-Vives and McCormick 2000; Sanchez-Vives et al. 2000a). Periods of increased activity and no activity could be present in the form of a particular pattern with Up states duration ranging between tens to hundred of milliseconds (DeWeese and Zador 2006; Luczak et al. 2009; Sakata and Harris 2009). Similarly, (DeWeese and Zador 2006) suggested that the Up states or bumps of population activity are generated by a large synchronized volley of action potentials of the presynaptic activity.

Different mechanisms have been suggested to this patterned activity like cognitive factors, and sensory influence of ascending pathways, although a major role have been attributed to the local cortical circuit (Luczak et al. 2009).

On the contrary, it could be the case that spontaneous activity lacks any structure. In our recordings of the awake animal we observed in Fig. 17 and in the autocorrelograms (figure not shown) that single units had no sequential structure as shown by (Luczak et al. 2009; Sakata and Harris 2009; Harris et al. 2010), possibly because we could not determine precisely the layer in which our recordings were performed which may be determinant (Sakata and Harris 2009). Further, our small number of samples (n=13) does not allow to draw conclusions on the spontaneous activity patterns of single units in auditory cortex. Moreover, cell type classification would be useful in order to differentiate spontaneous activity patterns.

Furthermore, we report (Fig. 17) that closely located recorded neurons exhibit very different patterns of interspike intervals. We also observed in several recordings that nearby neurons recorded within the same tetrode show very different degrees of response amplitude. Accordingly, (Rothschild et al. 2010) recently reported by means of calcium imaging that local populations of neurons tend to show higher heterogeneous firing to tones in the mouse auditory cortex than previously thought. Of particular relevance is the functional organization and dynamics of groups of neurons given that auditory cortex is suggested to show a rather sparse neuronal activity (Hromadka et al. 2008).

Further studies on the same single unit activity recorded in the awake and asleep state could provide interesting information on the patterns of spontaneous activity and its functionality. So far, any study have systematically reported how the activity of the same isolated single units changes depending on the brain state, i.e. awake versus REM or the later versus slow wave sleep. Only a single study reports that the activity recorded of the same single neuron during awake and slow wave

sleep presents an average of spontaneous activity of 2 and 0.5 Hz, respectively (Manunta and Edeline 1999).

## 5.3. Sustained neuronal firing in the auditory cortex of the passive listening and attentive animal

Some authors suggested that, in the anesthesized animal, sustained responses are responsible for temporal analysis of auditory stimuli and would code static auditory signals (Chimoto et al. 2002; Qin et al. 2003; Sakai et al. 2009b). Therefore, while tonic cells would process static spectral acoustic information, phasic cells would encode very rapid spectral cues.

Different, methods have been suggested to classify tonic versus phasic neurons. Although we used (epigraph 4.3.) a similar method to classify tonic and phasic neurons as to (Recanzone 2000; Chimoto et al. 2002) we did not find consistent differences in the response latency between phasic and tonic neurons, as previous authors did (Chimoto et al. 2002; Qin et al. 2003), possibly because of the different set of stimuli used. Additionally, similar proportions of tonic firing neurons have been reported in cats (28.5%)(Chimoto et al. 2002) and in monkey (29%)(Pfingst and O'Connor 1981); (30%) (Recanzone 2000). Some other authors (Lu et al. 2001; Qin et al. 2003) found even higher proportion sustained responsive neurons (59% and 89% respectively). It has also been suggested that phasic and tonic cells are not a clear cut division but a continuum (Recanzone 2000). Interestingly, within the neurons that we classified as phasic (78.57%) we found that several of them showed some tonic pattern. Moreover, it could be the case that the response pattern is influenced by stimulus intensity, or even by the attentional demands of the task performed by the monkey as in (Recanzone 2000), e.g. suppressive firing during stimulus presentation could occur once the animal detected the spatial location of the sound. Moreover, in the awake animal, neurotransmitters levels change during different brain states, like

arousal or attention, which would have a potential influence on the neuronal response pattern to long duration stimuli (Recanzone 2000).

The stimulus statistics of the stimuli ensemble could also determine the sustained firing (Ulanovsky et al. 2003; Maravall et al. 2007). Therefore, sustained stimulation could also induce a decrease in the sustained firing of auditory neurons, since sustained responses adapt in terms with the statistics of the stimuli ensemble (Ulanovsky et al. 2003). Additionally, some authors found that in the awake marmoset the neurons showing a preferred responsive frequency enhanced their sustained firing along with stimulus duration (Wang et al. 2005; Wang 2007). The same author suggests that temporal and rate neuronal coding provides the neuronal basis for fast and slow occurring auditory events, respectively (Lu et al. 2001). Accordingly, the stimuli we used in our study shown in Fig. 18B is coded in terms of oscillatory activity during the slow sustained stimulation. This pattern or sequential structure resembles the one reported by (Luczak et al. 2009; Sakata and Harris 2009; Harris et al. 2010) in the auditory cortex. It could be also the case that a interplay of short-term synaptic depression and spike frequency adaptation have an influence on this oscillatory neuronal behaviour during sustained activity (Puccini et al. 2006).

The influence of behaviourally relevant sustained stimulation on neuronal responses has been neglected so far. In the recordings that we obtained from the protocol shown in Fig. 19 we found that between the onset and offset of stimulus presentation some neurons showed whether an increased or decreased response. Enhanced inhibition or excitation at the onset and offset of stimulus presentation was also observed. These two response components at the onset/offset and between those may enhance the discrimination of stimulus duration. Accordingly, (Bartho et al. 2009) reported in the anesthetized animal significant onset responses of single units and population activity, while significantly reduced activity along stimulus duration (1 second) occurred around baseline activity. This difference of neuronal

activity between the onset and along stimulus duration may relate with the fact that recordings were conducted under anesthesia but also because sustained stimuli are usually irrelevant in the natural world. In contrast, in our study sustained stimulation was relevant and may explain the different response patterns of neuronal activity described in order to maintain the representation of the stimuli.

Classical studies of neuronal plasticity in auditory cortex have reported shifts in the preferred frequency of the neuron towards the relevant target stimuli in the context of associative learning paradigms (Weinberger 2004, 2007), while inhibition have never been attributed a major role, though. We found 4 neurons that showed a marked response decrease at the onset and offset of stimulus presentation and we suggest that this inhibition occurs as a result of a learning process. In this respect, increased inhibition has been recently attributed a major role in neuronal plasticity (Fritz et al. 2003; Galindo-Leon et al. 2009; Nelken 2009), such that increased inhibition may shape cortical responses to relevant stimuli. Increased inhibition away from the relevant stimuli may be of particular relevance, although we found increased inhibition at the key time windows of relevant stimuli during task performance (i.e. onset and offset).

## 5.4. Neuronal codes for temporal discrimination in the auditory cortex of the attentive animal

We wanted to explore within the auditory cortex the single cell basis of temporal categorization during decision making. We recorded from 86 neurons of the auditory cortex in the awake freely moving rat during behaviour and passive brain states (epigraph 4.4.). First, our results demonstrate that information content is higher to the relevant stimulus during task performance than in the passive state. Second, our results also evidence that a reduction in neuronal variability can account for the processing of relevant stimuli under attentive states with respect to passive listening. Finally, we report that evoked and spontaneous neuronal activity can be

slowly modulated depending on the attentional demands. In all, our results support the conclusion that the neuronal codes shown in the present study may be important to guide the decision making based on temporal aspects of auditory stimuli.

### 5.4.1. Information content in single units of auditory cortex

Quantification of information content in spike patterns has provided important insights in the understanding of key features of sensory processing (Imaizumi et al. 2010; DeWeese et al. 2003; Lu and Wang 2004; Nelken et al. 2005; Chechik et al. 2006; Nelken and Chechik 2007; Kayser et al. 2009). Previous studies (Imaizumi et al. 2010) have suggested that information content in multiunit activity of auditory cortex is higher in the interspike interval, as compared to the firing rate or event-locked spikes (Furukawa and Middlebrooks 2002), when repetitive stimulation is presented to the anesthesized animal. Similarly, (Kayser et al. 2009) quantified the information present in temporal spike patterns and the phase of population firing, suggesting that these combine information for encoding natural sounds in auditory cortex. Therefore, the combination of different neuronal codes could provide the most amount of information about auditory stimuli, being also robust to noise. In a further study, the same author reports the mutual information between the stimulus and neuronal response, quantifying the amount of information carried out within precise spike timing (few milliseconds) to the presentation of extended complex sounds (Kayser et al. 2010). The author shows that stimulus information is dependent on the spiking precision which enhances the encoding of information.

Other authors have also quantified the information present in spike count and spike timing, suggesting that in the auditory cortex the information can be represented by spike timing in case of sparse acoustic events, while firing rate-based representations encode rapidly occurring acoustic events (Lu and Wang 2004).

Moreover, intrinsic network rhythms could also encode stimuli information where the population oscillatory activity could provide a phase reference (Lisman 2005).

Interestingly, in the study by (Lemus et al. 2009) it was suggested that the auditory cortex may serve to encode information of sensory stimuli instead of having a cognitive function related with decision making or memory. In the task the monkey compared whether two auditory stimuli rates were different or equal. It was reported that stimulus-locked responses, and mostly firing rate, correlated with performance only during stimulus presentation while not in the delay periods as it would be if related to working memory or decision making. In all, the authors suggest that encoding of information, mostly by means of firing rate, occurs only during stimuli presentation. Our analysis shows that MI is particularly enhanced in the attentive task to the second stimulus with respect to the first. Our analysis of MI was performed though only during stimuli presentation and not in other time windows of the trial given that MI estimate could be affected by the low spontaneous neuronal firing. Further, we suggest that the increased amount of information during the relevant stimuli in the attentive task with respect to the passive one evidences the role of auditory cortex in temporal discrimination during a decision making task.

Despite these various studies, there is no evidence on the information content present during temporal discrimination in the auditory cortex of the awake animal. We show, by means of mutual information analysis, that information content is maximal during the attentive state when the target stimuli is presented (S2) with respect to the passive state of the animal. Hence, the enhancement of information content in the responses of single units in auditory cortex could contribute to an increase in the discrimination performance of the temporal category of auditory stimuli.

Alternatively, despite of the fact that MI could be potentially affected by neuronal adaptation in the auditory cortex (Lu and Wang 2004) other authors have

suggested (Maravall et al. 2007) that adaptation to stimulus statistics in the barrel cortex maintains and enhances information transmission and stimuli discriminability. Although the functional implications of neuronal adaptation and its underlying mechanisms remain unknown, adaptation could directly influence perceptual decisions (Maravall et al. 2007). Similarly, in the study of (Liu et al. 2010) where cats performed a "go" response to the deviant interstimulus interval, it was shown that the time course of neuronal adaptation strongly correlated with interval discrimination performance, where the perception of the auditory interval was explained by the degree of adapted response to the second stimulus. In all, our information-theoretic analysis suggests that adaptation per se constitutes the mechanism that augments information content related with the temporal category.

### 5.4.2. Firing variability of single units in auditory cortex

Sensory processing during attention has been linked to enhanced responses (Blake et al. 2002; Fritz et al. 2005; Blake et al. 2006; Atiani et al. 2009) or even decreased ones (Otazu et al. 2009). Increased inhibition has been also suggested to play an important role in the cortical responses to relevant stimuli (Galindo-Leon et al. 2009; Nelken 2009). Attention also induces tonotopic changes were an increased number of neurons respond to the target stimulus (Recanzone et al. 1993; Rutkowski and Weinberger 2005; Polley et al. 2006; Schreiner and Winer 2007; Hui et al. 2009; Bieszczad and Weinberger 2010) and tuning shifts of the same neurons towards the target stimulus (Brown et al. 2004). However, no study has tackled the issue of how attention affects response variability of single units in the auditory cortex of the behaving animal.

Earlier studies have suggested that a decline in response variability is a widespread phenomenon in the cortex that spans different areas, animal species and that always occurs to the onset of stimuli presented, irrespective of the brain state

of the animal (Churchland et al. 2010; Shadlen and Newsome 1998; Sussillo and Abbott 2009). Even more, the neuronal variability over trials declines in particular in situations where the encoded information serves to guide behaviour. This has been experimentally demonstrated in the recorded neurons of visual area V4 by Cohen and Maunsell (2009) and Mitchell et al. (2009) in the context of an attentional paradigm. These authors have shown that the mean-normalized variance (Fano Factor) of the spiking activity is reduced by attention, consequently, increasing the sensitivity of the neurons towards relevant aspects of stimuli. It remains unknown whether a decline in variability depends on the stimulus effectively driving the population to respond or on single units responsiveness. Neuronal response variability may also depend on the type of neuron, i.e. narrow of broad spiking (Mitchell et al. 2007). Moreover, stimulus-induced and state-dependent activity pattern of trial-to-trial variability may be explained by the same dynamics of ongoing spontaneous activity (Curto et al. 2009). Our results suggest that the external stimulation or the behavioural requirements of the task stabilize the dynamics in a controlled way such that the trial-to-trial neuronal variability is reduced. In this way, the signal-to-noise ratio is increased, yielding the basis for an improved encoding of the stimulus information.

The possible dependence of FF on firing rate deserves to be considered. It has been suggested that a reduction in neuronal response variability could be correlated with an increase in firing rate (Churchland et al. 2010; McAdams and Maunsell 1999; Kara et al. 2000; Mitchell et al. 2007). Some studies have tried to disentangle this issue and have shown that decreased variability is not due to an increase in firing rate (Churchland et al. 2010). Additionally, we have shown that in those bins where firing rate was equal between the passive and attentive states, variability was markedly reduced in the later. This is also consistent with the result reported by Mitchell 2007 (see Fig. S4) who matches those bins with similar firing rate between

the attended and unattended brain state, showing the presence of lower variability when equal firing rate exists in bin pairs.

### 5.4.3. Slow modulation of evoked and spontaneous activity during attention

Temporal response properties of auditory single neurons can be altered by attention (Fritz et al. 2003; Schnupp et al. 2006; Liu et al. 2010). Some other studies have focused on the response features to time-varying stimuli (Kilgard and Merzenich 1998; Lu et al. 2001). Indeed, auditory cortex is an ideal candidate for temporal information processing and modulations in the firing of single units of auditory cortex have been implicated in temporal expectation (Shinba et al. 1995; Coull 2009), memory (Sakurai 1994; Weinberger 2004), trial events association (Brosch et al. 2010; Quirk et al. 1997; Selezneva et al. 2006) or even the type of behavioural response (Villa et al. 1999). Such firing patterns may also increase the reliability of neuronal responses (Mitchell et al. 2007).

Importantly, most of the studies have shown modulation of phasic responses to code for relevant stimuli (Bao et al. 2004; Polley et al. 2006; Fritz et al. 2007; Lemus et al. 2009)). In this respect, (Selezneva et al. 2006) showed that phasic responsive neurons showed preferred firing to target stimuli and not to non-target ones. Importantly, sustained responses were reported to be decreased after presentation of target stimuli. These results were suggested to reflect the neuronal mechanisms underlying categorization of frequency steps. Importantly, the slow or sustained (up to several seconds) response pattern was analyzed in a further study and it was suggested (Brosch et al. 2010) to be a complement for the representation of timing of relevant events (e.g. stimuli, reinforcer or a particular behaviour) during the trial. A similar pattern was found in (Gottlieb et al. 1989). Therefore, sustained firing could provide a neuronal mechanism for anticipation and memory, reflecting a learning process where consecutive sensory and behavioural events are associated

with reinforcement. In all, slow modulation of firing could complement the representation of the timing of auditory stimuli as well as the codification of stimuli by means of phasic responses (Brosch et al. 2010). We suggest that the sustained firing that we report (epigraph 4.4.7.) after stimulus presentation is a result of learnt expectancy which increased the probability of detecting the relevant stimulus (S2). Additionally, we found neurons (data not shown) that showed progressive increase of firing rate after S2 presentation, which may reflect an association with the reward or motor activity. Further studies on information content during periods of modulation of spontaneous activity could provide additional information on the functional basis of such modulation. Together with the present results we found (data not shown) that some neurons markedly reduced their activity only during stimuli presentation. This inhibition may be a consequence of a learning process and may be important to code for relevant stimuli of the task at hand (Fritz et al. 2003; Weinberger 2004; Nelken 2009).

Some other studies showed that categorization of upward *versus* downward frequency modulated tones was reflected by changes in cortical spatial activity patterns (Ohl et al. 2001). Similarly, previous studies have shown persistent topographic changes induced by training (Ohl et al. 2001; Weinberger 2004; Polley et al. 2006). We believe that the training sessions might have induced topographic changes that lead to our current findings.

In all, given that the time course of slow modulation of firing was dependent on the behavioural state and the appearance of the relevant stimulus (S2) we suggest that this type of response pattern can have a predictive component on the appearance of S2 and may reflect the neuronal basis of category based decision making by means of tonic firing.

# 6. CONCLUSIONS

1. The adaptation of neuronal responses in the awake animal depends on the preceding history of stimulation. We conclude that the shorter the preceding interval between stimuli the more decrease occurs in response amplitude and the longer is the response delay to subsequent responses.

2. Longer preceding stimulus duration induces more neuronal adaptation on subsequent responses in the awake animal. Long auditory stimulation lowers the response amplitude and increases the response delay to subsequent responses.

3. Higher preceding stimulus intensity induces more neuronal adaptation on subsequent responses in the awake animal. High stimulus intensity lowers the response amplitude and increases the response delay to subsequent responses.

4. Postadaptation in the awake animal occurs more frequently following the second sound than the first one.

5. Potassium channels play an important role in fast and slow timescales of neuronal adaptation.

6. Neuronal adaptation occurs in the awake animal during long lasting stimulation. When sustained stimulation is presented there are mainly two neuronal classes: phasic responses which respond only at the beginning of stimulus presentation, and tonic which respond along stimulus duration.

7. Attentional demands during the discrimination of long-lasting stimuli duration modulate neuronal response to the onset and offset of the stimuli.

8. The information content of neuronal responses in auditory cortex during a temporal discrimination task is higher during attention to target stimuli than in the passive state of the animal.

9. Neuronal response variability preceding and during target stimuli is lower in attention than in the passive state of the animal.

10. Slow modulation of evoked and spontaneous activity occurs in the attentive state probably having a role in order to discriminate temporal information.

11. Intrinsic mechanisms play an important role in auditory adaptation. Moreover, processing in early auditory cortex is modulated by attention in different forms and the exact mechanisms underlying this phenomenon should be investigated in the future.

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# APPENDIX

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# FEATURE ARTICLE
# Cortical Auditory Adaptation in the Awake Rat and the Role of Potassium Currents

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**Responses to sound in the auditory cortex are influenced by the preceding history of firing. We studied the time course of auditory adaptation in primary auditory cortex (A1) from awake, freely moving rats. Two identical stimuli were delivered with different intervals ranging from 50 ms to 8 s. Single neuron recordings in the awake animal revealed that the response to a sound is influenced by sounds delivered even several seconds earlier, the second one usually yielding a weaker response. To understand the role of neuronal intrinsic properties in this phenomenon, we obtained intracellular recordings from rat A1 neurons in vitro and mimicked the same protocols of adaptation carried out in awake animals by means of depolarizing pulses of identical duration and intervals. The intensity of the pulses was adjusted such that the first pulse would evoke a similar number of spikes as its equivalent in vivo. A1 neurons in vitro adapted with a similar time course but less than in awake animals. At least two potassium currents participated in the in vitro adaptation: a $Na^+$-dependent $K^+$ current and an apamin-sensitive $K^+$ current. Our results suggest that potassium currents underlie at least part of cortical auditory adaptation during the awake state.**

**Keywords:** auditory circuits, auditory cortex, auditory processing, cortical slices, forward masking, forward suppression, intrinsic properties, in vitro cortex, potassium channels, sensory adaptation

## Introduction

Adaptation to repetitive stimulation is a common feature in sensory systems. Adaptation to known stimuli increases sensitivity toward new ones, acting as a gain control mechanism and influencing stimuli perception. Responses to sound in A1 are known to be affected by the preceding history of stimulation. Stimulus-specific adaptation has been described in the auditory cortex of the anesthetized animal (Brosch and Schreiner 1997; Ulanovsky et al. 2003, 2004; Pienkowski and Eggermont 2009). There the term "specific" is used in the sense that the cortex stops responding to repeated stimuli but remains responsive to rare infrequent sounds. However, not all adaptation is specific to the preceding stimulus (Bartlett and Wang 2005).

Although adaptation was believed to be mostly cortical, it has been recently shown that auditory adaptation also exists in the inferior colliculus (Perez-Gonzalez et al. 2005) and in the auditory thalamus (Anderson et al. 2009; Malmierca et al. 2009). Some of the adaptation observed in the cortex may be transmitted from lower nuclei, and conversely, part of the adaptation observed in those areas may be of descending cortical origin.

A number of different mechanisms that could underlie cortical adaptation (hereby considered as the decreased response amplitude to a second with respect to a first stimulus) have been previously considered: synaptic depression (Wehr and Zador 2005), decreased excitation or lateral inhibition (Qin and Sato 2004), increased inhibition (Zhang et al. 2003), or excitatory–inhibitory imbalance (De Ribaupierre et al. 1972a; Volkov and Galazjuk 1991; Ojima and Murakami 2002; Oswald et al. 2006). Each of these mechanisms involves a network of interneuronal connections. Until now, the role of ionic currents on auditory adaptation has been largely ignored. One of the reasons is that the currents that usually underlie adaptation processes, namely $K^+$ currents, have not been characterized in the auditory cortex. Potassium currents have been thoroughly studied in other cortical areas, their activation is often activity-dependent, and they can maintain neurons hyperpolarized ranging from tens of milliseconds to tens of seconds (for reviews, see Schwindt, Spain, Foehring, Stafstrom, et al. 1988; Sah 1996; Vergara et al. 1998; Bhattacharjee and Kaczmarek 2005). Potassium channels play a role in sensory adaptation in other sensory areas (Schwindt, Spain, Foehring, Chubb, and Crill 1988; Sanchez-Vives et al. 2000a; Diaz-Quesada and Maravall 2008; Kuznetsova et al. 2008). Depolarization and high frequency firing during sensory responses induce an intracellular increase of ions such as $Ca^{2+}$ or $Na^+$ that activate ion-dependent $K^+$ channels and also membrane depolarization can directly activate voltage-dependent $K^+$ channels. The activation of potassium currents hyperpolarizes the membrane potential and decreases neuronal responsiveness to subsequent inputs. This can happen even in the absence of spikes, via ions entering the cell through α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors (Nanou et al. 2008).

The above-mentioned studies and some other recent ones (Hildebrandt et al. 2009; Sakai et al. 2009; Szymanski et al. 2009; von der Behrens et al. 2009) have improved insight into the cortical versus subcortical nature of neuronal adaptation, the dependence of neural adaptation on anesthesia, and differences on adaptation between animal species, sound stimulation, and cortical layers. The contribution of intrinsic mechanisms to auditory cortical adaptation remains, however, an open question. In this study, we aimed at exploring the time course of auditory adaptation at the single unit level in the primary auditory cortex (A1) of the freely moving rat. Additionally, we were interested in finding out to what extent auditory adaptation could be supported by the activation of

hyperpolarizing currents. To accomplish this, we mimicked the protocols performed in the awake animals in rat A1 in vitro.

## Materials and Methods

### *Recordings from Chronically Implanted Freely Moving Animals*

Recordings were obtained from 6 Lister Hooded rats (300–450 g) chronically implanted with tetrodes in the primary auditory cortex (Doron et al. 2002).

*Surgical Procedure*

Anesthesia was induced using intraperitoneal injections of ketamine (80 mg/kg) and xylazine (10 mg/kg). The animal was then mounted in a stereotaxic apparatus, and the skull was exposed. During the surgery, the animal was deeply anesthetized using a mixture of isoflurane (0.5–1.2%) and oxygen (1.5 L/min). A trephine was used to make a 3-mm diameter craniotomy over somatosensory cortex (S2) between −3.6 and −5.2 mm A-P and 6.6–7 mm M-L (Paxinos and Watson 1998). These coordinates were used in order to position the microdrive dorsally, which made it more stable than entering laterally right over A1. Body temperature was monitored through a rectal thermometer and maintained (36–38°) by means of an electric blanket. Heart rate and blood oxygen levels were monitored. Reflexes were regularly checked during surgery to assure deep anesthesia. Other drugs were given during surgery and the recovery period in order to prevent infection, inflammation, and for analgesia: antibiotics (enrofloxacin; 10 mg/kg; subcutaneous [s.c.]) and topical application of neomycin and bacitracin in powder (Cicatrin), analgesic (buprenorphine; 0.05 mg/kg; s.c.), anti-inflamatory (methylprednisolone; 1 mg/kg; intraperitoneally) and atropine (0.05 mg/kg, s.c.) to prevent secretions during surgery. Rats were cared for and treated in accordance with the Spanish regulatory laws (Boletin Oficial del Estado 256; 25 October 1990), which comply with the European Union guidelines on protection of vertebrates used for experimentation (Strasbourg; 18 March 1986).

*Tetrodes and Microdrives*

Each tetrode was made from 4 twisted strands of HM-L-coated 90% platinum-10% iridium wire of 17 and 25 μm diameters (California Fine Wire). If the wire used was 17 μm diameter, gold plating decreased their impedance to ~300 KΩ. Four tetrodes were held by a cannula that was attached to a microdrive supplied by Axona Ltd. This microdrive allowed for dorsal to ventral movement of the tetrodes to search for new units. Microdrives were attached to the skull with dental cement and 7 stainless steel screws. The auditory cortex was reached by vertical descent, and the tetrodes were lowered 100 μm during the surgery. Vertical descent performed after surgery was of 50–75 μm per day until an auditory response was observed. All the recordings included in this study corresponded to A1 (Doron et al. 2002). This estimation is based on the depth of the included recordings and on the anatomical reconstructions of the electrode track. The auditory latencies were 10–20 ms, which are as well characteristic of A1 (Ojima and Murakami 2002; Malmierca 2003; Nelken et al. 2003).

*Electrophysiological Recordings*

Animals lived in large cages of 70 × 45 × 31 cm (Rody Cavia; SAVIC) in a rich environment, under a 12:12 h light:dark cycle, water, and food ad libitum. After a week of postoperative recovery period, animals were habituated to the recording chamber. The electrode wires were AC-coupled to unity gain buffer amplifiers. Lightweight hearing aid wires (2–3 m) connected these to a preamplifier (gain of 1000) and then to the filters and amplifiers of the recording system (Axona). Signals were amplified (×15 000–40 000), high-pass filtered (360 Hz), and acquired using software from Axona Ltd. Each channel was continuously monitored at a sampling rate of 48 kHz, and action potentials were stored as 48 points per channel (1 ms; 200 μs prethreshold; 800 μs postthreshold) whenever the signal from any of the prespecified recording channels exceeded a given threshold set by the experimenter for subsequent off-line spike sorting (OFS) analysis. Before every experimental session, tetrodes were screened for neuronal activity. Once spikes could be well isolated from background noise the experimental protocol started.

*Experimental Set Up*

The loudspeaker was located 53 cm above the bottom of the recording chamber. The box was built in black acrylic and had a surface of 22 by 27 cm and the walls had a height of 65 cm. The walls of the box were covered with corrugated cardboard (4 mm thickness) for sound-resonance suppression. Measurements were taken of the sound from different points of the recording chamber, and no differences in the intensity of the sound were detected. During the recordings, the rat was freely moving within the limited space of the chamber. Recordings were obtained in darkness, and the experiment was filmed with an infrared camera placed above the recording chamber.

*Presentation of Sound Stimuli*

Protocols of stimulation were controlled through Matlab and a National Instrument card and breakout box (FS 300 kHz). Sound triggers had microsecond precision. A WAV-file containing white noise was generated. We first observed the effect of the interstimulus interval on subsequent responses. The sound stimuli had a duration of 50 ms and an intensity of 80–90 dB, being identical for both the first and second stimulus. Pairs of same intensity white noise stimuli were separated by different interstimulus intervals randomly presented and ranging between 50 ms and 8 s (generally 0.05, 0.1, 0.3, 0.5, 0.75, 2, 5, and 8 s). Intertrial intervals varied randomly between 8 and 15 s before the beginning of a new trial. In order to obtain reliable values in the adaptation curve, one complete session of 50–100 trials was carried out for each of the intervals. Animals were continuously recorded for sessions of up to 2 h. The time course of adaptation recovery was fitted by a logistic function. For display purposes, we use a logarithmic scale that expands the early part of the recovery time course.

Once the dependence of auditory responses with respect to the time elapsed since the previous stimuli was studied, we explored to what extent auditory responses varied depending on the intensity or the duration of the preceding stimuli. With that purpose, durations and intensities of the first stimulus were randomly varied between 0.05 and 0.7 s and 70 to 103 dB, respectively.

*Spike Analysis*

Cluster cutting (isolating single units from the multiunit recording data) was performed using an OFS (Plexon) (Supplementary Fig. S1). Waveforms were first sorted into units by using the valley-seeking algorithm (Koontz and Fukunaga 1972). Waveforms were considered to have been generated by a single neuron when they occurred simultaneously in the 4 electrodes that defined a discrete cluster in 3D principal component (or peak to peak) space distinct from clusters for other units using a multivariate analysis of variance test. Single units exhibited a recognizable refractory period (>1 ms) in their ISI histograms and had a characteristic and distinct waveform shape and peak-to-peak amplitude when compared with other spikes. Single-unit spike analysis consisted of peristimulus histograms (PSTH) and rasters around the time of occurrence of the sensory stimulation.

### *Recordings from In Vitro Cortical Slices from Rat Auditory Cortex*

*Slice Preparation*

The methods for preparing cortical slices were similar to those described previously (Descalzo et al. 2005). Briefly, rat cortical slices were prepared from 250 g rats that were deeply anesthetized with sodium pentobarbital (40 mg/kg) and decapitated. Four hundred-micrometer-thick coronal slices of the auditory cortex (−3.5 to 5.5 mm) (Paxinos and Watson 1998) were obtained. A modification of the technique developed by Aghajanian and Rasmussen (1989) was used to increase tissue viability. After preparation, slices were placed in an interface-style recording chamber (Fine Sciences Tools) and bathed in artificial cerebrospinal fluid containing (in mM): NaCl, 124; KCl, 2.5; $MgSO_4$, 2; $NaHPO_4$, 1.25; $CaCl_2$, 2; $NaHCO_3$, 26; and dextrose, 10 and were aerated with 95% $O_2$, 5% $CO_2$ to a final pH of 7.4. Bath temperature was maintained at 34–35 °C.

*Recordings and Stimulation*

Sharp intracellular recording electrodes were formed on a Sutter Instruments P-97 micropipette puller from medium-walled glass and beveled to final resistances of 50–100 MΩ. Micropipettes were filled with 2 M potassium acetate. Recordings were digitized, acquired, and analyzed using a data acquisition interface and software from Cambridge Electronic Design. In order to induce a discharge pattern similar to that evoked in the awake animal by auditory stimulation, depolarizing current pulses of the same duration (50 ms) were given. The intensity of the current injection (0.4–1 nA) was adjusted in order to obtain a spike discharge similar to that evoked in the awake animal with 50 ms of white noise and 90 dB, a train of 3–5 action potentials. Pairs of stimuli were separated by intervals between 40 ms and 5 s.

*Analysis of Spike Frequency Adaptation*

In the awake preparation, a Peristimulus histograms (PSTH) and rasters were generated and the peak value of the response was taken for a 10 ms bin. This method could not be applied in vitro because, given the absence of synaptic noise, the spikes evoked by the same pulse always occurred at the same time. The method then used was to measure the exact time that pulses 1 and 2 took to generate the same number of spikes, most often 2–3 spikes. The measure was similar to the one used to calculate frequency in McCormick et al. (1985). Given that both measures (in vivo / in vitro) refer to frequency (Spikes/s) and both are relative responses (Response2/Response1), they can be compared. In the intracellular recordings, R2/R1 was (Spikes/time R2) / (Spikes/time R1). Since the number of spikes considered was the same (see above) Time 1 / Time 2 was measured. In the extracellular recordings R2/R1 was also (Spikes/time R2) / (Spikes/time R1). Here the time was the same (10 ms per bin) and thus adaptation was measured as Spikes R2 / Spikes R1.

Intrinsically bursty neurons (8 of the in vitro sample) were not included in the analysis of adaptation because the method to calculate their adaptation should have been different and thus not comparable with the rest of the sample. We opted for a more homogeneous sample that only included regular spiking neurons.

## Results

Recordings from 76 isolated neurons were obtained in the primary auditory cortex of awake freely moving rats. Among these, detectable auditory responses were evoked in 54 neurons that are included in the study. Similar proportions of responsive neurons have been found by other authors (50% in Hromadka et al. 2008). In addition, recordings from 98 neurons in rat primary auditory cortex in vitro were also included for the second part of the study.

### *Single Neuron Recordings in A1 from Freely Moving Rats*

Single units were identified by means of tetrode recording and subsequent cluster cutting from the freely moving rat (see Materials and Methods). We were concerned to ascertain that the animal was awake during the whole protocol. Experiments were carried out in the dark, where rats have been reported to be awake during 54–66% of the time, around 5% in rapid eye moment (REM) sleep and the rest in non-REM sleep (Bertorelli et al. 1996; Stephenson et al. 2009). Those studies refer to a quiet environment and without sound stimulation, which was not the case here. Indeed, sound has been used before in protocols of sleep deprivation (Franken et al. 1995). In addition, we carried out analysis on the local field potential recordings obtained along with the single units. The analysis followed that described in (Gervasoni et al. 2004). In all analyzed cases ($n = 17$) periods of low frequencies (see Fig. 1*B* in Gervasoni et al. 2004) were rarely observed, while those of theta, normally associated to movement, predominated.

Under these conditions, the mean spontaneous activity was 5.07 ± 3.25 Hz (mean ± standard deviation), ranging between 0.6 and 16 Hz. The distribution of values is illustrated in Supplementary Figure S2A. Mean and standard deviation were similar to reported data from cell-attached recordings in head-fixed animals (Hromadka et al. 2008). The spontaneous frequency was calculated by averaging the firing rate value of each 10 ms bin during a 300 ms time window preceding auditory stimuli (between 50 and 100 trials, 15–30 s intertrial interval). The peak frequency (2 ms bins) of the subsequently evoked auditory responses (50 ms, 80–90 dB) was also measured. A significant positive relationship was found between the spontaneous frequency and the maximum frequency evoked by the stimuli (Supplementary Fig. S2B–E).

*Interstimulus Interval and Adaptation*

Auditory responses to pairs of 50 ms sounds (white noise) separated by interstimulus intervals spanning between 50 ms and 8 s (from the end of stimulus 1 (S1) to beginning of stimulus 2 (S2); see Materials and Methods) were tested in a total of 30 neurons. After averaging the response to 50–100 trials of stimulation for each interval, we observed that most neurons (83.3%) showed some degree of adaptation in the form of a decreased response to the second identical sound, the adaptation being larger for shorter intervals. The remaining neurons showed either no difference between the two responses (13.3%) or an increased response of the second response with respect to the first (3.4%). Figure 1 illustrates the peristimulus time histogram (PSTH) of the response (spike/s) to 50 trials (bottom) and raster plots (top) in each panel. The responses correspond to the intervals 100, 300, 500, and 2000 ms for one neuron (Fig. 1*A–D*). The response to the second stimulus (R2) was in all cases smaller than the response to the first one (R1). The time course of adaptation is represented for this particular case (Fig. 1*E*). For a 50 ms interstimulus interval, the R2 was 0.35 the peak amplitude of R1. When the interval was increased to 1 s, R2 was on the average 0.80 of R1. In this neuron, a total recovery of the amplitude of R2 with respect to R1 was not completely achieved until an interval of 3 s had elapsed.

The average adaptation time course for 30 neurons is represented in Figure 1*F*. In these cases, for a 1-s interval between the 2 auditory stimuli, R2 had approximately 0.80 the amplitude of R1 and was completely recovered for intervals between 2 and 5 s. We conclude that stimuli occurring 1 or more seconds earlier have an influence on the amplitude of subsequent responses.

*Duration of the First Stimulus and Adaptation*

In 21 neurons recorded in A1 of the awake rat, the duration of the first auditory stimulus was randomly varied (50, 300, 500, and 700 ms), while the duration of the second stimulus (50 ms) was maintained (Fig. 2*A–C*). The objective was to determine to what extent the adaptation of R2 was contingent on the duration of R1. This was tested for intervals of 300, 400, 500, 750 and 1500 ms between R1 and R2. In 62% of the observations we found an effect of S1 duration, the longer duration of the first stimulus further decreasing R2. In the remaining cases adaptation either remained the same (19%) or there was less adaptation than for shorter stimuli (19%).

*Intensity of the First Stimulus and Adaptation*

The influence of the intensity of the first stimulus (70–103 dB) in the response to S2 was also explored in 21 neurons for

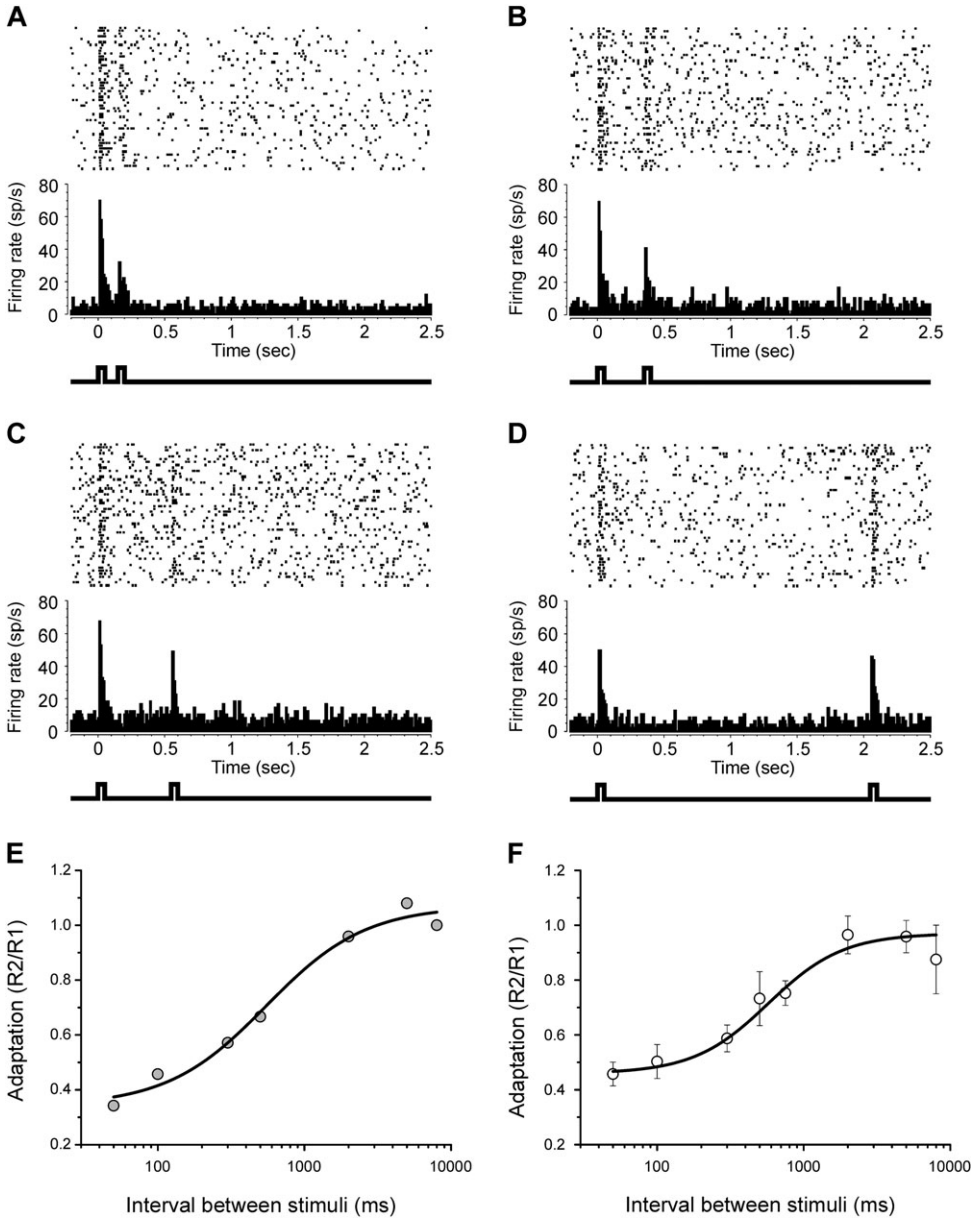


**Figure 1.** Auditory adaptation in single neuron recordings from A1 neurons in the awake freely moving rat. (*A–D*) Responses to 2 identical sounds (50 ms; 90 dB; white noise) separated by 100, 300, 500, 2000, ms intervals (*A*, *B*, *C*, *D*, respectively). PSTHs of the response (spike/s) to 50 trials (bottom) and raster plots (top) in each panel. (*E*) Relative amplitudes of the peak responses to the second with respect to the first stimulus illustrating the time course of adaptation. The neuron is the same as in panels (*A–D*). A sigmoid was fitted and $R^2 = 0.98$. (*F*) Plot of the relative amplitude of the response to the second sound with respect to the first one for different intervals for an average of 30 neurons. $R^2 = 0.99$. Error bars are SEM. Note that in average 2 s are needed for the second response to have an amplitude which is the same as the first one.

various intervals (50, 100, 200, 300, 400, 500, 750, 1000, and 1500 ms). The intensity of S1 was varied randomly (see Materials and Methods). In 50% of neurons an influence of the intensity of S1 was observed on R2, such that higher intensity of S1 induced stronger adaptation (Fig. 2*D–F*). Of the remaining neurons, the adaptation of 40% remained constant independently from S1 intensity. Finally, the remaining 10% of neurons showed less adaptation for higher than for lower intensities of S1.

### *Latency of Auditory Responses and Adaptation*

As reported by others (Eggermont 1999), the occurrence of adaptation often not only affects the magnitude of responses but also their timing. Here, the timing of responses was first quantified at the response's threshold. The mean firing frequency and the 95% of the confidence interval during the 300 ms preceding the stimulation were calculated. The response to multiple trials (80–100) was represented in a PSTH in 2 ms bins. After 10 ms of stimulus presentation, the first bin that crossed the 95% confidence interval was taken as the onset of the auditory response, and the exact time taken was the mid value of a 2 ms bin (Supplementary Fig. S3).

Auditory responses following the shortest interstimulus intervals (<0.3 s) often did not cross the 95% confidence interval and thus their latency could not be determined. In order to detect possible changes in latency with adaptation, interstimulus intervals were classified into short ISIs (0.05, 0.1, 0.3, 0.4, and 0.5 s) and long ISIs (0.75, 2, 5, and 8 s). Auditory

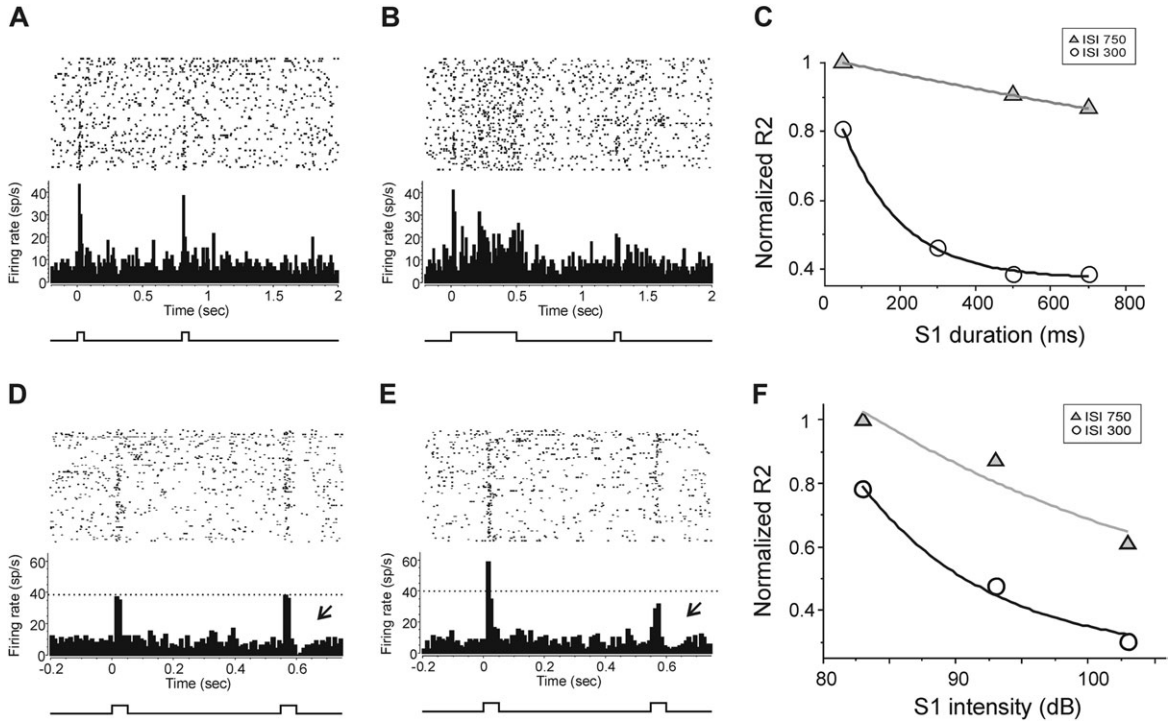


**Figure 2.** Relative adaptation of the second auditory response with respect to the first one in A1 of the awake rat. (*A*) Plot of the PSTH for one A1 cell to 60 trials of two 50 ms white noise sounds (90 dB) presented at 750-ms interval. On top, raster plots. (*B*) PSTH shows a decrease of the response to the second sound when the duration of the first is prolonged up to 500 ms. (*C*) Normalized response to the second sound (R2; 50 ms) (R2 divided by the value of the maximum R2, which occurs when the duration of S1 equals that of S2) represented against the duration of the first one for 2 intervals (300 and 750 ms). (*D*) Plot of the PSTH for one A1 cell to 100 trials of two 50 ms white noise sounds (90 dB) presented with 500-ms interval. (*E*) PSTH of the responses to both stimuli when the intensity of the first stimulus has been increased to 100 dB. Note that the response R1 increases, while R2 decreases. An arrow points to the postadaptation following the second stimulus (*D*, *E*). (*F*) Normalized response to the second sound (R2) (as in *C*) for different intensities of the first stimulus and for 2 different intervals (300 and 750 ms).

responses following short ISIs had significantly longer delays (12 ± 1 ms) than those following long ISIs (11 ± 2 ms; $n = 26$) ($P < 0.0003$), although the difference was small. It is possible that this difference was underestimated given that the most adapted responses (ISI < 0.3 s) were not included.

Auditory responses to S2 following long duration S1 (500 ms) had also significantly longer response delays (mean: 12 ± 2 ms; $P < 0.003$) than those following shorter stimuli (50 ms, mean: 11 ± 1 ms) ($n = 20$). Latency was also significantly ($P < 0.01$) longer for S2 stimuli following louder S1 stimuli (95 dB) (S1 = 11.9 ms, S2 = 13 ms). Whereas for louder S1 (95 dB), the response's latency was significantly shorter (11 ± 1 ms) than for 70 dB S1 (13 ± 2 ms; $P < 0.023$; $n = 21$) as reported earlier (Polley et al. 2006). For details on statistical analysis of response delays, see Table 1 in Supplementary Material.

The difference in latency was consistent across individual cases, and for that reason it was statistically significant in spite of the small latency difference (around 1 ms). An increased latency of the first evoked spike was also detected in the adapted responses while in vitro (see below). Even when the measure used here has been used by other authors, a decreased response amplitude may result in an artifactual increase of the latency when detected at the response's threshold (Bair et al. 2003). We therefore repeated the latency measures at the peak of the response and at 50% and 25% of the peak. In that case, no significant differences in the latency between the first and the second responses were detected (Supplementary Table S1). To conclude, the consistent but small differences in latency detected at the threshold of the adapted responses were not robust enough to persist when measured at the 25%, 50%, or 100% of the response's peak amplitude.

### *Postadaptation Following Auditory Responses*

We refer to "postadaptation" as the decay in the spontaneous activity firing following the end of an auditory response (Fig. 2*D*,*E*, see arrows). PSTHs (10 ms bins) were generated in order to quantify postadaptation. A 95% confidence interval was determined, and the first bin following the auditory response that was below the 95% confidence interval was considered postadaptation (Supplementary Fig. S4). Postadaptation was detected in 79% of cases following S2 and in 59% of cases following S1 ($n = 26$ neurons).

We observed more postadaptation following S2 when S1 was of longer (≥500 ms) duration (89%) than with short (<500 ms) duration stimuli (70%) (e.g., Supplementary Fig. S4*B*). Finally, we found (Fig. 2*D*,*E*) more postadaptation after S2 when it was preceded by a short ISI (<0.3 s) (71%) than by a longer (>0.3 s) ISI (60%).

## *Mechanisms of Neuronal Adaptation in Auditory Cortex In Vitro*

In order to explore to what extent the process of adaptation could be generated by activation of intrinsic membrane currents, 98 neurons recorded from rat A1 slices were included in the study ($n = 34$ rats). All neurons included were recorded from layers 2/3 and 4 and they were regular spiking. Eight additional neurons classified as intrinsically bursting (Nowak et al. 2003) were not included (see Materials and Methods). The average input resistance was 50.6 MΩ ($n = 89$).

*Time Course of Adaptation*

In order to evaluate the time course of adaptation of these neurons, a paradigm for neuronal stimulation was used that mimicked the one presented to the awake animal in the form of sounds. The paradigm used in vivo consisted of 2 sounds of 50 ms duration that were separated by different time intervals (ranging from 50 ms to 8 s), and it was replicated in vitro by means of current injection. Thus, pairs of square pulses of 50 ms duration separated by time intervals ranging from 50 ms to 5 s were injected. The intensity of the pulse was adjusted such that the first stimulus would induce a neuronal response of 3–5 action potentials, similarly to the response that a 50 ms sound stimulus evoked in the awake animal (Fig. 1). No spontaneous activity occurred in the slices, either supra or subthreshold, and therefore the observed events could be exclusively attributed to the activation of intrinsic membrane currents and not to activation of synaptic conductance. In addition, 6 of the recordings were performed under local application of 100 μM 6-cyano-7-nitroquinoxaline-2,3-dione, which did not affect adaptation or the subsequent afterhyperpolarization (AHP).

Adaptation defined as a decrease in the action potential frequency evoked by the second pulse with respect to the first pulse was detected in all cases (Fig. 3). The amount of adaptation and the time course of its recovery were evaluated in a total of 36 neurons. The time course of adaptation was averaged for all neurons (Fig. 3*D*). For the intensities used, the response evoked by the second pulse was still diminished with respect to the first one for intervals of 1 s between stimuli, while for intervals of 2 s the response was almost totally recovered. Still, there is a large heterogeneity across neurons. In Figure 3*C*, the normalized values of adaptation for 36 neurons are shown. For the sake of intelligibility of the graph, only the fitted sigmoidal curves have been displayed. In Figure 3*D*, the average adaptation recorded in the awake animal and that in the slices have been overlapped. There we can observe that the adaptation is larger in awake animals than in the slices. Hence, the maximum adaptation that occurred for the 50-ms interval was to 0.46 in awake animals and to 0.70 in the slice.

*Duration of the First Stimulus and Adaptation*

In 11 neurons, the influence of the duration of the first square pulse on the spike frequency adaptation was explored (Fig. 4*A–C*). The protocols carried out in awake animals by means of sound stimulation were mimicked in vitro by using current injection, and the duration of the first pulse was varied between 100, 200, 300, 500, and 700 ms, while the second pulse was of

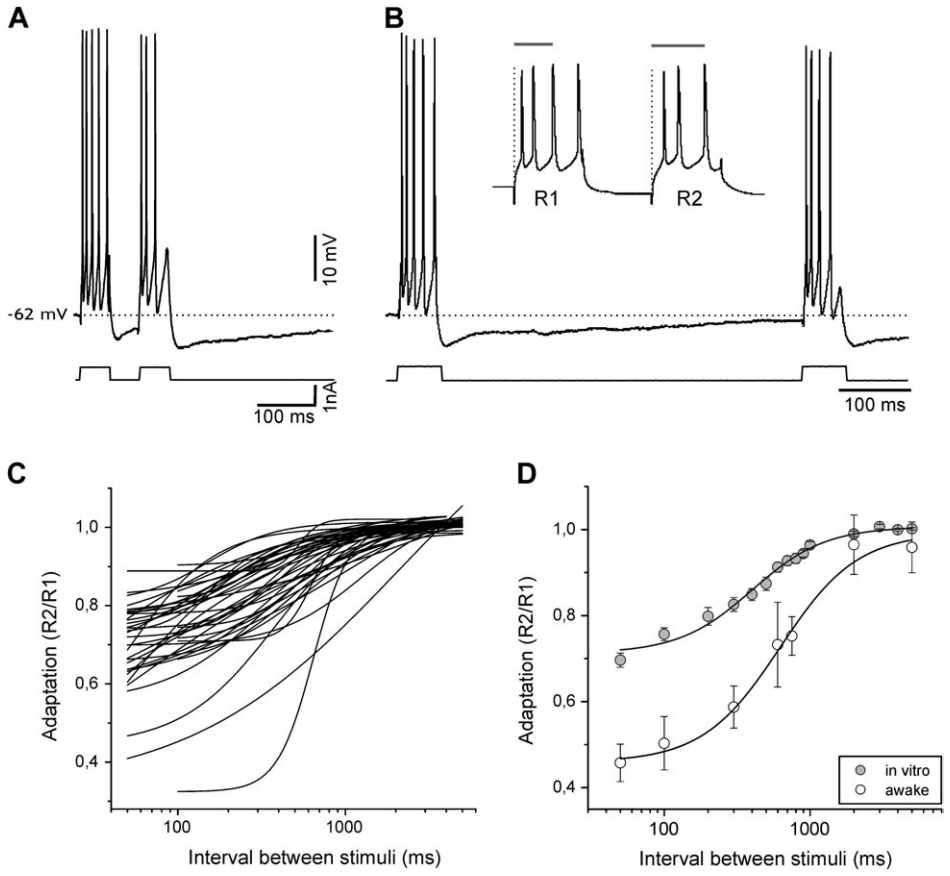


**Figure 3.** Time course of adaptation in A1 cortical slices and its comparison with adaptation in vivo. (*A*) Intracellular recordings illustrating the injection of two 50 ms pulses of 0.9 nA separated by a 50 ms interval. The intensity was adjusted to evoke 4–5 spikes with the first stimulus to mimic the sound stimulation in vivo. At the bottom trace, the current. (*B*) Recording from the same neuron as in A, but now the interval is 500 ms. The inset illustrates how the spike rate computed as responses (R1, R2) was calculated. It was taken as the time for the same number of spikes to be fired in R1 and R2 (for details see Materials and Methods). The 3 spikes shown in the inset in Figure 3*B* took, from time 0 (represented with a discontinuous line) 28.52 ms and 39.07 ms. (*C*) The recovery from adaptation in 36 neurons is represented, the protocol being that in panels A–B. For the sake of clarity, the sigmoidal fits are represented in order to illustrate the relative heterogeneity across cells and how in most cases adaptation was recovered in 2 s. (*D*) Relative frequency rate of the second response with respect to the first one for different intervals (50 ms–5 s) between identical 50 ms pulses of current injection as in *A* and *B* were averaged for 36 A1 neurons in vitro (gray circles). In order to compare with the adaptation evoked by a similar protocol in vivo, data from in vivo that has been plotted in Figure 1*F* is also displayed. A logistic function has been fitted (see Materials and Methods) and $x_0$ is 622 ms awake and 400 ms in vitro. In both cases $R^2 > 0.99$. Error bars are SEM.

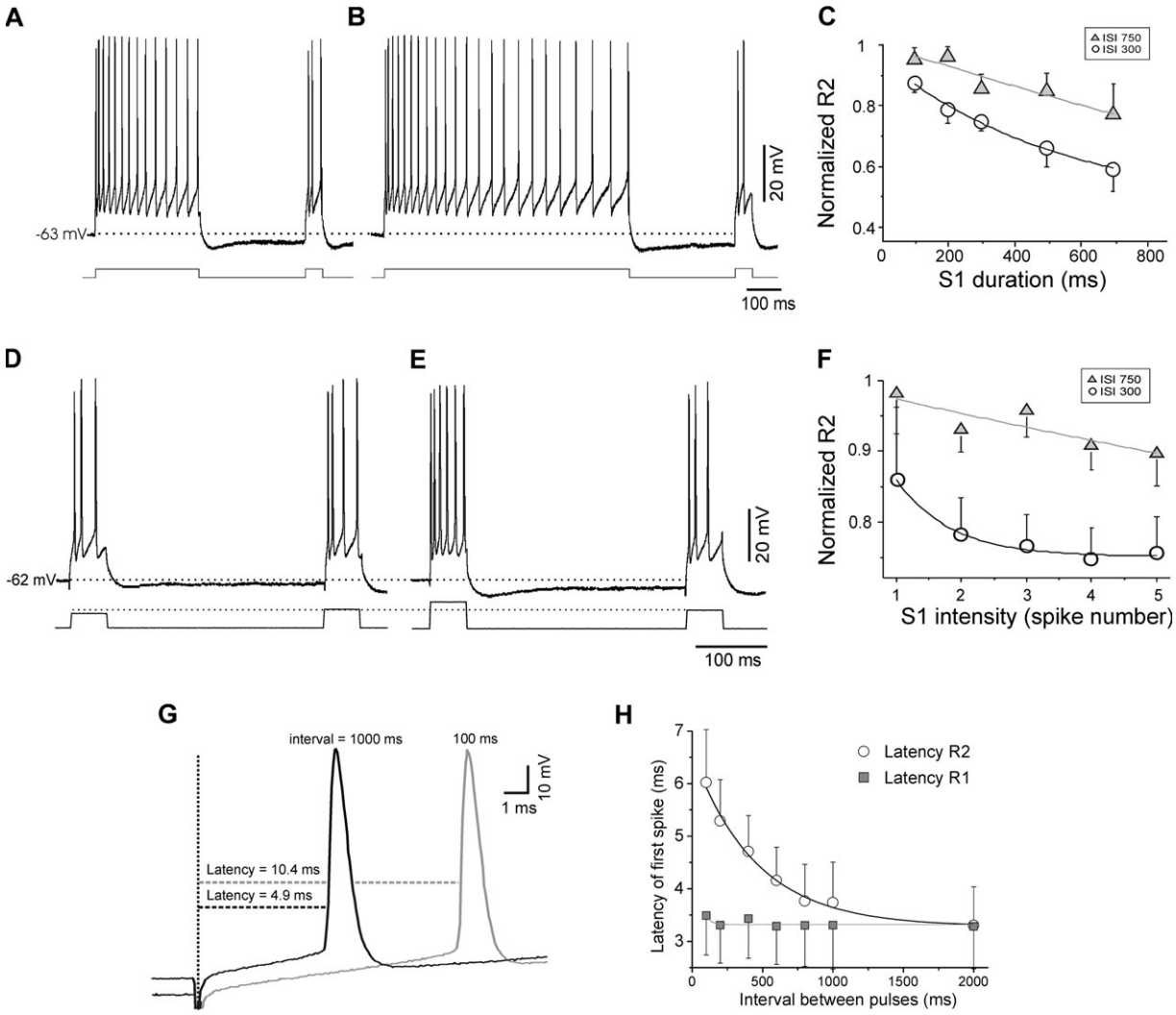


**Figure 4.** Attenuation of the response to the second pulse depends on the intensity and duration of the first pulse. (*A*) Intracellular recordings of a neuron while the duration of the first stimulus (S1) was varied between 100 and 700 ms. In the panel S1 = 300 ms. S2 was of the same intensity as the first one (0.7 nA) but only 50 ms duration. (*B*) Same recording but here the duration of S1 was 700 ms. (*C*) Average (n=11) normalized response (response to stimulus 2 divided by the response to an identical isolated stimulus, which has the maximum evoked response amplitude) is represented for different durations of S1. Normalized exponential fits at two different intervals (750 and 300 ms) show that response to S2 decreased when S1 was longer. (*D*),(*E*) Intracellular recordings of the responses to two 50 ms pulses while the intensity of the first pulse is varied between 0.4 nA (*D*) and 1.5 nA (*E*). The intensity of S1 affected R2 response. (*F*) Average ($n$ = 14) normalized response 2 (as in *C*) (for different intensities of S1 and two intervals (300 and 750 ms). (*G*) First spike latency for S2 separated from S1 by 1000 ms (4.9 ms) and 100 ms (10.4 ms). (*H*) Average (n = 7) latency of first spike (ms) represented against the interval between pulses (50 to 2000 ms).

50-ms duration (Fig. 4*A*,*B*). As in vivo, 2 intervals between the stimuli were tested, at 300 and 750 ms. Figure 4*C* represents the average adaptation for both intervals. When compared with the one in the awake (Fig. 2*C*), we see that the adaptation detected when the interval between pulses is 750 ms was similar in both cases. However, for shorter intervals (300 ms), the adaptation was larger in the neurons of awake animals.

### *Intensity of the First Stimulus and Adaptation*

In 14 neurons, we explored the influence of the intensity of the first pulse on the spike frequency adaptation to the second pulse (Fig. 4*D*–*F*). The maximum number of spikes evoked with a 50 ms pulse in regular spiking neurons was usually 4–5. The intensity of the first pulse was adjusted in order to evoke a number of spikes between 1 and 5 (usually between 0.4 and 1.3 nA). The intensity of the second pulse was constant and adjusted in each cell to evoke 4 spikes (0.4–0.8 nA). Figure 4*F* represents the average adaptation ($n$ = 14) for 2 different intervals between the pulses, 300 and 750 ms, which again is less to the one evoked by a similar protocol in the awake animal (Fig. 2*F*).

### *Latencies of the Spike Response*

Not only does the amplitude of the spike response vary but also the latencies of the spikes evoked by the second pulse varied with the time interval since occurrence of the first pulse (Fig. 4*G*). Figure 4*H* illustrates how the average latency remained constant for the response to the first pulse R1 around 3.3 ms, increasing to 6 ms for 100 ms intervals and then decaying progressively for longer intervals all the way to 3.3 ms.

Intrinsic properties may thus account for the increased delay that we observed as well as the one reported by others both in the anesthetized (Brosch and Schreiner 1997; Kilgard and Merzenich 1999; Chimoto et al. 2002; Ter-Mikaelian et al. 2007)

and the awake animal (Brosch and Schreiner 1997; Kilgard and Merzenich 1999; Ter-Mikaelian et al. 2007).

*Spike Frequency Adaptation and AHP*

In order to understand the basis of the spike frequency adaptation in the A1 cortical neurons in vitro, we explored the underlying mechanisms. An AHP followed the spike trains in all cases (Fig. 3*A*,*B*). The average AHP following a 50 ms pulse that evoked 4–5 action potentials (the average "first pulse" during the protocol) had a mean amplitude of 6.5 ± 0.4 mV when measured from a membrane potential of –60 ± 2 mV, and a mean duration of 1678.2 ± 107.1 ms ($n$ = 36; $x$ ± standard error of the mean [SEM]) (Fig. 3*A*,*B*). The amplitude and duration of the AHP increased with the number of spikes (Fig. 5*A*) up to a plateau (Fig. 5*D*,*E*). During the AHP, there was a decrease in the excitability of the neuron. Figure 5*C* illustrates the silencing of a neuron's tonic firing for 700 ms coinciding with the AHP that follows a 100 ms spike train. When the amplitude of the AHP was measured at different times (between 50 ms and 5 s) following a 50 ms depolarizing pulse, the repolarization from the AHP followed a very similar time course to the recovery of spike frequency adaptation (Fig. 5*B*). This is very suggestive of a relationship between the AHP following the first pulse and the spike frequency adaptation during the second pulse. The significant correlation between AHP duration and adaptation in the population (for 50 and 500 ms intervals) is illustrated in Supplementary Figure S5.

To explore the ionic basis of the AHP, two $K^+$ currents that for their time courses could be involved in the process were studied: apamin-sensitive $Ca^{2+}$-dependent $K^+$ current (Pennefather et al. 1985) and $Na^+$-dependent $K^+$ current (Schwindt et al. 1989). These two currents have been found to influence the time course of neuronal responses in the cat (Schwindt et al. 1988).

The application of apamin (500 nM; local application) partially blocked the AHP following a 50 ms depolarizing pulse and spike train (Fig. 6*A*,*B*). The partial blockade was measured as the reduction of the AHP peak amplitude, which occurred in all cases ($n$ = 20; Fig. 6*C*, inset) and consisted of an average decrease of the peak to 62% of the amplitude. Not only was the AHP partially blocked by apamin but the adaptation of the response to the second stimulus was also consistently reduced (Fig. 6*C*; $n$ = 20). For each of the 20 cells, the pairs of pulses separated by different intervals were given at the same membrane potential before and after the application of apamin. Adaptation was less in the presence of apamin, especially for those pulses separated by the shortest intervals (50 and 100 ms). This finding supports that apamin-sensitive $Ca^{2+}$-dependent $K^+$ current underlies at least the earlier adaptation (<200 ms) following a 50 ms spike discharge.

Apamin did not block the slower part of the AHP (Fig. 6*B*). We next explored to what extent $Na^+$-dependent $K^+$ current, a current of a slower time course (Schwindt et al. 1989) was playing a role on spike frequency adaptation in A1. To this end, $[Na^+]$ was reduced from 152 to 26–42 mM by replacing NaCl with choline chloride in the bath. To prevent the action of choline on muscarinic receptors, we include the muscarinic antagonist scopolamine (10 μM) in the bath. Scopolamine per se does not have a direct effect on the AHP (Uchimura et al. 1990; Sanchez-Vives et al. 2000a), and this is in agreement with what we observed here. In these conditions, the size and duration of the AHP were significantly reduced ($n$ = 11). Indeed, when $[Na^+]$ was decreased following apamin application, the AHP often was totally blocked ($n$ = 7; Fig. 6*D*). Spike

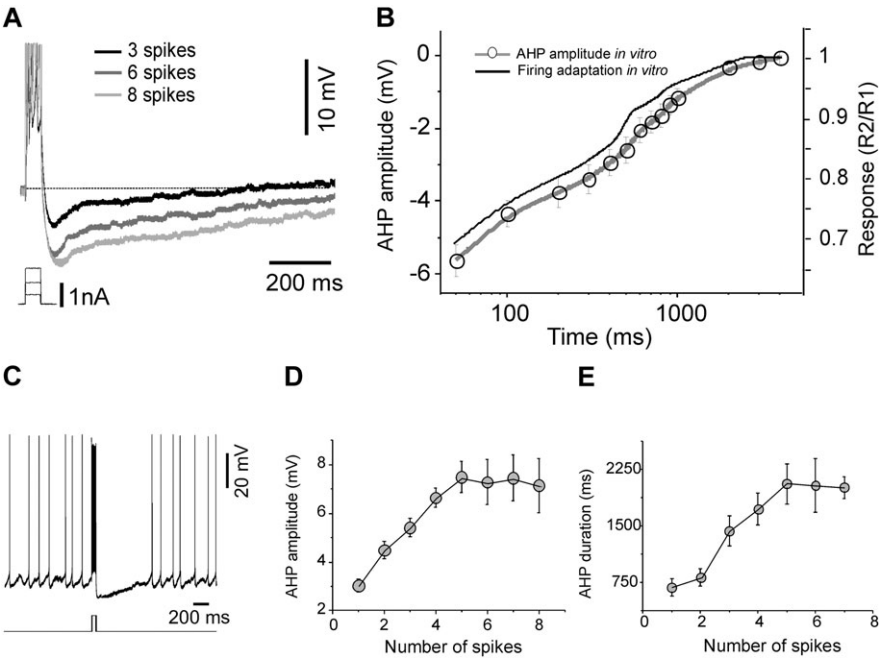


**Figure 5.** AHP in rat A1 neurons in vitro. (*A*) A suprathreshold 50 ms pulse activates an AHP that increases with the number of action potentials. Pulses of 0.5, 1, and 1.5 nA evoked 3, 6, and 8 spikes and were followed by AHPs of 600 ms, 1 s and 1.3 s, respectively. Top trace is Vm, the schematics of the injected current are represented below. (*B*) This graph represents an AHP following a 50 ms pulse, and the attenuation of R2 firing frequency with respect to R1 in the same neuron. (*C*) Intracellular recording during the tonic firing of a cortical neuron (−54 mV). A pulse of 0.9 nA has been given and the subsequent AHP maintains the neuron silent for 700 ms. (*D*) AHP amplitude at the peak increases with the number of spikes evoked by a 50 ms pulse ($n$ = 24). (*E*) AHP duration increases with the number of spikes evoked by a 50 ms pulse ($n$ = 20).

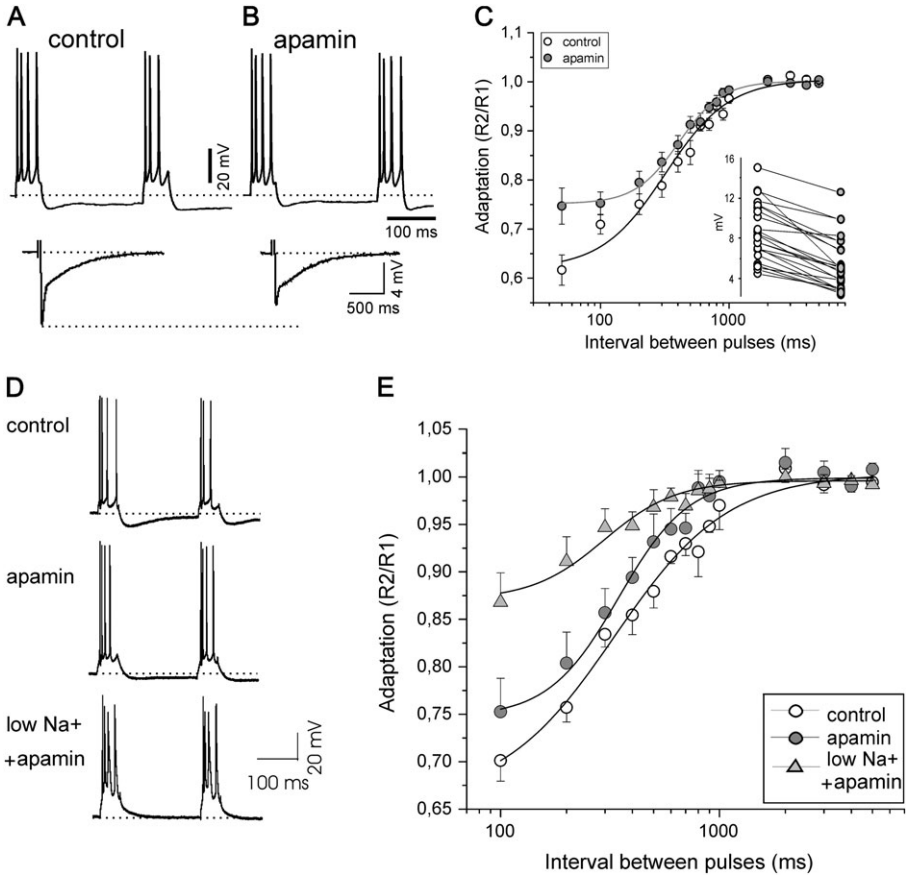


**Figure 6.** Role of $Ca^{2+}$ and $Na^{+}$ dependent $K^{+}$ channels on adaptation. (*A*) Two 0.9 nA pulses separated by a 200-ms interval evoke 4 and 3 action potentials, respectively. The AHP following the first pulse is displayed below, having an amplitude of 12.7 mV. (*B*) Apamin 1 μM was locally applied. Note that now the second pulse evokes also 4 action potentials as does the first one, plus the AHP amplitude has been reduced to 5 mV. (*C*) Time course of the adaptation recovery in control and in apamin in 20 neurons. Note that adaptation is reduced in apamin, particularly for shorter intervals between pulses. In the inset, the peak amplitude of the AHP in control and in apamin. A logistic function was fitted to the recovery from adaptation (see Materials and Methods). Control, $x_0 = 318$ ms; A2 = 0.62; $R^2 = 0.97$; apamin, $x_0 = 406$ ms; A2 = 0.75; $R^2 = 0.99$; (*D*) Two pulses of 0.9 nA separated by 200 ms in control, apamin, and low sodium plus apamin. (*E*) Time course of the adaptation recovery in control, apamin, and low sodium plus apamin in 7 neurons. Note that adaptation is reduced in apamin and in low sodium. The parameters of the fitted logistic function were: control, $x_0 = 332$ ms; A2 = 0.66; $R^2 = 0.99$; apamin, $x_0 = 354$ ms; A2 = 0.76; $R^2 = 0.95$; low $Na^{+}$, $x_0 = 296$ ms; A2 = 0.87; $R^2 = 0.92$.

frequency adaptation was again tested with the same protocol as above while in apamin plus low $[Na^{+}]$. In low $[Na^{+}]$, action potentials were still generated, although their amplitude was reduced and their duration increased. This was tested in 7 neurons and the resulting average adaptation has been represented in Figure 6*E*. Lowering $[Na^{+}]$ further blocked the adaptation already reduced by apamin. Still, a certain level of spike frequency adaptation remained, probably partly the result of the remaining $Na^{+}$-dependent $K^{+}$ current given that $[Na^{+}]$ was not totally eliminated from the bath. Reduction of sodium has a similar effect to replacement with lithium (Franceschetti et al. 2003) and has been used before to study $Na^{+}$-dependent $K^{+}$ currents (Sanchez-Vives et al. 2000a). Still, the caveat of reducing the size of action potentials is that it may affect the activation of other channels.

### *Adaptation in Neurons with Background Activity*

One of the differences that exist between the awake animal and the slice is that neurons in the slices were silent, while in the awake animal there was ongoing activity. Ongoing firing may have an influence on the intrinsic adaptation itself, setting neurons in a different adaptive state. To explore the effect of ongoing activity on adaptation, spontaneous activity of one neuron in the awake animal was recorded for 20 min in the absence of auditory stimulation (Fig. 7*A*). A period of 10 s was selected, the average firing frequency during this period being 5.5 Hz. At the time of occurrence of each spike, a brief pulse of 2.5–4 ms was injected, adjusting the intensity such that it would evoke just one spike per pulse (2.4–3 nA) (Fig. 7*B,C*). This fake "spontaneous" firing also induced the activation of AHPs that would follow action potentials (Fig. 7*D*), and an average membrane potentials that was in average 3.01 ± 1.92 mV ($n$ = 9) more hyperpolarized than that in silent neurons. The stimulation (background activity) was looping continuously over which the whole protocol of stimulation was carried out. The same protocols of adaptation used above were repeated, following what was described earlier for silent slices (Fig. 7*E*). The intensity of S1 was adjusted such that the first pulse would evoke 3–5 action potentials. The degree and time course of adaptation were calculated for 9 neurons (Fig. 7*F*). The standard deviation of each of the points (SEM in the error bars) was larger than for the adaptation than in silent slices due to the noise added by the background activity. The adaptation curve and its time course in neurons with background activity

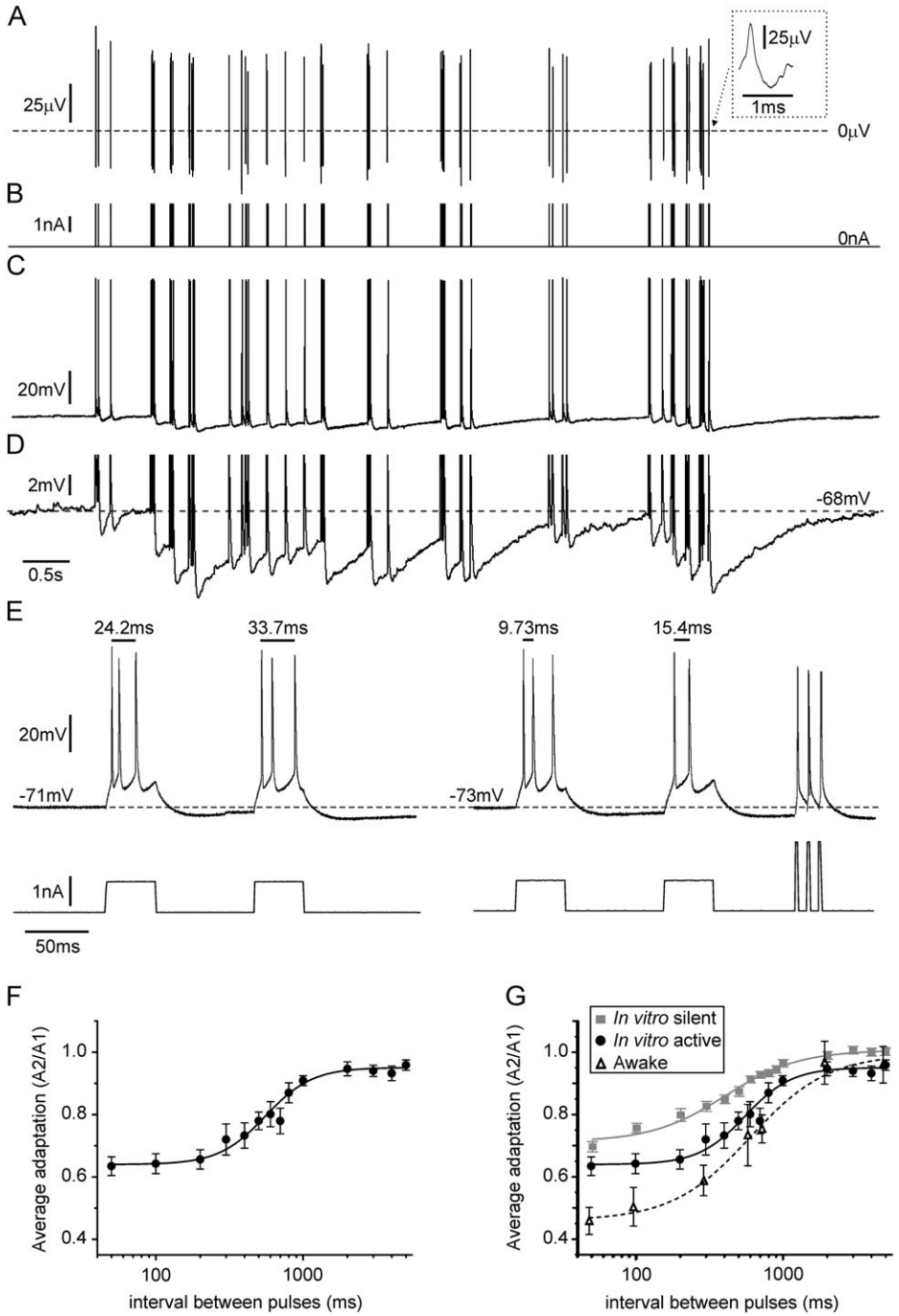


**Figure 7.** Adaptation R2/R1 in neurons with background activity mimicking the one in the awake animal. (*A*) Raw trace of single spikes recorded in the awake rat. The inset shows an expanded spike. (*B*) Current square pulses (2.5–4 ms) following time pattern of the firing in A. The pulses we reinjected in silent neurons to mimic the firing in the awake animal. (*C*) Intracellularly recorded neuron firing one action potential per injected pulse. (*D*) Expanded trace of intracellular recording shown in (*C*) to illustrate membrane potential changes as well as the increases in AHP in response to spiking activity. The dash line marks the resting Vm while in silence. (*E*) Two raw traces of the intracellular recording (top) to illustrate the neuronal response to two 50 ms square pulses (ISI = 100 ms) (bottom). Left and right trace; neuron without and with "fake" spontaneous firing in response to short pulses, respectively. Note that the Vm is more hyperpolarized in the active neuron and its adaptation is larger. (*F*) Time course of adaptation recovery average in neurons with "fake" spontaneous firing. A logistic function has been fitted (see Materials and Methods, $R^2 = 0.91$, $N = 9$). (*G*) Summary of the average adaptation time course in in vitro silent, in vitro active, and in awake preparations.

(Fig. 7*F*) were then represented along with the average one in the awake state (Fig. 1*F*) and in the silent slice (Fig. 3*D*) in Figure 7*G*. Interestingly, intrinsic adaptation evoked in neurons with "fake" background activity was larger than that in silent neurons (Fig. 7*E*,*G*) and hence closer to that in the awake animal.

## Discussion

We have used a combined in vivo (awake) and in vitro approach to study the functional mechanisms that mediate cortical auditory adaptation and with this we have demonstrated that part of the adaptation observed in awake animals

can be explained by the activation of potassium currents. The results reveal auditory adaptation using pairs of auditory stimuli in single neurons in the awake freely moving rats. Heterogeneity across cells with respect to the degree of adaptation and its time course were found, the time courses ranging from hundreds of milliseconds to several seconds. A replica of the stimulation protocol used in the awake animal was reproduced by means of current injection in A1 cortical slices. In this case a possible underlying mechanism of auditory adaptation, so far overlooked, was explored in detail: the participation of $K^+$ currents.

### *Auditory Adaptation in the Anesthetized and the Awake Preparations*

Studies in anesthetized animals have been relevant for our current understanding of auditory adaptation. However, anesthesia induces changes in neuronal excitability, facilitates oscillatory activity, modifies spontaneous activity, or increases neuronal frequency tuning sharpness (Gaese and Ostwald 2001). The effects vary with different anesthesias, affecting phenomena such as sensory adaptation (Wehr and Zador 2005; Moshitch et al. 2006; Rennaker et al. 2007). Wehr and Zador (2005) showed that pentobarbital anesthesia in the rat prolonged the very slow component of adaptation (several hundreds of milliseconds in that case), while ketamine has been reported to reduce the maximum rate of responses to repeated clicks (Rennaker et al. 2007). Cortical auditory adaptation in anesthetized cats has been described for different anesthesias and protocols displaying a variety of time courses including 50–1600 ms (Hocherman and Gilat 1981), or up to 400 ms (Calford and Semple 1995; Brosch and Schreiner 1997), and even reaching tens of seconds (Ulanovsky et al. 2004; Pienkowski and Eggermont 2009).

Awake animal preparations bypass the problems associated with anesthesia. Additionally, in this study, we have found that the impact of a 50 ms sound on subsequent responses to an identical stimulus persists for several seconds in the awake rat. This effect was normally expressed through attenuation of the response to the second stimulus. Larger responses induced by the first stimulus due to longer or more intense stimuli provoke a larger attenuation of R2 (Fig. 2). Similarly, previous studies found adaptation in the responses to the second sound in a 2-sound sequence for intervals up to 1–2 s in the anesthetized cat (Hocherman and Gilat 1981; Calford and Semple 1995; Brosch and Schreiner 1997; Reale and Brugge 2000) or up to 300 ms in the awake cat (Fitzpatrick et al. 1999). Neuronal adaptation in the passive listening primate was found to last no more than 2 s (Bartlett and Wang 2005), while in the primate performing a task has been reported to last up to 5 s (Werner-Reiss et al. 2006), suggesting that the brain state may have an impact on the duration of adaptation. The fact that cortical adaptation also exists in the awake restrained rat was also observed by Anderson et al. (2006). Using a different conceptual framework, different stimulation paradigms (broadband 80 μs clicks), and a different recording technique (multiunit recordings), Anderson et al. (2006) focused on the detection of the maximum stimulus rate that A1 responses can track (synchronization boundary). In spite of these differences, some of the reported findings in Anderson et al. are in agreement with those presented here, specifically, the decrease in mean response rate and duration with the stimulation rate, as well as the description of postadaptation.

The motivation to characterize auditory adaptation in the awake rat done in this study was first to obtain data on adaptation in freely moving animals, given that most of the previous studies were done in anesthetized preparations. Besides, doing these experiments allowed us to design a specific protocol that we could replicate in the auditory cortex in vitro by means of intracellular current injection in order to study the possible participation of potassium channels on auditory adaptation.

### *Cellular and Network Mechanisms of Cortical Auditory Adaptation*

Different mechanisms that could underlie cortical auditory adaptation have been proposed. Adaptation occurring at lower levels, in the inferior colliculus (Malmierca et al. 2009) or the auditory thalamus (Anderson et al. 2009; Antunes et al. 2010), could contribute to what is observed in the cortex. Still, adaptation appears to be larger in cortical layers that do not receive subcortical input (Szymanski et al. 2009), suggesting that part of it is being generated through the cortical circuitry.

Synaptic depression, both thalamocortical and intracortical, has been proposed as a suitable stimulus-specific mechanism since it can be associated to specific inputs to the neuron (Ulanovsky et al. 2004; Percaccio et al. 2005) and it can provide a variety of time scales (Varela et al. 1997; Carandini et al. 2002). Still, synaptic depression in vivo is less than that in cortical slices, showing an inverse relationship with the occurrence of ongoing activity in the cortical network (Reig et al. 2006). Inhibition could also play a role in auditory adaptation, a mechanism that would provide stimulus specificity (Zhang et al. 2003), as proposed by Eytan et al. (2003) based on ex vivo networks. Intracellular recordings in the cortex of the anesthetized rat (Wehr and Zador 2005) revealed that inhibition plays a role in the adaptation during the first 50–100 ms after the stimulus. However, this does not explain the adaptation of slower time course spanning from 100 ms to tens of seconds, for which $GABA_B$ receptors were proposed by some authors (Buonomano and Merzenich 1998) but found not to be involved by others (Wehr and Zador 2005). AHPs following auditory responses were described already in the first intracellular recordings from this region (De Ribaupierre et al. 1972b), being mostly attributed to IPSPs (De Ribaupierre et al. 1972b; Tan et al. 2004; Wehr and Zador 2005). Our data suggest that at least part of these AHPs may be due to the activation of potassium currents.

### *Intrinsic Mechanisms and Cortical Adaptation*

Most of the studies dealing with adaptation or forward masking/suppression have ruled out intrinsic mechanisms as a possible underpinning mechanism. Hyperpolarizing membrane currents have been found to play an important role in sensory adaptation in other sensory systems (Sanchez-Vives et al. 2000a, 2000b; Diaz-Quesada and Maravall 2008; Kuznetsova et al. 2008). Their activation depends on neuronal depolarization and eventually firing (Nanou et al. 2008), independently of the origin of the depolarization. This lack of dependence on the input is the reason why ionic currents have been largely ruled out as a suitable player in auditory adaptation, which is stimulus–frequency specific (Ulanovsky et al. 2003, 2004; Wehr and Zador 2005).

So, how could $K^+$ currents support frequency-specific adaptation? First, not all auditory adaptation is stimulus-specific

(Bartlett and Wang 2005). Second, in A1 different frequencies follow a tonotopic map (Doron et al. 2002) and usually neurons in the same electrode track share the same preferred stimulation frequency (Read et al. 2002). Adaptation is very similar in each particular cortical column, with no differentiation according to layers (Ulanovsky et al. 2004 but see Szymanski et al. 2009) but across columns. In a highly interconnected network such as a cortical column, the activation of hyperpolarizing currents in even a small percentage of neurons reverberates in the local circuits, inducing modulation of activity in the whole circuit (Compte et al. 2003). Given the predominance of vertical versus horizontal connectivity, adaptation would not necessarily propagate to adjacent areas of different preferred frequency. In this way, intrinsically mediated adaptation could also become stimulus-specific.

A role for potassium currents in adaptation is compatible with the participation of other mechanisms with which intrinsic properties would interact. Hence, the combination of spike frequency adaptation with synaptic depression in the network allows the computation of the rate of change of the stimulus (Puccini et al. 2007) and a higher detectability of unexpected stimulus (Puccini et al. 2006), a property described in the auditory cortex (Ulanovsky et al. 2003).

### *$K^+$ Current-Mediated Spike Frequency Adaptation in the Awake Animals*

A similar time course of adaptation was found in this study between in vitro and in awake but the attenuation of the responses was larger in the awake animal (Fig. 3*D*). One possible factor contributing to this difference is the heterogeneity of neuronal classes recorded in the awake animal while in vitro only regular spiking neurons were included. Intrinsically bursting neurons recorded in vitro were not included given the difficulty to measure adaptation in a way comparable with that in regular spiking neurons. In any event, cortical intrinsically bursting neurons in rat sensorimotor cortex have been described to have as well sodium-dependent potassium currents contributing to their AHPs (Franceschetti et al. 2003).

Ongoing firing in the awake animal is probably responsible at least in part for the lesser adaptation observed in vitro than in the awake animal. Adaptation in vitro increased when neurons were not silent, but awake-like background firing was artificially induced (Fig. 7). Still, adaptation in vitro remained less than in the awake animal (Fig. 7*G*). These findings suggest that intrinsic properties could underlie at least part of the sound adaptation existing in awake animals for intervals between 50 ms and 2 s, while leaving room for additional mechanisms. Other adaptation features found in the awake state were also observed in slices. These were the dependence of response (R2) on the duration and intensity of the first stimulus and the increased delay of adapted responses.

There is an additional possible bias that should be considered when comparing populations of recorded neurons both in vivo and in vitro: while recordings in vitro were intracellular and thus they did not select neurons on the basis of activity, those in vivo were extracellular and thus biased toward spiking neurons (Shoham et al. 2006).

Potassium currents have been thoroughly studied in vitro, however, their role in the awake, functioning brain is not well known. Studies of ionic currents require the use of techniques that are not fully viable in the awake freely moving animal, where only relatively short intracellular recordings are possible (Lee et al. 2006). The study of ionic channels in vitro is based on the idea that those currents must exist in the brain tissue in situ. However, it is difficult to extrapolate the knowledge obtained from in vitro, or even from the anesthetized preparations, to the awake and functioning brain. Here, we have concentrated on the activation of potassium currents by spikes. But it should be taken into account that in vivo, in addition to the changes in conductance and membrane potential induced by potassium channels activation, there are conductance and membrane potential changes due to excitatory and inhibitory inputs to neurons. The last ones were not mimicked in our in vitro simulations of in vivo discharge patterns.

The ionic currents that we describe here in A1 slices must exist in the brain of the awake rat, given that both species and brain area were the same. Furthermore, the slices studied here were obtained from adult animals and recorded at 34–35 °C. Ongoing spontaneous activity was generally absent in our slices while we report an average of 5 Hz firing rate in awake animals (Supplementary Fig. S2). Ongoing activity in the awake may induce $K^+$ currents activation even in the absence of stimulation, thus neurons could have a basal "preadaptation." We show that even if a neuron in vitro is tonically firing at a similar rate to spontaneous activity in vivo (Figs 5*C* and 7*D*) an AHP and decreased excitability follow a spike discharge. And indeed, we found that neurons with spontaneous activity display larger adaptation than silent ones (Fig. 7*G*). Still, ionic currents may be up or down regulated in the awake brain. On the one hand, norepinephrine blocks both $Ca^{2+}$ and $Na^+$-dependent $K^+$ currents (Foehring et al. 1989), while acetylcholine does the same on the $Na^+$-dependent $K^+$ current (Schwindt et al. 1989) hence they neuromodulate depending on the brain state.

$K^+$ currents in vivo could on the other hand be amplified in the network by reverberation in cortical circuits (Lorente de Nó 1949). In an active interconnected network, the decreased firing induced by $K^+$ currents is transmitted to connected neurons as decreased synaptic activity. The observation that $Na^+$ entering the cells through AMPA receptors is enough to activate $Ca^{2+}$ and $Na^+$-dependent $K^+$ current (Nanou et al. 2008) would contribute to the even further amplification of its effect at the network level.

In conclusion, our results indicate that intrinsic properties of auditory cortical neurons probably participate as functional mechanisms mediating adaptation to sounds. The modulation of $K^+$ channels through adrenergic or cholinergic neurotransmitters (Foehring et al. 1989; Schwindt et al. 1989) could thus be a key mechanism in the top-down modulation of auditory adaptation through attention (Fritz et al. 2007).

## Supplementary Material

Supplementary material can be found at: http://www.cercor.oxfordjournals.org/

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