Variability and information content in auditory cortex spike trains during an interval-discrimination task

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A RELEVANT ASPECT OF AUDITORY perception is the analysis of temporal information. Different studies have shown how single units of auditory cortex discriminate relevant temporal information for behavior (Bao et al. 2004; Fritz et al. 2007; Lemus et al. 2009; Polley et al. 2006). Temporal processing in auditory cortex has been previously studied by means of a number of experimental manipulations. Some studies have focused on interval production tasks (Merchant et al. 2008), discrimination of stimuli repetition rate (Bao et al. 2004; Fritz et al. 2007; Lemus et al. 2009; Polley et al. 2006), or frequency categorization of tones (Ohl et al. 2001; Selezneva et al. 2006). However, the mechanisms of temporal discrimination at the level of the cortical single unit are not well known. This issue was explored in a recent study where monkeys had to compare two acoustic flutter stimuli (Lemus et al. 2009). In that study, an absence of modulation of neuronal firing related to working memory or to decision components of the task was found. We were interested to explore the task-modulation of neuronal discharges to identical stimulation patterns, in particular in a task where the interval between stimuli would be behaviorally relevant. For that purpose, we devised an interval-discrimination task where the same patterns of auditory stimulation were carried out during engaged (correct choices provided reward) and in idle states (performance not required), and studied spontaneous and evoked firing patterns of single auditory cortical neurons.

Studies of neural mechanisms of auditory processing in auditory cortex have reported both an increased excitability and response to the stimulus (Hromadka and Zador 2007; Lemus et al. 2009; Otazu et al. 2009; Polley et al. 2006; Weinberger 2004), and also a suppression of auditory responses (Otazu et al. 2009) with task-engagement. Here we studied the modulatory influences of task-engagement on single-unit firing focusing on two aspects: the spike firing variability and the mutual information (MI) in spike trains. Recent studies have emphasized the importance of reduced variability (Churchland et al. 2010, 2011; Cohen and Maunsell 2009; Hussar and Pasternak 2010; Mitchell et al. 2007, 2009) to increase the signal-to-noise ratio, yielding the basis for an improved encoding of the stimulus information. However, neuronal response variability in auditory cortex has so far mainly been studied under anesthesia (Curto et al. 2009; DeWeese et al. 2003), although some recent studies have reported changes in variability in the awake state (Grana et al. 2009; Zhou and Wang 2010). It is not known to what extent variability of neuronal auditory responses in the behaving animal actually contributes to temporal discrimination. In this study, we carried out Fano factor (Ff) variability analysis along with information-theory measurements of the neuronal encoding during discriminatory behavior. We combined both measures since changes in variability suggest changes in information content, e.g., reduced variability suggests that information is increased, but it is not sufficient per se to quantify such change. MI analysis has been used in a few in vivo preparations to measure the stimulus-response relationship, to detect whether neuronal activity is stimulus-selective or not (Chechik et al. 2006; Lu and Wang 2004; Nelken and Chechik 2007). We quantified the information content in behaviorally relevant auditory responses during an interval-discrimination task by comparing the MI of firing patterns in auditory cortex during engagement vs. idle brain states.

METHODS

Ethics approval. The project was approved by the animal Ethics Committee of the University of Barcelona. Rats were cared for and treated in accordance with the Spanish regulatory laws (BOE 256; 25-10-1990), which comply with the European Union guidelines on protection of vertebrates used for experimentation (EUVD 86/609/EEC).

Surgical procedure. Recordings were obtained from two Lister Hooded rats (250–350 g) that were chronically implanted with te-
tetrodes in their primary auditory cortex. Animals were trained for 21
days. After 1 wk of water and food ad libitum, a microdrive holding
the tetrodes was implanted. To perform the surgery, anesthesia was
induced using intraperitoneal injections of ketamine (60 mg/kg) and
medetomidine (0.5 mg/kg). The animals were then mounted in a
stereotaxic frame, and their skulls exposed. A 3-mm-diameter crani-
otomy was made, with its center at −5.3 mm anterior-posterior, and
6.6–7 mm medial-lateral from bregma (Paxinos and Watson 1998).
These coordinates were used to position the microdrive dorsally,
which made it more stable than entering laterally over the auditory
cortex. Body temperature was monitored through a rectal thermometer
and maintained (36–38°C) using 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 recovery period to prevent infection, inflamma-
tion, and as analgesia: antibiotics (enrofloxacin; 10 mg/kg sc) and
topical application of neomycin and bacitracin in powder (Cicatrin),
algesic (buprenorphine; 0.05 mg/kg sc), anti-inflammatory (methy-
lprednisolone; 10 mg/kg ip), and atropine (0.05 mg/kg sc) to prevent
secretions during surgery. Once the animals went through all exper-
imental sessions, humane killing was performed by means of an
overdose of pentobarbital (0.8 ml).

**Tetrodes and microdrives.** Each tetrode was made from four twisted
strands of HM-L-coated 90% platinum-10% iridium wire of 17 diameters
(California Fine Wire, Grover Beach, CA). Gold plating decreased their
impedance to ca. 300–500 KΩ. Four tetrodes were held by a cannula
attached to a microdrive supplied by Axona (St. Albans, UK). This
microdrive allowed for dorsal to ventral tetrode movement to search
for new units. Microdrives were attached to the skull with dental
cement and seven stainless steel screws. The auditory cortex was
reached by vertical descent, and the tetrodes were lowered 300 μm
during the surgery. Vertical descent performed after surgery was of 50
μm/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
histological reconstruction of the electrode’s tracks. The auditory
latencies were typically 10–20 ms, which are also characteristic of A1
(Malmierca 2003; Nelken et al. 2003; Ojima and Murakami 2002).

**Electrophysiological recordings from awake, freely moving rats.**
During the training period, animals lived in large cages of 28 × 42 × 30
cm (Charles River) in a rich environment, under a 12:12-h
light-dark cycle, and with food ad libitum and water restriction.
Before training and after 1 wk of postoperative recovery period, the
animals were accustomed 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
1,000), and to the filters and amplifiers of the recording system
(Axona, St. Albans, UK). Signals were amplified (×15,000–40,000),
high-pass filtered (360 Hz), and acquired using software from Axona
(St. Albans, UK). Each channel was continuously monitored at a
high-pass filtered (360 Hz), and acquired using software from Axona
(Axona, St. Albans, UK). Signals were amplified (×15,000–40,000),
high-pass filtered (360 Hz), and acquired using software from Axona
(St. Albans, UK). Each channel was continuously monitored at a
sampling rate of 48 kHz. Action potentials were stored as 50 points
per channel (1 ms; 200 μs prethreshold; 800 μs postthreshold)
whenever the signal from any of the prespecified recording channels
exceeded a threshold set by the experimenter for subsequent offline
spike sorting analysis. Data were excluded if any drift was detected.
Before each experimental session, tetrodes were screened for neuronal
activity. Once spikes could be well isolated from background noise,
the experimental protocol started.

**Experiment.** The recordings were performed inside a box
built in black acrylic of 22 × 25.5 × 35 cm. This box was placed
inside two wooden boxes placed one inside the other. Between each
box, two isolating foam rubbers (4 and 2 cm thick) were placed to
soundproof for low and high frequencies. A wooden cover and
soundproof foams closed the entire recording chamber, with only a
hole to allow the entry of a recording wire (2 mm thick) connected
to the preamplifier. Water valves were placed outside the recording
chamber. The animals poked their noses into three different sockets (2
cm wide and separated by 3 cm each, and with no cover in the top part
to avoid being hit by the microdrive). Recordings were obtained in
darkness, and the experiment was filmed with an infrared camera
placed above the recording chamber.

**Behavioral protocol.** The behavioral protocol consisted of four
different recording stages with a total duration of ca. 2.5 h. The
animals only went through the whole session once a day. The idle
listening recording stage (ca. 17 min) was performed before and after
the engagement stage (ca. 40 min). The final stage (ca. 40 min)
comprised an idle recording with reward delivery after each stimulus
pair was presented. The aim of the idle stages was to compare the
neuronal responses while the idle animal heard stimulus presentation
with respect to the engaged brain state during task performance. Later,
the animals were trained to poke their noses into the center socket,
which immediately triggered the onset of two identical stimuli (80 dB,
5,322 Hz, 50-ms duration). The animals had to remain in the center
socket until the end of the stimuli presentation. They had to discrimi-
nate whether the two stimuli were separated [from the end of stimulus 1
(S1) to beginning of stimulus 2 (S2)] by 150 or 300 ms. This
required a left or right poke respectively, to get the water reward.
In the behavioral 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 sound
pressure level (SPL)]. All trials during task performance were self-
initiated by the animals. During the “initial-idle” and “idle-post”
recordings, the animals freely moved around the recording box (with
occluded sockets) while listening to stimulus presentation. Finally,
in the last idle recording stage (idle + reward), the left and right sockets
were occluded while not the central one, where the animal repeatedly
entered and received a water drop 0.3 s after having listened to the
same stimuli as in the engagement stage. Note that there was move-
ment in all phases of the experiment, either toward the sockets or
around the cage.

**Presentation of sound stimuli.** The protocols of stimulation were
controlled through MATLAB, a National Instrument card (BNC-2110),
and a breakout box (FS 300 kHz). Sound triggers had microsecond
precision. Sound stimuli were delivered through earphones (ER.6i Isola-
tor, Etymotic Research), which were screwed in each recording session to
the earphone holders, chronically attached to the animal skull with dental
cement. The earphones were adjusted inside the ear with silicone tips
with a separating distance of 1 mm from the ear canal. Similarly sound
 calibration was performed inside the acoustic isolation box with a
microphone (MM1, Beyerdynamic) placed 1 mm away from the
earphone and using a preamplifier (USB Dual Pre, Applied Research
and Technology). The sound stimuli during idle and engaged record-
ing stages had a duration of 50 ms, with an intensity of 80-dB SPL
pure tones of 5,322 Hz, and a 6-ms rise/fall cosine ramps. It was
identical for both the first and second stimulus. Interstimuli intervals
(ISIs) were 150 or 300 ms, and both had the same amount of trials
(180–200). Similarly, the total number of correct trials in the engage-
ment stage was the same as in the idle one (180–200). The intertrial
interval also had a similar duration in the engagement and idle stages
(2–3 s).

**Data analysis.** Cluster cutting (isolating single units from the
multunit recording data) was performed using an Off-Line Spike
Sorter (OFSS, Plexon). Waveforms were sorted as in (Abolafia et al.
2011b). Single units exhibited a recognizable refractory period (>1
ms) in their ISI histograms.

Analysis of peristimulus histogram (PSTH) were performed using
10-ms bins to estimate responses to auditory events accurately. Fre-
quency response histograms were obtained by averaging the spiking
activity within each bin during the whole recording. The onset of each
stimulus presentation was aligned to zero. Raster plots illustrate the
timings of individual spikes in individual trials during the whole
recording. Only correct trials were selected, comprising 180–200
responses per each side.

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We refer generally to “spontaneous activity” in this paper to that firing rate of the neuron occurring whenever there was no auditory stimulation. Therefore, we include under the term “spontaneous activity” neuronal firing rate that may as well correspond to prolonged responses to the stimuli or to modulation due to cognitive and behavioral states (expectation, engagement, attention, etc.).

The Ff was computed as the ratio between spike count variance across trials and mean spike count. Ff was calculated in 10-ms bins along the trial duration (from −200 ms to S2 + 600–750 ms) for correct trials (180–200 at each response side). For each cell, we compared the minimum Ff value during the ISI (−200 ms to 0) and the minimum Ff value in the interval from 0 to S2 + 600–750 ms. We defined a decreased Ff when a neuron had three consecutive bins with Ff lower than the minimum value obtained during the ISI (whether short or long ISI).

Sparseness of neuronal activity has been shown to affect information theoretic measurements (Nelken and Cheechik 2007). To avoid this, we selected neurons with the highest firing rate while the rat performed correct trials. In all cases, a minimum of 180 trials per side were considered. Given that the animal made few errors, plus the fact that the firing was sparse, there were not enough spikes fired during wrong trials to allow for independent analysis of correct vs. incorrect trials. The response to all correct trials was represented in a PSTH in 10-ms bins. The mean firing frequency and its standard deviation during the 200 ms preceding S1 was calculated. Neurons were considered to have significant evoked-spiking responses if after S1 onset the evoked firing during the 50-ms stimulation was 5 standard deviations over the spontaneous frequency (Recanzone et al. 2000; Sakai et al. 2009). Twenty-one neurons crossed the threshold during S1 presentation (50 ms).

To estimate the information content carried by the neuron’s firing rate, we performed MI analysis, which measures the strength of association between two variables, in our case “spike rate” and “category of ISI.” The MI (I) was calculated as:

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

where I is information; s is spike rate, r is category of the ISI (either short or long), \( p(s) \), \( p(r) \) are the marginal distributions, and \( p(s,r) \) is the joint distribution. The MI has a zero value if the two variables are independent. Our calculations were based on frequency estimates of the probabilities \( p(r,s) \), \( p(s) \), and \( p(r) \) by using spike counts in 50-ms time windows during each stimulus presentation. We estimated the value of the MI by using the direct method for the MI estimate and the Panzeri et al. (1996) method for the bias correction, since the computation of the information content is subject to statistical errors given that the MI is based on the estimation of probabilities. This method corrects for the bias by means of decomposing the MI in different factors and then removing the ones affected by the bias or noise.

To establish the significance of the estimated MI, we applied the surrogates method (Schreiber and Schmitz 2000). We tested if the estimated MI significantly rejected the null hypothesis, i.e., noninformation content \( [I(R;S) = 0] \). We tested this null hypothesis by generating 1,000 surrogates of the spike activity during stimulus duration, which by construction should not contain information. Thus each surrogate is generated by shuffling the assignment between stimulus and response. We computed for each surrogate the MI value, and we compared all of these bootstrapped information values with the real value of the original data. We calculated the statistical significance of the estimated MI by computing the area in the null hypothesis distribution (MI of the surrogate) below the MI value corresponding to the original data. We considered that, if the area of the null hypothesis was larger than 85% of the total area, the estimated MI value of the original data was significant. This criterion means that the null hypothesis (no significant MI) can be rejected with an 85% probability, i.e., the estimated MI is significant at 0.15 level \( P < 0.15 \). Let us note that even for a smaller \( P \) value of 0.05, still seven neurons passed the test. Nevertheless, we took a \( P \) value of 0.15 to increase the amount of neurons that passed the test and have a more reliable statistics. All of the calculated MI values for both original data and surrogates were bias corrected using this method.

**RESULTS**

Single-unit recordings were obtained from the auditory cortex of two awake, freely moving rats chronically implanted with tetrodes. Auditory stimulation was given through earphones (see methods). We isolated 86 single units, which, when classified according to their auditory responses following Recanzone (2000), consisted of the following: onset (26%), onset + offset (13%), offset (2%), nonresponsive (43%), suppressive (13%), and other (3%). The percentage of nonresponsive neurons was similar to the one reported by Hromadka et al. (2008) while using cell-attached recordings in head-fixed awake animals.

Our objective was to study the effects of task-engagement, not only on auditory evoked responses during correct trials, but also during the ISI. In order for these intervals without stimulation to be behaviorally relevant, we designed an interval-discrimination task, where the rat had to go to the left (or to the right), depending on the duration of the interval between stimuli (Fig. 1B). The experimental procedure consisted of a sequence of different recording stages with a total duration of 2.5 h (Fig. 1A). Two idle recording stages, “initial-idle” and “idle-post” were recorded before and after the task-engagement, respectively. During the task, the animals had to enter in the central socket which triggered the presentation of two identical stimuli separated by an ISI of 150 or 300 ms (Fig. 1B). The rats had to categorize the two different ISIs, 150 and 300 ms, by going to the left or to the right and nose poking to obtain a reward. The last stage was the “idle + reward” recording stage, where the animals were rewarded in the center socket independently of the stimuli being presented (Fig. 1A).

The aim of these recording sequences was to track the activity of a single unit and compare response patterns between engaged vs. idle brain states. The three idle stages (initial-idle, idle-post, and idle + reward) had the same amount of trials (180–200 trials each), stimuli (50 ms; 80 dB; 5.3 kHz), ISIs (150 and 300 ms), and intertrial intervals (2 to 3 s) as the task-engagement stage. As soon as the animals reached 70% of behavioral performance (Fig. 1, C and E), they were implanted. After the last recording, we obtained a psychometric curve to further evaluate the perceptual and behavioral effects of the short and long ISIs being presented to the animal during the behavioral task. During the psychometric curve, the short ISI (150 ms) became longer, while the long ISI (300 ms) became shorter, allowing us to test the perceptual threshold of ISI discrimination (Fig. 1, D and F).

Engagement diminishes variability during and after stimulation in auditory cortex. Both enhanced (Atiani et al. 2009; Blake et al. 2006; Fritz et al. 2005) and reduced responses (Oizuru et al. 2009) to stimulation have been observed while processing behaviorally relevant auditory stimuli. We first explored the effect of engagement on the firing rate of 33 neurons that comprised the onset and offset-offset ones. In most cases, engagement significantly increased the spike firing (see methods) during stimuli-evoked responses \( n = 22; \) Fig. 2), while the opposite trend was less common \( n = 11 \). The
response to the second stimulus was typically decreased as a result of auditory adaptation processes (Abolafia et al. 2011b; Otazu et al. 2009; Ulansky et al. 2004). Substantial adaptation was observed both in onset responses (Fig. 2A) as well as offset ones (Fig. 2B) (n = 13). There was a trend for the average adaptation to the second stimulus to be lower in the engaged than in the idle state (n = 10), although the difference was not statistically significant (P = 0.23 and P = 0.37 for short and long ISIs, respectively; Wilcoxon; Fig. 2C). In the remaining three cases, adaptation increased during engagement.

Next we studied whether engagement altered neuronal response variability in the auditory cortex of the behaving animal. We calculated the Ff (spike-count variance divided by spike-count mean) to test how neuronal variability changes on a trial-by-trial basis. Since the evoked auditory responses were mostly phasic, we found that 10-ms bins showed the best time resolution and reflected the most accurately the changes in neuronal activity during the ISI (Fig. 3, A and D). Accordingly, neuronal activity variability during task-engagement for short and long ISIs compared with the idle state was not only observed in one neuron. Statistical comparisons (Wilcoxon) showed a significant difference between the variability during S2 in the engaged state vs. that in initial idle (P < 0.008) or idle-post (0.008). However, there was no significance (P > 0.7) when the two idle states were compared.

Out of these 14 neurons with significantly modulated Ff during engagement, 9 neurons showed a reduction of variability in the 200 ms preceding a stimulus presentation, while 8 neurons showed a reduction of variability during the ISI. In all, we observed a significant reduction of variability along the trial duration for all studied neurons during engagement compared
with the idle brain state, and this was enhanced during stimuli presentation.

It has been shown by other authors that Ff is not contingent on the firing rate (Churchland et al. 2010; Kara et al. 2000; McAdams and Maunsell 1999; Mitchell et al. 2007). We also tested this, and, for that purpose, we selected bins with similar firing rate (<5% difference) from engaged and idle trials. We plotted for each selected bin the Ff value in the idle vs. the engaged state for each neuron (n = 14; see Fig. 4). Bins were matched according to the same time location of the trial in the different brain states (Fig. 4A) and also to different time location (Fig. 4B). Figure 4 shows that most of the values remain above the x/y main diagonal, indicating that Ff values are larger in the idle than in the engaged state than what would be expected by a change in firing rate. We also computed the mean distance of the values with respect to the x/y main diagonal, which reflects the difference between the Ff-idle and Ff-engaged. We found that the positive values of the difference was 0.14 (std: 0.05) and 0.09 (std: 0.07) (A and B, respectively), while for negative values was 0.08 (std: 0.06) and 0.05 (std: 0.03) (A and B, respectively). Thus the Ff is nearly two times larger in the idle compared with the engaged state, and, therefore a decrease in Ff during engagement is not a mere artifact of an increase in firing rate. Additionally, the number of values above the diagonal are 64% and 67% (A and B, respectively), while the ones below are 36% and 32% (A and B, respectively). Finally, we compared the statistical significance (Wilcoxon) between the values above and below the x/y main diagonal (i.e., engagement vs. idle). We found no statistical significance for Fig. 4A (P < 0.3), while the opposite was found in Fig. 4B (P < 0.00), possibly due to the increased number of values in the later. Thus from this section we conclude that, during engagement, there is a reduction in variability. To evaluate if this decrease in Ff is associated with an increased encoding capability, we proceeded to use information theory to estimate MI.

**MI is increased during engagement.** MI analysis has been previously used to estimate the information content present in spike trains generated by neurons from the auditory cortex in both anesthetized (Lu and Wang 2004; Nelken et al. 2005) and awake animals (Kayser et al. 2009). Here, we performed the MI analysis to find out whether single units in auditory cortex of the awake behaving animal encode information related to interval-discrimination of auditory stimuli. In our interval-discrimination task, the animals had to decide whether two identical stimuli were separated by 150 or 300 ms. In that task, the key stimulus that determines whether the ISI category is “short” or “long” is the second one. MI between the variable “spike count” and the variable “ISI category” (150 or 300 ms) was calculated. Hence, we compared the MI value in the response to the first stimulus vs. that to the second stimulus, in both idle and engaged states.

To compute the MI value, we used here the bias-corrected method of Panzeri et al. (2007), as described in METHODS. Furthermore, to evaluate the statistical significance of these MI values, we used the surrogates method (see also METHODS). Given the sparse activity and the requirements for the calculation of MI (Nelken and Chechik 2007), we found necessary to use 50-ms bins which comprised the stimulus duration (S1/S2). MI was calculated in 21 neurons with an average firing rate during the 50-ms duration of the auditory stimulus (S1) that was significant, as defined in METHODS. Of these 21 neurons, 10 successfully passed the surrogates test of the spike count during S2 in the engaged brain state. One example neuron of these 10 is shown in Fig. 5A, showing a raster plot and PSTH, for ISIs of 150 ms and 300 ms. In this case, MI values were higher during the response to S2 than to S1 in the engaged state (S2: 0.016; S1: 0.0), while it was not in the
initial-idle stage (S2: 0.0; S1: 0.0), idle-post (S2: 0.0; S1: 0.0), or idle 
/reward (S2: 0.0004; S1: 0.0). Furthermore, we tested 
the surrogates significance of these MI values, and we found 
that, in the engaged state, the MI value during S2 significantly 
(0.007) passed the surrogates test.

Mean MI values during responses to S1 vs. S2 stimuli (N = 10) in the initial-idle (S2: 0.007), idle-post (S2: 0.005), and 
idle + reward (S2: 0.010) were lower than in the engaged state 
(S2: 0.028) (see Table 1). Even though these MI values could 
be interpreted as rather low, similar MI values have been found 
in the auditory cortex (Brasselet et al. 2012). The surrogate test 
was only passed in the engaged brain state, and these results 
suggest that the engaged state carries more information than 
the first one.

Statistical comparisons (Wilcoxon) of the MI were also 
performed between S1 and S2 for each brain state (see Table 
1). We observed significant differences between MI values 
during S1 and S2 in engagement, but not during the idle brain 
states (see Table 1). Additionally, S2 values of MI were 
compared (Wilcoxon) among brain states (see also Table 1), 
and we also observed a significant difference of MI in S2 
during engagement compared with the idle states. Therefore, 
information content of spike trains evoked by auditory re-
sponses is augmented during the engagement in an interval-
discrimination task.

A response profile of another example neuron showing an 
"onset-offset" pattern is illustrated in Fig. 6, A and B. In this 
case, spontaneous activity is increased during the time interval 
preceding stimulus presentation during task-engagement. The 
MI value in the response to S2 was 0.0465, with a surrogate 
significance of 0.002, during task-engagement, while MI value

Fig. 3. Response variability is reduced during 
engaged brain states. A and B: Fano factor (FF) of 
an example neuron during short (A) and long (B) 
ISI and for the engaged (red) vs. idle stages (blue). 
Gray bars indicate the presentation of stimulus 1 
(S1) and S2. A reduction in FF during the engaged 
compared with the idle state can be observed 
during trials of both short (A) and long (B) ISI. Not 
only during stimulation but also during the ISI, 
and during the spontaneous activity period preceding 
stimulation (~0.2–0 s), variability was decreased 
in the engaged with respect to the idle state of 
the animal. C and D: same as in A and B for 
another example neuron with an onset-offset re-
sponse pattern. In the engaged state, there was a 
reduction in FF during the onset and offset com-
pared with the idle state. Similarly, during the 
spontaneous activity period preceding stimulation 
(~0.2–0 s), there was a decreased variability in 
the engaged (C and D) compared with the idle 
state of the animal. E and F: the difference in the 
FF value for each bin of the trial was obtained 
between the engaged state and the average idle 
recordings. The obtained differences were aver-
ged, and the SEM errors are displayed with the 
gray shadow. Most of the values are negative, 
indicating lower variability in the engaged condi-
tion with respect to the idle one. Enhanced differ-
ces in variability are observed during stimuli 
presentation.

Fig. 4. The FF is not dependent on the firing rate. A: the FF value of idle 
recordings (Y-axis) is plotted against the FF value of engaged ones (X-axis). 
The FF value was calculated for each pair of bins with equal spike count (~5%) 
between the idle and engaged states. Each pair of bins with equal firing rate had 
the same position in the trial in the different brain states. B: each pair of bins 
with equal firing rate had different position in the trial. Only neurons with 
significant FF were included (n = 14) for A and B. A significantly larger 
amount of the values remained above the x/y main diagonal for A and B.

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was lower during the idle ones (initial-idle: 0.0001; idle-post: 0.0065; idle + reward: 0.0001) (Fig. 6C, left). This neuron further shows that the engaged state of animals has an effect on the information content in spike trains evoked by behaviorally relevant stimuli.

We also explored the information contained in the offset responses evoked once the stimulus was terminated. Seven neurons showed offset responses to auditory stimulation, while four of them showed additionally onset responses (e.g., Fig. 6, A and B). 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 MI during a time window of the same duration as the one used to calculate MI during stimuli presentation (50 ms). The 50-ms window was taken around the peak of the offset response (25 ms before and after the peak) of S2. MI values of the population mean of offset neurons (Fig. 6C, right) were significantly higher in the engaged state (0.0310) compared with the idle (initial-idle: 0.0021; idle-post: 0.0017; idle + reward: 0.0067) as evidenced with the surrogate test (see Table 1). To test the significance of that MI value, statistical comparisons (Wilcoxon) of the MI were again performed between S1 and S2 for each brain state (see Table 1). As in the other case, we observed significant differences between MI values during S1 and S2 in engagement (0.0468), while not during the idle brain states (see Table 1). When we compared S2 values of MI (Wilcoxon) among brain states, we again observed a significant difference of MI in S2 during engagement compared with the idle states (see Table 1). These results suggest that offset neuronal response after S2 termination not only carries information, but carries a similar amount of information about the category of the ISI carried by the one of the responses of onset neurons during S2 presentation.

**Table 1.** MI values during responses to S1 vs. S2 stimuli

<table>
<thead>
<tr>
<th>Summary Statistics State</th>
<th>MI in S1</th>
<th>Surrogate in S1</th>
<th>MI in S2</th>
<th>Surrogate in S2</th>
<th>$P$ Value Between S1 and S2</th>
<th>$P$ Value Between S2 Engaged vs. Idle</th>
<th>MI Value in S2 Offset</th>
<th>Surrogate in S2 Offset</th>
<th>$P$ Value Between S2 Offset Engaged vs. Idle</th>
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<tr>
<td>II</td>
<td>0.002</td>
<td>0.56</td>
<td>0.007</td>
<td>0.53</td>
<td>0.313</td>
<td>0.027*</td>
<td>0.0021</td>
<td>0.677</td>
<td>0.0082*</td>
</tr>
<tr>
<td>E</td>
<td>0.011</td>
<td>0.28</td>
<td>0.028</td>
<td>0.04*</td>
<td>0.010*</td>
<td>0.0310</td>
<td>0.0468*</td>
<td>0.7124</td>
<td>0.0047*</td>
</tr>
<tr>
<td>IP</td>
<td>0.002</td>
<td>0.50</td>
<td>0.005</td>
<td>0.51</td>
<td>0.562</td>
<td>0.039*</td>
<td>0.0017</td>
<td>0.7124</td>
<td>0.0047*</td>
</tr>
<tr>
<td>I+R</td>
<td>0.0002</td>
<td>0.70</td>
<td>0.013</td>
<td>0.60</td>
<td>0.250</td>
<td>0.031*</td>
<td>0.0067</td>
<td>0.2630</td>
<td>0.0156*</td>
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</table>

II, initial idle; E, engagement; IP, idle-post; I+R, idle + reward. MI in S1, mean mutual information (MI) value in *stimulus 1* (S1) (bits). Surrogate in S1, mean value of the surrogate significance test in S1. MI in S2, mean MI value in *stimulus 2* (S2) (bits). Surrogate in S2, mean value of the surrogate significance test in S2. $P$ value engaged vs. idle, $P$ value between S2 in engagement and S2 in idle. MI value in S2 offset, mean MI value in S2 offset (bits). Surrogate in S2 offset, mean value of the surrogate significance test in S2 offset. $P$ value between S2 offset engaged vs. idle, $P$ value between S2 offset in engagement and S2 offset idle. *$P < 0.05$.

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Relationship between $F_f$ and $MI$. To study the relationship between $F_f$ and $MI$, more systematically we developed a theoretical toy model that parameterized the experimental data. The toy model was defined by generating artificial spike train datasets whose interspike intervals follow a gamma distribution with a given mean firing rate (Fig. 7B) as in the real data (Fig. 7A) to test the relationship between $F_f$ and $MI$ (Fig. 7C).

Artificial datasets were modeled by gamma point processes, where $F_f$ and the firing rate can be controlled. This model has been successively used to model spiking data (Baker and Lemon 2000; Ponce-Alvarez et al. 2010). In this model, interspike intervals are independently drawn from a gamma distribution (see equation below) that has two parameters: a scale parameter, $r$, that controls the intensity of the process (firing rate), and a shape parameter, $\alpha$, that controls the variance of the distribution. Indeed (Nawrot et al. 2008) showed that $F_f = 1/\alpha$.

For a given pair of $r$ and $\alpha$, we generated a spike train composed of 1,000 consecutive interspike intervals. The length of the spike train ($T$) was divided into short non-overlapping time bins of 50 ms (equal to the stimulus period in the experiments), and the spike count ($N$) was calculated in each time bin. To avoid border effects, we left aside the first 10 time bins, and we stored the spike counts of the following 200 bins. As a result, we obtained a spike count distribution for a given set of $r$ and $\alpha$, noted $f_{r,\alpha}(N)$.

Using this procedure, we generated, for a given $\alpha$ and for two fixed values of the rate parameter, denoted $r_1$ and $r_2$, two spike count distributions, $f_{r_1,\alpha}(N)$ and $f_{r_2,\alpha}(N)$, and computed the MI between the parameter $r$ and the $N$, $MI$ (Abeles et al. 1994; $\{N\}$).

In our case, $r_1$ corresponds to the mean firing rate during S2 in short ISI trials, while $r_2$ corresponds to the mean firing rate during S2 in long ISI trials for a certain neuron. According to $F_f = 1/\alpha$, we varied $\alpha$ between 0.3 and 1.1, while keeping $r_1$ and $r_2$ fixed. Then we calculated the MI for the pair ($r_1$, $r_2$), for all the $\alpha$, between the stimulus and the response. This procedure was repeated 1,000 times to estimate the error. As shown in Fig. 7C, the MI of the simulated distribution (Fig. 7B) increased for decreasing values of $F_f$. We found then a negative correlation between these parameters (corr: $-0.77$; $P < 0.05$) such that, for lower $F_f$, $MI$ increased. In conclusion, this toy model has been successfully used to model spiking data (Baker and Lemon 2000; Ponce-Alvarez et al. 2010).
model demonstrated that a parametric decrease of the Ff systematically increased the MI, generalizing therefore the experimental observations.

DISCUSSION

We studied neuronal responses in rat auditory cortex during a decision-making task where intervals between auditory stimuli were categorized. Neuronal responses during and after evoked activity were compared in engaged vs. idle states. Their firing rate, MI, and variability were also quantified. Out of 86 neurons recorded in the auditory cortex of the awake, freely moving rat, auditory responses were evoked in 49 neurons, a proportion similar to that in Hromadka et al. (2008). We refer to task-engagement since we consider that the animal needs to be engaged to do the task correctly. However, during trials of engagement, we cannot rule out the participation of other mechanisms like expectation (Jaramillo and Zador 2011). We found that neuronal firing rate during engagement was more often up- than downregulated during auditory responses. We cannot rule out a bias of extracellular recordings toward more active neurons, influencing our observed impact of task-engagement on the firing rate. Ongoing activity recorded in the intervals in between auditory stimuli during the same task is in some cases also significantly modulated by engagement, being usually increased (Abolafia et al. 2011a). A prominent decrease in neuronal variability during both sensory-evoked and nonevoked activity was detected during engaged vs. idle listening. Finally, information content in auditory-evoked spike trains was higher in engaged than in idle states, in particular in those evoked by the task-relevant stimulus.

In general, Ff reduction can be associated with increased encoding capabilities only under strong assumptions. Indeed, a neuronal network can have a very low Ff (almost identical spike trains in multiple trials), but zero coding precision (identical spike trains for multiple stimuli). We showed that, in our case, the reduction of the Ff is indeed directly associated with an increment of the encoding/processing of the discrimination capability evidenced in the behavioral response. Furthermore, MI is more powerful because it is defined by the measurement of different sources of variability, namely an entropy term that characterizes the neuronal variability in general, and another “conditional” entropy that measures the specific variability observed for a given condition (or behavioral response). Let us note that the increase of MI observed is not only due to a decrease of the conditional entropy term, but to the combination of both total response entropy and conditional noise entropy terms. Indeed, the total response entropy term increases too, so that the increased information acts synergistically with changes in the neural representation. To complement this view, we also studied the direct reduction of the variability per se. We thus studied the Ff reduction for a specific condition. Ff is particularly useful because, contrary to the MI, it can be computed in small sliding windows during the whole trial. Indeed, by doing this, we were able to show for the first time that a reduction of variability is observed in the absence of external stimulation (between the stimuli), but also in a relevant time region.

In our study, we have demonstrated that the reduction of variability (Ff) observed during stimulus presentation (including the interval between both stimuli), in particular the larger reduction due to engagement, is in fact associated with increased encoding capabilities for discrimination. We show this by complementing the Ff variability measurement with a direct information-theoretical measurement of encoding capabilities via MI between neuronal activity and behavioral responses.

Firing variability of single units in auditory cortex. Sensory processing during the processing of task relevant information has been linked to enhanced responses (Atiani et al. 2009; Blake et al. 2006; Fritz et al. 2005) and also to decreased ones (Otazu et al. 2009). Moreover, evoked responses in primary auditory cortex can be modulated as a result of temporal expectation (Jaramillo and Zador 2011). Increased inhibition
has also been suggested to play an important role in cortical responses to relevant stimuli (Galindo-Leon et al. 2009; Nelken 2009). Task-engagement also induces tonotopic changes (Biesczad and Weinberger 2010; Polley et al. 2006; Rutkowski and Weinberger 2005; Schreiner and Winer 2007) and tuning shifts of the same neurons toward the target stimulus (Brown et al. 2004). However, there are no studies describing how engagement 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, 2011; Shadlen and Newsome 1998; Sussillo and Abbott 2009). Furthermore, the neuronal variability over trials declines in particular in situations where the encoded information serves to guide behavior (Churchland et al. 2006, 2010, 2011; Hussar and Pasternak 2010). This has been experimentally demonstrated in recorded neurons of the visual area V4 (Cohen and Maunsell 2009; Mitchell et al. 2009) in the context of an attentional paradigm. In these studies it has been shown that the mean-normalized variance (Ff) of the spiking activity is reduced by attention, consequently increasing the sensitivity of neurons toward relevant aspects of stimuli. Neuronal response variability may also depend on the type of neuron, i.e., narrow or broad spiking (Mitchell et al. 2007), while some authors suggest that the attentional effects on variability may reflect an intrinsic property of neural circuits (Deco and Hugues 2012). Moreover, stimulus-induced 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 behavioral requirements of an interval-discrimination task stabilize the dynamics in a controlled way, such that neuronal variability is reduced. Thus 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; Kara et al. 2000; McAdams and Maunsell 1999; Mitchell et al. 2007). Some studies have shown that decreased variability is not due to an increase in firing rate (Churchland et al. 2010). In our current study, we find that, in bins where the firing rate was equal between the idle and engaged states, variability was still reduced in the later (Fig. 3). This is consistent with the result reported by Mitchell et al. (2007) in visual cortex (V4), where lower variability during engagement was observed when bins with equal firing rate were compared.

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 (Chechik et al. 2006; DeWeese et al. 2003; Gehr et al. 2000; Imaizumi et al. 2010; Kayser et al. 2009; Lu and Wang 2004; Nelken and Chechik 2007; Nelken et al. 2005). Previous studies (Imaizumi et al. 2010) have suggested that information content in multunit activity of auditory cortex is higher in the interspike interval than during the firing rate or event-locked spikes (Furukawa and Middlebrooks 2002) when repetitive stimulation is presented to anesthetized animals. 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 the auditory cortex. Therefore, the combination of different neuronal codes could enrich auditory stimuli representation and increase robustness against noise. Looking into the MI between the stimulus and neuronal response, Kayser et al. (2010) suggested that spike precision enhances the encoding of information about extended complex sounds. Also, information can be carried by spike timing in case of sparse acoustic events, while firing rate-based representations encode rapidly occurring acoustic events (Lu and Wang 2004).

The study of auditory activity during a task where a monkey compared the relative frequency of two auditory stimuli showed that stimulus-locked responses, and in particular firing rate, only correlated with performance during stimulus presentation (Lemus et al. 2009). This was not the case though during delay periods, as it would be the case if it were related to working memory or decision making. The authors suggested that the auditory cortex may serve to encode information of sensory stimuli, mostly by means of firing rate, with no cognitive function related to decision making or memory. Our analysis shows that MI is particularly enhanced to the relevant stimulus during task-engagement, although we do not definitely demonstrate its association to performance. Altogether, we think that this is an evidence of the role of auditory cortex in temporal discrimination during a decision-making task, even when its origin may be a top-down influence.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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