Supplementary Information

Auditory cortex mediates the perceptual effects of acoustic temporal expectation

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Supplementary Fig. 1: Reaction time on difficult trials was greatly influenced by expectation. Reaction time on trials with expected and unexpected early targets grouped by difficulty (same subject from Fig. 2a). The top panel comprises trials with the three easiest detection difficulties. The lower panel, the three most difficult levels. Triangles indicate when late targets could appear. Unexpected difficult trials show a bimodal distribution, with fast responses consistent with the subject reacting to the target, whereas slow responses consistent with missed detections (and occurring around the time where the animal expected a late target).
Supplementary Fig. 2: Slow behavioral responses were associated with error trials. Percentage of correct trials grouped according to reaction time. Data from the same animal in Fig. 2a. The band surrounding the average plot represents 95% confidence interval on the estimates. Dashed line indicates chance level.

Supplementary Fig. 3: Late targets were always expected. Percentage of correct trials as a function of difficulty (same subject as in Fig. 2c) for trials with late targets. Error bars correspond to the 95% confidence intervals on estimates. These results suggest that late targets in expect-early blocks are still expected by the subject. In these trials, once the subject realizes an early target will not appear, he will expect the target to occur late. These results are consistent with those from human subjects (Nobre, 2001).
**Supplementary Fig. 4: Control measurements for alternative behavioral strategies.** To further test that temporal expectation affected sensory processing and not just motor preparedness, we trained animals on the same task with a slightly modified stimulus. In this case, two tones were simultaneously presented at each time slot as shown on the left panel. In the original version of the task, subjects can adopt a strategy whereby they do not listen for a modulated sound, but simply choose a reward port based on the carrier frequency of the sound at the expected time. The modified stimulus presented here discourages that strategy by requiring a detection of a modulated sound at all difficulties. The right panel shows the performance for one animal as in **Fig. 2c.** Stars indicate significance levels (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).
Supplementary Fig. 5: Inactivation of auditory cortex completely impaired performance in some animals. Performance under muscimol inactivation and saline control sessions for an animal that was completely impaired by inactivation. Performance on muscimol sessions was at chance even for the easiest difficulty tested. Error bars correspond to the 95% confidence intervals on estimates.

Supplementary Fig. 6: Animals performed hundreds of trials during inactivation sessions. Average number of trials per 2-hour session under each condition (inactivation with muscimol or saline control), for each of the 5 animals from the inactivation experiment. Error bars indicate s.e.m. across sessions.
Supplementary Fig. 7: Examples of modulation of single cell responses. Spike raster and PSTH, as in Fig. 4a, for two example cells, but with trials in chronological order. By showing each block of trials separately, we verify that modulation of responses is due to expectation and not to non-stationarity of the recordings. The cell on the left panel shows an onset response, while the cell on the right shows a delayed/offset response to pure tones.

Supplementary Fig. 8: Modulation of spontaneous firing rates by temporal expectation. Modulation index for spontaneous firing of each responsive cell included in the study (N=102). Histogram as in Fig. 4b, but calculated for spontaneous firing rates over a period of 200 ms preceding the stimulus. Cells with significant modulation are shown in black. There was no significant modulation for the population of cells ($p = 0.24$, paired Wilcoxon signed-rank test). A similar lack of modulation was observed when we analyzed the population of cells from each of the two experiments separately.
Supplementary Fig. 9: Neuronal responses to target sounds. Evoked spiking activity elicited by target sounds, on expected and unexpected conditions, for the 26 cells that fulfilled our selection criteria (same used for Fig. 4b, but using responses to target sounds). We quantified the onset of the evoked response (5-30 ms window) and selected the target sound that elicited the strongest response for each cell. Black circles show cells with significant modulation ($p < 0.05$, Wilcoxon rank-sum test). The population did not show a significant effect ($p = 0.6$, paired Wilcoxon signed-rank test). It should be noted that our task design was less sensitive to neuronal responses elicited by the target. Because subjects were allowed to withdraw from the stimulus port at any time after the onset of the target (stopping the presentation of the sound), the actual duration of the target sound was variable across trials. The evaluation of evoked responses was therefore restricted to the onset period. In addition to the difference in target durations, the number of presentations of a given unexpected target sound was very low (by definition).
Supplementary Fig. 10: Frequency tuning was estimated by randomizing the frequency of the third tone. Spike raster of cell from Fig. 4a. Trials are grouped by the frequency of the third tone as indicated on the left. Fig. 4a shows only trials for the preferred frequency (24 kHz). Trials in blue are from expect-early blocks, in red from expect-late. Each tone is 100 ms long.
Supplementary Fig. 11: Modulation of response to tones did not depend on the relation between preferred and target frequencies. One could posit that neurons with preferred frequencies around those of the targets in this particular task (6.5 and 31 kHz) would display a stronger modulation compared to other cells. We did not observe a systematic difference in modulation strength depending on preferred frequency ($p=0.38$, Kruskal-Wallis one-way ANOVA). The figure shows the modulation index as a function of preferred frequency for each cell. Cells with significant modulation are shown in black. Average modulation index for each frequency is indicated by the purple line.
Supplementary Fig. 12: Preferred frequency was not affected by temporal expectation. One could hypothesize a shift of the frequency tuning towards the most relevant frequencies as the time of the target approaches. For the frequency resolution used in our study, we did not observe systematic changes in preferred frequency. In this figure, each dot indicates the preferred frequency for one cell under each of the two expectation conditions. Small random noise was added on each coordinate to avoid overlap. The color of each dot represents the average firing rate evoked by the preferred stimulus of the cell across all trials. Most cells with reliable firing rates fall along the diagonal, indicating no change in preferred frequency.
Supplementary Fig. 13: Modulation of neuronal responses to target sounds. Change in evoked response to the target sound between expectation conditions (expected-unexpected) as a function of the mismatch between the preferred frequency of each cell and that of the target. The data is the same as in Supplementary Fig. 9: each gray circle represents the modulation for one cell for the target that elicited the largest response, and black circles show cells with significant modulation ($p < 0.05$, Wilcoxon rank-sum test). The preferred frequency for each cell was estimated from the responses to tones preceding late targets. When the data are sorted in this fashion, a positive modulation emerges, but only for those cells with preferred frequency close to that of the target. From those neurons with tuning close to the target frequency (less than one octave apart), 7 out of 10 showed an increase in evoked response. Additionally, there was a significant correlation ($\rho = -0.6$, $p = 0.001$, t-test on transformed correlation) between the change in response and the mismatch between the frequency of the target and the preferred frequency of each cell. We also observed a significant negative correlation ($\rho = -0.3$, $p = 0.012$) when performing a similar analysis on responses from these cells to each tone preceding the expected early targets.
Supplementary Fig. 14: Recording sites were confirmed by histology. Coronal slice from one of the animals implanted with a microdrive. Electrolytic lesions, seen here as darker spots, were used to verify the location of the tetrodes. Areas according to Paxinos and Watson (2005). Au1: primary auditory cortex, AuD/AuV: secondary auditory cortex dorsal/ventral.
Supplementary Equations

The waveform of frequency-modulated targets $s(t)$ was generated as:

\[ s(t) = A \sin(2\pi ft + m(t)) \]  
  \( m(t) = \text{TMD} \frac{f}{f_m} \sin(2\pi f_m t), \quad f_m = 15 \text{ Hz} \)

where $A$ is the amplitude, $f$ is the carrier frequency, $f_m$ is the modulation frequency, and TMD is the target modulation depth.

The sigmoid function for fitting psychometric data was defined as:

\[ P = q_1 + \frac{q_2}{1 + \exp \left( \frac{\log(\text{TMD}) - q_3}{q_4} \right)} \]

where $q_i$ are fitting parameters and TMD is the target modulation depth that defines difficulty.

The root mean square power of local field potentials (LFP) for each condition $i$ was calculated as:

\[ \text{LFP}_i = \sqrt{\frac{1}{50} \int_{310}^{360} [\bar{\phi}_i(t)]^2 \, dt} \]

where $\bar{\phi}_i$ is the average waveform LFP over trials from condition $i$. 

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