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Auditory cortical and hippocampal local-field potentials to frequency deviant tones in urethane-anesthetized rats: An unexpected role of the sound frequencies themselves

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Abstract

The human brain can automatically detect auditory changes, as indexed by the mismatch negativity of event-related potentials. The mechanisms that underlie this response are poorly understood. We recorded primary auditory cortical and hippocampal (dentate gyrus, CA1) local-field potentials to serial tones in urethane-anesthetized rats. In an oddball condition, a rare (deviant) tone ($p = 0.11$) randomly replaced a repeated (standard) tone. The deviant tone was either lower (2200, 2700, 3200, 3700 Hz) or higher (4300, 4800, 5300, 5800 Hz) in frequency than the standard tone (4000 Hz). In an equiprobability control condition, all nine tones were presented at random ($p = 0.11$). Differential responses to deviant tones relative to the standard tone were found in the auditory cortex and the dentate gyrus but not in CA1. Only in the dentate gyrus, the responses were found to be standard- (i.e., oddball condition-) specific. In the auditory cortex, the sound frequencies themselves sufficed to explain their generation. These findings tentatively suggest dissociation among non-contextual afferent, contextual afferent and auditory change detection processes. Most importantly, they remind us about the importance of strict control of physical sound features in mismatch negativity studies in animals.

Keywords: change detection; acoustic frequency; hippocampus; local-field potentials; primary auditory cortex.

1. Introduction

Any change in invariant attributes of the auditory past is of potential importance for survival. The rapid and effortless auditory change detection is, therefore, essential.

Indeed, auditory change detection seems to be automatic. In adult humans, who may be voluntarily attending to a non-visual modality, detection of auditory changes is accompanied by an electrical brain response, termed the mismatch negativity (MMN). MMN is a component of negative polarity of scalp-recorded event-related potentials (ERPs) at about 150-200 ms post-stimulus (Näätänen et al., 1978; Näätänen, 1990). MMNs observed even in comatose (Kane et al., 1996) and generally anesthetized (Koelsch et al., 2006) adults as well as sleeping infants (e.g., Alho et al., 1986) suggest that MMN is also reasonably independent from the awake behavioral state.

MMN can be observed in a so-called passive oddball condition as a response to a (‘deviant’) tone that rarely replaces a repeated (‘standard’) tone. Its connection to a deviant tone as a change in the repetitiveness of a standard tone (Näätänen et al., 2005) rather than merely as a rare tone in the series (Jääskeläinen et al., 2004; May and Tiitinen, 2009) is suggested by the disappearance of MMN when the standard tone is replaced with silence (deviant-alone condition, Näätänen et al., 1989) or with physically heterogeneous tones that each occurs as often as the deviant tone would do (equiprobability condition, Jacobsen and Schröger, 2001).

Deviant tones also elicit differential brain responses (higher-amplitude brain responses to deviant than standard tones) in awake (e.g., Javitt et al., 1994; Ruusuvirta et al., 1995, 2010; Pincze et al., 2001), sleeping (e.g., Csépe et al, 1987) and anesthetized (e.g., Kraus et al., 1994; Ruusuvirta et al., 1996; Ahmed et al., 2011; Astikainen et al., 2011; Nakamura et al., 2011; Tikhonravov et al.,

2008) animals (for negative findings in anesthetized rats see, however, Erikson & Villa, 2005; Lazar & Metherate, 2003). Furthermore, similarly to MMN (Jacobsen and Schröger, 2001), differential brain responses in animals have been attributed to deviant tones as changes in the repetitiveness of a standard tone rather than as rare tones relative to the standard tone (e.g., Ruusuvirta et al., 1998; Nakamura et al., 2011; Taaseh et al., 2011; Jung et al., 2013; Shiramatsu et al., 2013; Harms et al., 2014; Malmierca et al., 2014). Together, these findings suggest that the mechanisms for automatic auditory change detection are not limited to the human brain.

If a deviant tone is physically different from a standard tone and if differential brain responses to the deviant tone are not standard-specific (i.e., the removal of standard stimuli does not make differential responses to disappear), one cannot directly attribute these responses to different presentation rates of the deviant tone than the standard tone. Before doing that, one must exclude the possibility that the physical deviant-standard difference itself suffices to explain the responses.

With two tones, one assigned to the deviant and the other standard stimulus category, physical features of the tones is reasonably easy to control for. One can simply counterbalance the assignments of the tones to their categories across the sample. With a larger number of physical deviant variants, counterbalancing would not be feasible. Then one option is to statistically test the extent that the physical features alone of the tones account for the deviant-related effects in brain responses.

The present study capitalized on the previous observation in urethane-anesthetized rats of auditory-cortical and hippocampal differential brain responses to temporally deviant tones (Ruusuvirta et al., 2013). Using the same preparation, we applied deviant tones with different

sound frequency levels. The equiprobability condition (Jacobsen & Schröger, 2001) was used to test the standard-specificity of differential brain responses and, if not observed, also whether these responses are simply to the different sound frequencies themselves.

2. Materials and methods

2.1. Animals and surgery

The experiments were approved by the Finnish National Animal Experiment Board (Permit code: ESLH-2007-00662). They were carried out in accordance with the European Communities Council Directive (86/609/EEC) on the care and use of animals in experimental procedures.

Eleven adult Sprague Dawley rats were used in the experiment (weight 305–375 g). They were housed in groups in cages with water and feed ad libitum. For the surgery and acute recordings, the animals were anaesthetized with urethane (Sigma-Aldrich, St. Louis, MO, USA; 24 g/100 ml-solution) i.p. (1.2 g/kg). The level of anesthesia was controlled by regular testing of pedal withdrawal reflex, and if required, extra doses of urethane were given (0.1-0.2 ml). When in full anesthesia, the animal was placed in a stereotaxic instrument using blunt ear bars. Under additional local anesthesia (Lidocain 20%, Orion Pharma, Espoo, Finland) skin and muscle tissue above the skull over electrodes' target areas were removed. For the reference electrode, a hole was drilled in the skull over the right side of the cerebellum and a small insulin needle (BD Lo-Dose syringe, USA) was inserted in the cerebellum (AP −10 mm, ML: 2–3 mm and DV: 2 mm). A needle (18G, Terumo, Somerset, NJ, USA) inserted subcutaneously into the neck served as the ground electrode.

For the hippocampal recordings (Figure 1), intracranial electrodes were implanted (Formvar-insulated stainless steel wire, diameter 50 µm, California Fine Wire Company Co, Grover Beach, CA, USA) through a small hole drilled in the skull. Electrodes with 800-µm tip separation were lowered to the polymorphic layer of dentate gyrus (PoDG) and to the radiatum layer of cornu ammonis 1 (CA1, rad) in the dorsal hippocampus (AP: −3.1 mm, ML: 1.7 mm and DV -3.6 mm). Next, skull was removed over a 2×2 mm region in the left primary auditory cortex (from bregma anterior posterior (AP): −4.5–(−6.5) mm, dorsoventral (DV) 3–5 mm lateral to the bone edge of the upper skull surface), but the dura was left intact.

A tip of a Teflon-insulated stainless steel wire (diameter 200 µm, A-M Systems, Carlsberg, WA, USA) was placed on the surface of the dura above the auditory cortex as guided by local-field potentials on-line recorded in response to 4000 Hz stimuli (presented as the standard tone in the actual experiment).

2.2. Stimuli and procedure

Sinusoidal tones were 50 ms in duration (including 10-ms onset- and offset-ramps). The sound pressure level was measured with a sound level meter (type 2235, Bruel & Kjaer, Nærum Denmark) with C-weighting (optimized for 40-100 dB measurement). The sound pressure level was about 80 dB (SPL) at the location of the animal's right pinna. The stimulation was controlled by E-prime software (Pittsburg, PA, USA) and the tones delivered via a passive loudspeaker placed at 20 cm from and directed to the right ear of the animal.

Figure 2 shows the experimental stimuli. In the oddball condition, eight tones of different frequencies (2200, 2700, 3200, 3700, 4300, 4800, 5300, 5800 Hz) were used as deviant tones (P = 1/9) that were interspersed with a standard tone of 4000 Hz (P = 8/9). The serial order of the

tones was random except that there were always at least two occurrences of the standard tone between consecutive occurrences of a deviant tone. The onset-to-onset interval between the tones in a series was 425 ms. The oddball condition comprised a total of 5760 tones. In the equiprobability condition, the 9 frequencies occurred randomly (i.e., P = 0.11 for each frequency). The tones in the equiprobability condition are hence termed as control-deviant tones. There were 80 repetitions of each 9 tone, resulting in 560 tones in the equiprobability condition.

2.3. LFP recordings

After surgery, the right ear bar was removed and recording started. Local-field-potentials were first amplified 10-fold with the MPA8I preamplifier (Multi Channel Systems MCS GmbH, Reutlingen, Germany), high-pass filtered at 0.1 Hz, low-pass filtered at 5000 Hz, and 50-fold amplified with an FA32I filter amplifier (Multi Channel Systems MCS GmbH), low-pass filtered at 400 Hz with a CyberAmp 380 filter amplifier (Molecular Devices Corporation, Sunnyvale, CA, USA), and finally sampled with 16-bit precision at 2 kHz (DigiData 1320A, Molecular Devices Corporation). The data were stored on a computer hard disk with Axoscope 9.0 data acquisition software (Molecular Devices Corporation, Sunnyvale, CA, USA).

2.3.3. Off-line data analysis

Offline data analyses were performed using Vision Analyzer software (Brain Products, Gilching, Germany). First, data segments with artifacts were removed from the data. The artifacts referred to voltage steps larger than 300 μV/ms. The removals comprised the signal from 200 ms before to 200 ms after the artifact. Visual inspection of the data indicated no other types of artifacts in the data.

The data were offline-filtered (0.1–30 Hz, 24 dB/octave, Butterworth Zero Phase filters), segmented for each type deviant tone and its immediately preceding standard tone in oddball condition, and for each type of control-deviant tone in equiprobability condition. Data segments were baseline corrected against the mean of the signal during a 50-ms time window prior to tone onset.

Finally, the artifact-free data segments were averaged for each animal separately for different deviant tone types, standard tones and control-deviant tones. At least 64 sweeps were included in an average, the mean number of sweeps per average being 77.8 for the standard tones and 78.3 for the deviant tones.

2.3.4. Histology

After the recordings, the locations of the tips of the intracranial electrodes were electrically marked in the tissue (anodal 30-µA 5-s current). The animals still in anesthesia were sacrificed by cervical dislocation. Their brains were removed from the skull for immersion post-fixation for 4 h in 4 % paraformaldehyde (PFA) solution followed by 30 % sucrose solution for two days. The brains were stored in the 30 % ethylene glycol solution −20°C until slicing. Coronal sections (thickness 35 µm) were cut with a freezing slide microtome. The electrode locations were verified from the sections by cresyl violet and Prussian blue staining and the exact locations of the electrode tips were confirmed by microscope observation (Figure 1). The number animals with successfully implanted electrodes to the target areas were 11 for the auditory cortex, 10 for the CA1, and seven for the dentate gyrus.

2.3.5. Statistical analyses

LFPs were averaged for the deviant tone of each frequency, the control-deviant tone of each frequency, the standard tone preceding the deviant tone of each frequency and as interspersed among the control-deviant tones in each animal and brain location. From these averages, mean amplitudes were calculated for the time windows of 25-74.5 ms, 75-124.5 ms, 125-174.5 ms, and 175-224.5 ms.

The statistical analyses were performed with SPSS for Windows (SPSS Inc., Chicago, IL, USA).

To assess whether differential responses were elicited by the deviant tones, a repeated measures of analysis of variance (ANOVA) was performed for the mean amplitude values of each time window with factors stimulus type (deviant vs. standard), deviance direction (ascending vs. descending), and deviance magnitude (±300, ±800, ±1300 vs. ±1800 Hz). Paired t-tests (two-tailed) were used as post-hoc tests for breaking down interactions of stimulus type with other factors.

Furthermore, to assess the standard-specificity of differential responses, if observed, in a specific time window to the deviant tones, repeated measures ANOVA models, or paired t-tests (two tailed), were used to compare the response amplitudes between the control-deviant tones and the deviant tones (Astikainen et al., 2011) and between the control-deviant tones and the standard tone (Ruusuvirta et al., 1998). To indicate the standard-specificity, the former comparison was expected to yield a significant difference (higher response amplitudes for the deviant tones than the control-deviant tones). The latter comparison, in turn, was expected to reveal a non-significant result (no differential responses to the control-deviant tones relative to the standard tone) to indicate this specificity. These comparisons were also made on a sample-point-by-sample-point basis using paired t-tests (two tailed).

Moreover, to assess the contributions of both the occurring probabilities and the frequencies of the tones to response amplitudes, a repeated measures ANOVA was performed for a given time window values with sound-frequency (standard frequency, deviant frequency) and stimulus type (oddball condition, equiprobability condition) as factors.

To counteract Type 1 error due to multiple comparisons in sample-point-by-sample-point comparisons, an alpha level of .05 had to be reached for at least 10 consecutive data points to consider a robust response amplitude difference to exist. Huynh-Feldt-corrected (if not otherwise stated) degrees of freedom were used whenever the sphericity assumption was violated. The P values were reported as corrected but the degrees of freedom as uncorrected. Partial eta squared (η_p^2) was used as an index of an effect size estimates for ANOVA and Cohen's *d* for t-tests.

--- Insert Figure 1 about here ---

--- Insert Figure 2 about here ---

3. Results

Figure 3 illustrates responses to the deviant tones and the standard tone (oddball condition), and to the control-deviant tones (equiprobability condition) in each recording site. Overall, the responses peaked at about 35 ms from stimulus-onset and descended back to the baseline (or below) by about 175 ms from stimulus onset. The polarity of the peak was positive in the auditory cortex and negative in the hippocampal sites.

3.1. The primary auditory cortex

3.1.1. Deviant vs. standard -comparisons

In the time window of 25-74.5 ms, neither significant main effect of stimulus type nor its significant interactions with other main effects were found.

In the time window of 75-124.5 ms, there was a stimulus type (deviant, standard) × deviance direction (below-standard frequencies, above-standard frequencies) -interaction, $F(1,10) = 5.80$, $p = 0.037$, $\eta_p^2 = 0.37$. Subsequent paired- t-tests (two-tailed) revealed a significant deviant-standard response amplitude difference for the ascending, $t(10) = 3.22$, $p = .009$, $d = 0.80$, but not for the descending, $t(10) = 0.33$, $p = .752$, $d = 0.08$, deviant tones.

Also, a significant stimulus type × deviance direction × deviance magnitude (±300, ±800, ±1300, ±1800 Hz) –interaction was found, $F(3,30) = 4.05$, $p = 0.016$, $\eta_p^2 = 0.28$. Subsequent paired t-tests further indicated higher-amplitude responses to the 5300-Hz deviant tones, $t(10) = 4.25$, $p = .002$, $d = 1.11$, and to the 5800-Hz deviant tones, $t(10) = 3.15$, $p = .010$, $d = 0.92$, than to the standard tone, the remaining comparisons indicating no significant differences ($p \geq 0.181$) (Figure 4).

In the time windows of 125-174.5 ms and 175-224.5 ms, neither the main effect of stimulus type nor its interactions with other main effects were found significant.

The comparisons described above, thus, indicated differential responses to the deviant tones in the time window of 75-124.5 ms in the auditory cortex.

3.1.2. Control-deviant vs. deviant and control-deviant vs. standard -comparisons

In the time window of 75-124.5 ms, in which differentia responses were observed to the deviant tones relative to the standard tone, a repeated measures ANOVA with stimulus type (deviant vs. control-deviant) and deviance direction (below-standard frequencies, above-standard frequencies) as factors indicated neither a main effect of stimulus type, $F(1,10) = 0.53$, $p = 0.482$, $\eta_p^2 = 0.05$,

nor its interaction with deviance direction, $F(1,10) = 0.10$, $p = 0.761$, $\eta_p^2 = 0.10$. A repeated measures ANOVA with stimulus type (control-deviant vs. standard) and deviance direction as factors indicated no significant main effect of stimulus type, $F(1,10) = 0.30$, $p = 0.597$, $\eta_p^2 = 0.03$, but its trend-level interaction with deviance direction, $F(1,10) = 4.45$, $p = 0.060$, $\eta_p^2 = 0.31$.

Paired t-tests indicated no significant response amplitude differences ($p \geq 0.232$) between the 5300-Hz as well as 5800-Hz deviant tones and their corresponding control-deviant tones. Consistently, both the 5300-Hz control-deviant tones, $t(10) = 3.24$, $p = 0.009$, $d = 0.99$, and the 5800-Hz control-deviant tones, $t(10) = 2.75$, $p = 0.021$, $d = 0.80$ differed in response amplitude from the standard tone.

Thus, according to the comparisons described above, differential responses to the deviant tones could not be regarded as standard-specific because such responses were also observable to the control deviant tones not preceded by the standard tone and because responses to the deviant tones were not higher in amplitude in comparison to the control-deviant tones.

3.1.3. Test for stimulus condition × sound frequency -interaction

In the time window of 75-124.5 ms, there was a main effect of sound frequency (below-standard frequencies, standard frequency, above-standard frequencies), $F(2,20) = 10.80$, $p = 0.003$, $\eta_p^2 = 0.519$, but not of stimulus condition (equiprobability, oddball), $F(1,10) = 1.93$, $p = 0.195$, $\eta_p^2 = 0.161$ or the interaction between the two, $F(2,20) = 1.31$, $p = 0.292$, $\eta_p^2 = 0.116$.

In the time window of 75-124.5 ms, a repeated measures ANOVA with sound frequency (4000 Hz, 5300 Hz) and stimulus condition as factors indicated a main effect of sound frequency,

$F(1,10) = 20.76$, $p = 0.001$, $\eta_p^2 = 0.675$, but no significant interaction between the two, $F(1,10) = 0.08$, $p = 0.781$, $\eta_p^2 = 0.008$. Likewise, a repeated measures ANOVA with sound frequency (4000 Hz, 5800 Hz) and stimulus condition as factors indicated a main effect of sound-frequency, $F(1,10) = 16.19$, $p = 0.002$, $\eta_p^2 = 0.618$, but, again, no interaction between the two, $F(1,10) = 1.38$, $p = 0.267$, $\eta_p^2 = 0.121$ (Figure 5).

According to the tests described above, differential responses in the auditory cortex to the deviant tones relative to the standard tone indicated different frequencies of these tones with no observable role of their occurring probabilities.

--- Insert Figure 3 about here ---

--- Insert Figure 4 about here ---

--- Insert Figure 5 about here ---

3.2. CA1 of the hippocampus

3.2.1. Deviant vs. standard -comparisons

In the time window of 25-74.5 ms, a repeated measures ANOVA with stimulus type (deviant, standard), deviance direction (below-standard frequencies, above-standard frequencies) and deviance magnitude (±300, ±800, ±1300, ±1800 Hz) as factors indicated a marginally significant three-way interaction, $F(3,27) = 2.86$, $p = 0.056$, $\eta_p^2 = 0.24$.

In the other time windows, neither a main effect of stimulus type nor its interactions with the other main effects were significant ($p \geq 0.172$).

The three-way interaction described above was at trend level, and it concerned an unusually early latency range for differential responses (e.g., Astikainen et al., 2011) that also preceded such responses observed in the auditory cortex (Ruusuvirta et al., 2013). Therefore, it is unlikely to indicate differential responses to the deviant tones.

3.3. Dentate gyrus of the hippocampus

3.3.1. Deviant vs. standard -comparisons

In the time window of 25-74.5 ms, no main effects or their interactions were found ($p \geq 0.268$).

In the time window of 75-124.5 ms, a stimulus type (deviant, standard) × deviance direction (below-standard frequencies, above-standard frequencies) -interaction was only marginally significant, $F(1,6) = 5.63$, $p = 0.055$, $\eta_p^2 = 0.48$.

In the time window of 125-174.5 ms, stimulus type × deviance direction interaction was significant, $F(1,6) = 6.35$, $p = 0.045$, $\eta_p^2 = 0.51$. Post-hoc paired t tests (two-tailed) further indicated that the deviant-standard amplitude difference was at trend level with the ascending, $t(6) = 2.02$, $p = 0.090$, $d = 0.62$, but non-significant with descending, $t(6) = 1.35$, $p = 0.225$, $d = 0.88$, deviant tones.

In the time window of 175-224.5 ms, there was a significant stimulus type × deviance direction interaction, $F(1,6) = 6.64$, $p = 0.042$, $\eta_p^2 = 0.53$. No significant standard-deviant amplitude

differences were separately found for the ascending, t(6) = 1.58, p = 0.166, *d* = 0.67, or descending, t(6) = 1.44, p = 0.199, *d* = 0.94, deviant tones.

The comparisons described above, thus, indicated the generation of differential responses in the time window of 125-224.5 ms in the dentate gyrus to the deviant tones.

3.3.2. Control-deviant vs. deviant and control-deviant vs. standard -comparisons

In the time window of 125-174.5 ms, a repeated measures ANOVA with stimulus type (deviant vs. control-deviant) and deviance direction (below-standard frequencies, above-standard frequencies) as factors indicated neither a main effect of stimulus type, F(1,6) = 0.096, p = 0.767, η_p^2 = 0.016 nor its interaction with deviance direction, F(1,6) = 3.72, p = 0.102, η_p^2 = 0.382. A repeated measures ANOVA with stimulus type (control-deviant vs. standard) and deviance direction as factors indicated neither a main effect of stimulus type, F(1,6) = 0.86, p = 0.390, η_p^2 = 0.125 nor their interaction, F(1,6) = 0.60, p = 0.467, η_p^2 = 0.091.

In the time window of 175-224.5 ms, a repeated measures ANOVA with stimulus type (deviant vs. control-deviant) and deviance direction as factors indicated neither main effect of stimulus type, F(1,6) = 0.073, p = 0.796, η_p^2 = 0.012 nor its interaction with deviance direction, F(1,6) = 3.43, p = 0.113, ηp2 = 0.364. A repeated measures ANOVA with stimulus type (control-deviant vs. standard) and deviance direction as factors indicated a trend for a main effect of stimulus type, F(1,6) = 4.75, p = 0.072, ηp2 = 0.442. Their interaction was non-significant, F(1,6) = 0.265, p = 0.625, ηp2 = 0.042.

Due to the negative findings from control-deviant vs. standard comparisons described above, differential responses to the deviant tones could be regarded as standard-specific in the dentate

gyrus in the time window of 125-174.5 ms (although not of 175-224.5 ms due to the marginally significant finding for that window).

3.3.3. Test for stimulus condition × sound frequency -interaction

In the time window of 125-174.5 ms, a repeated measures ANOVA with sound frequency (below-standard frequencies, standard frequency, above -standard frequencies) and stimulus condition (oddball, equiprobability) as factors indicated no main effect of sound frequency, $F(2,12) = 0.64$, $p = 0.515$, $\eta p2 = 0.096$, stimulus condition, $F(1,6) = 0.88$, $p = 0.386$, $\eta p2 = 0.127$, or their interaction, $F(2,12) = 1.92$, $p = 0.197$, $\eta p2 = 0.243$.

In the time window of 175-224.5 ms, a repeated measures ANOVA with sound frequency and stimulus condition as factors indicated no main effect of sound frequency, $F(2,12) = 1.23$, $p = 0.323$, $\eta p2 = 0.170$, stimulus condition, $F(1,6) = 0.72$, $p = 0.797$, $\eta p2 = 0.012$, or their interaction, $F(2,12) = 2.66$, $p = 0.116$, $\eta p2 = 0.307$.

The comparisons described above, thus, did not indicate the effect of the frequencies themselves of the tones or of the occurring probabilities of the tones of these frequencies on responses to the tones.

3.4. Control-deviant vs. deviant and control-deviant vs. standard –comparisons on a sample point-by-sample point basis

Deviant vs. standard and control-deviant vs. standard comparisons were also performed on a sample point-by-sample point basis (Table 1). These comparisons could only be made for the auditory cortical recordings as the positive results from the dentate gyrus recordings solely relied on a stimulus type × deviance direction in a repeated measures ANOVA for time-window

average values with no significant findings in post-hoc comparisons. The auditory cortical comparisons indicated that the longest time period of standard-specific differential brain responses to the deviant tones (as indexed by a significant response amplitude difference between the deviant tones and the standard tone and, in the same latency range, the absence of such a difference between the control-deviant tones and the standard tone) was too short (8.5 ms between 109.0 ms and 117.5 ms response time points) to indicate the plausible existence of any functionally distinct brain process dedicated to auditory change.

----Insert Table 1 about here---

Discussion

We found differential responses to the frequency-deviant tones relative to the standard tone in the time window of 75-124.5 ms in the auditory cortex of urethane anesthetized rats. In the dentate gyrus, such responses were observed in the time window of 125-224.5 ms. No concomitant differential responses were observed in CA1. In the dentate gyrus, differential responses to the deviant tones were found to be specific to the standard tone in the time window of 125-174.5 ms as such responses were not observable to the control-deviant tones (not preceded by the standard tone). In the auditory cortex, differential responses did not similarly show standard specificity despite this specificity was tested in two alternative ways (analyses with time-window amplitude averages and with values of individual sample points). Most importantly, these responses could be fully explained by the frequencies themselves of the tones, the occurring probabilities of the tones playing no observable role.

Our findings are, in many respects, in contrast to previous findings in rats. First, differential responses were observed in the dentate gyrus but they were not accompanied by the activity in

CA1 (Ruusuvirta et al., 2013). There was only trend-level CA1 activity in the time window of 25-74.5 ms, and this activity was observed in an earlier latency range than expected (Astikainen et al., 2011). It was also not simultaneous with the dentate activity (Ruusuvirta et al., 2013) observed not until in the time window of 125-174.5 ms. Second, differential responses in the auditory cortex unexpectedly appeared to precede those in the dentate gyrus (Ruusuvirta et al., 2013). Third, differential responses in the auditory cortex were not found to be affected by the removal of the standard tone from the series, i.e., by the switch from the oddball condition to the equiprobability condition (Ruusuvirta et al., 1998; Ahmed et al., 2011; for freely moving rats, see, Harms et al., 2015). Fourth, and most importantly, these responses could not even be linked to the rarity of the deviant tones relative to the standard tone but merely to the fact that these tones were of different sound frequencies.

The explanation of these unexpected findings remains to elucidated. Even the fact that a high number of frequency deviant variants (8) was used in the present study is unlikely to account for our findings given that urethane anesthetized rats appear to tolerate deviance-irrelevant physical variability in an oddball series at least with higher-order auditory deviants (e.g., for frequency-intensity combinations, see, Astikainen et al., 2006; Astikainen et al., 2014). Note also that the present data were obtained from a small number of animals, which obviously restricts the conclusions derivable from the data.

Our finding that auditory cortical response amplitudes simply increased towards higher sound frequencies is most likely due to the increasing sensitivity of the rat auditory system towards higher sound frequencies (e.g., Heffner et al., 1994). In the present study, even the electrode position on the dura could hardly play a role as it was chosen on the basis of the maximum

amplitude responses to a tone in the middle (4000 Hz) of the sound frequency range used, that is, to a tone used as the standard tone in the actual experiments.

If replicable, this finding also suggests a dissociation of non-contextual auditory processing (not sensitive to stimulus occurring probability) from contextual auditory processing (sensitive to stimulus occurring probability, Jääskeläinen et al., 2004; von der Behrens et al., 2009; May and Tiitinen, 2009; Taaseh et al., 2011) and further from auditory change detection per se (Näätänen, 1990; Farley et al., 2010). Such non-contextual mechanisms might play a key role to allow the attentional tuning of the auditory cortex to specific frequency content (da Costa et al., 2013) free from degradation by the repetition of a sound carrying this content.

As standard-specific differential responses were only found in the dentate gyrus, it would be tempting to attribute the hippocampus as an active source of such responses. However, one cannot exclude a possibility of other auditory cortical areas, such as AII (Pincze et al., 2001), generating the responses cortically and even being thereby necessary for the hippocampal response to emerge.

All in all, the main lesson to be learned from our findings is obviously that in MMN studies in animals, a strict control for confounding by physical sound features is always needed. This need becomes particularly important when a given relative deviant-standard difference is construed from high absolute physical feature values that each may activate the brain differently.

To conclude, as the main finding, we found that auditory cortical brain responses to tones can be altered by the frequencies of the tones even without a contribution from their different occurring probabilities despite standard-specific responses can be observed in the hippocampus. This finding remains to be verified and explained in future studies. Nevertheless, it suggests, although

only tentatively, a novel type of dissociation between the non-contextual afferent registration of a tone and the detection of the rarity, or change, of the tone relative to the preceding tones. Most importantly, it emphasizes a need for careful experimental control over physical auditory characteristics in MMN studies in animals.

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Figure legends

Figure 1. Electrode locations. The representative histological cresyl violet and Prussian blue stained sections and a drawing of a coronal section of a rat brain -3.1 mm from bregma illustrate the locations of electrodes in the CA1 radiatum layer (rad), in the polymorphic layer of the dentate gyrus (PoDG). Adapted from Paxinos and Watson (2007).

Figure 2. Experimental stimuli. In the oddball condition, the deviant tones of 8 different non-standard frequencies rarely (p = 0.11) replaced the repetitive standard tone. In the equiprobability condition, control stimuli of 9 different frequencies were presented with the same probability (p = 0.11).

Figure 3. Grand-averaged LFP responses from the auditory cortex (ACx) and the hippocampus (CA1 and DG). The left panels show responses to ascending and descending deviant tones (across their different deviance magnitudes), their corresponding standard tones, and control-deviant tones (the deviant tones presented in the equiprobability condition). The right panels show deviant-standard and control-deviant-standard differential waveforms calculated from the responses depicted in the left panels. Black rectangles below the x-axes refer to the tone. The four consecutive 50-ms time segments for calculating average response amplitudes are illustrated in each panel.

Figure 4. Grand-averaged LFP responses in the auditory cortex to each type of deviant tones and to the standard tones (a response averaged across all deviant-specific sets of standard tones).

Figure 5. Grand-averaged LFP responses in the auditory cortex to the tones of 4000, 5300 and 5800 Hz presented in the oddball condition (OB) and the equiprobability condition (EQ). The LFP response for the OB standard is calculated as the mean amplitude values for the responses immediately preceding deviant tones of 5300 Hz and 5800 Hz. The rectangle refers to the time window of 75-124.5 ms for calculating average response amplitude.

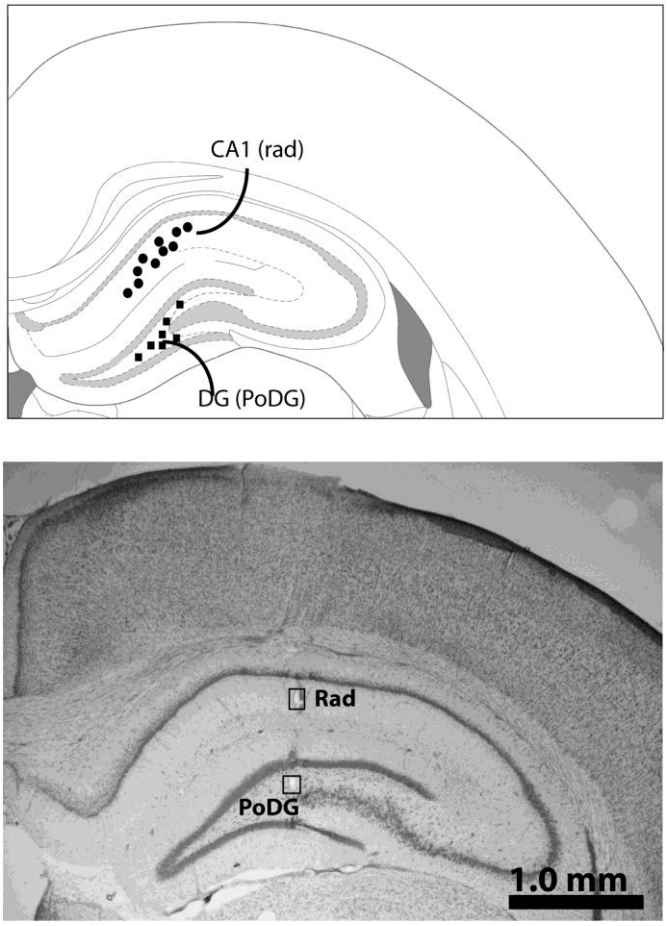

Figure 1

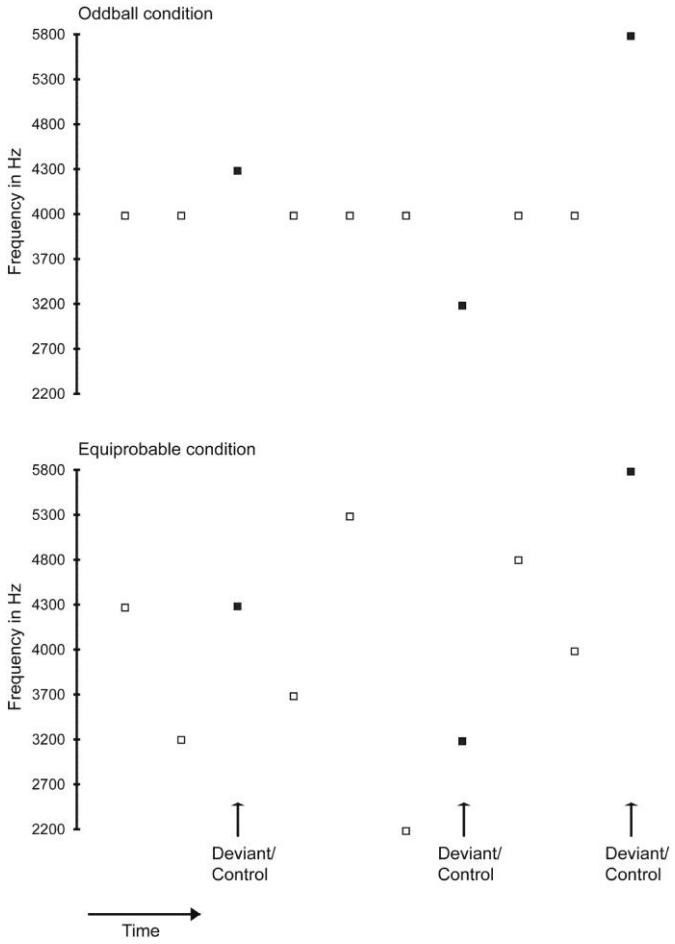

Figure 2

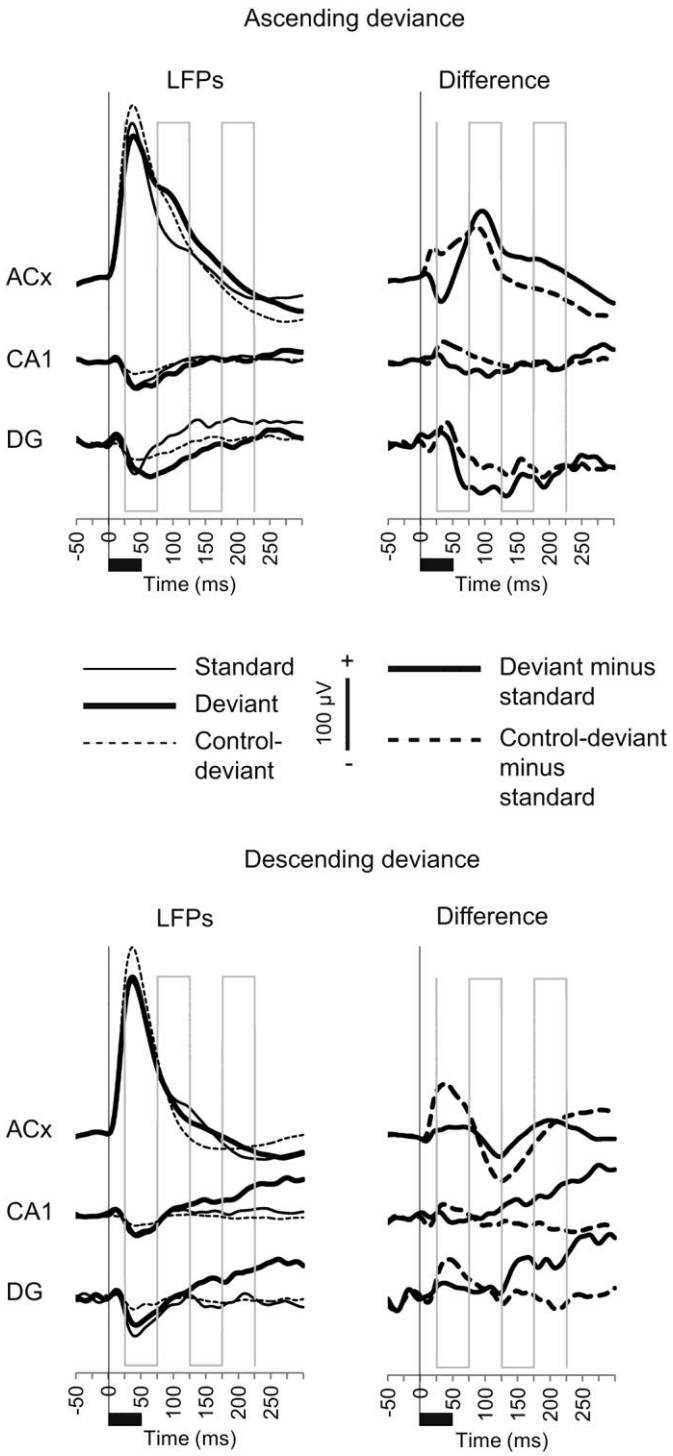

Figure 3

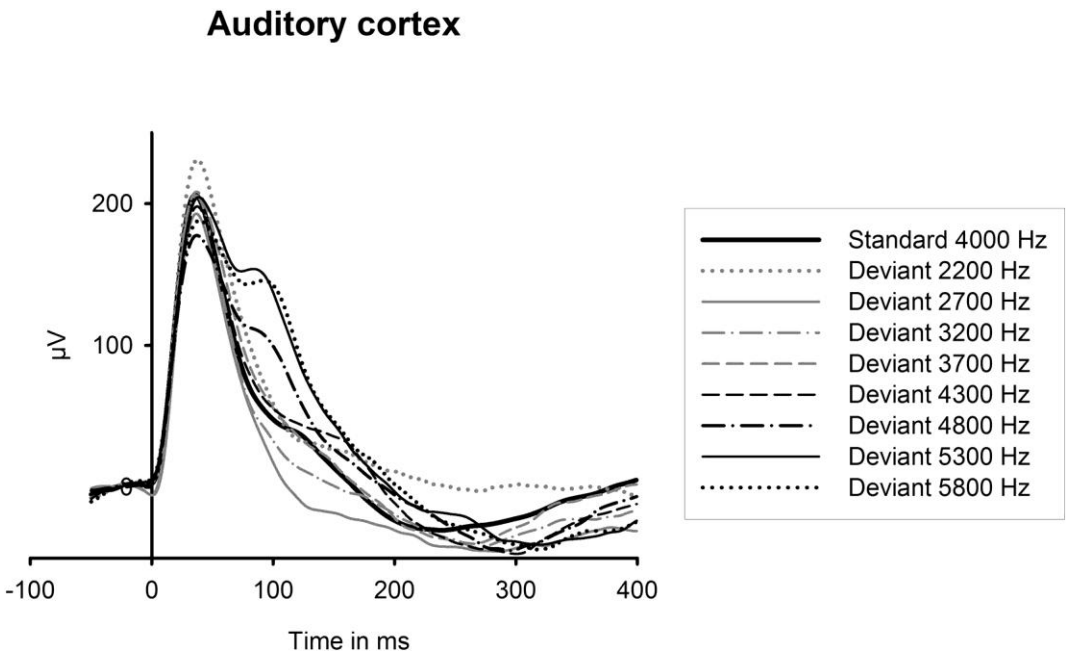

Figure 4

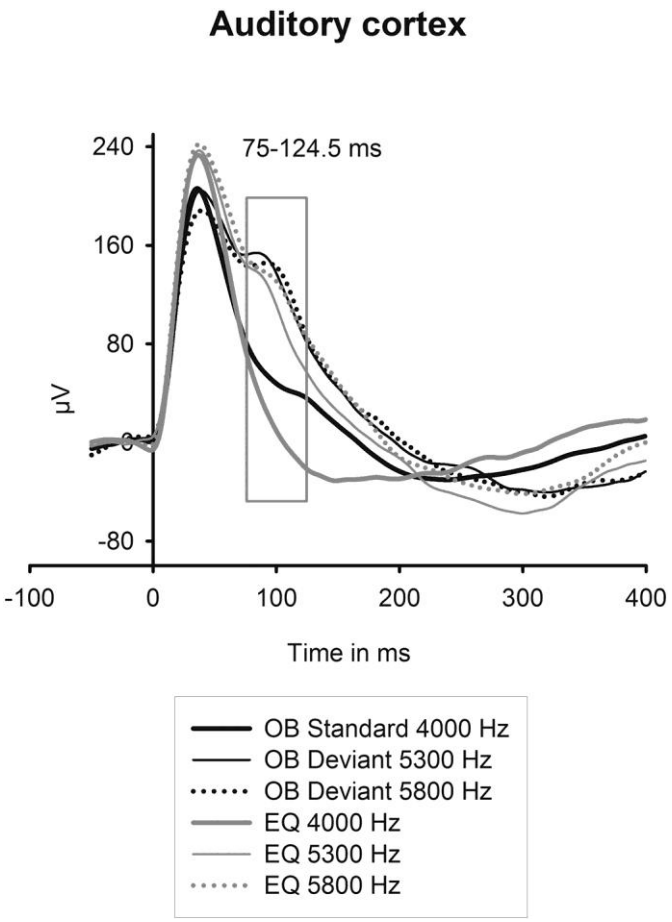

Figure 5

Table 1. Point-by-point paired t-tests on auditory cortical response amplitudes. The latency range of differential responses refers to a significant amplitude difference (for at least 10 consecutive sample points) between the deviant tones and the immediately preceding standard tones. The latency range of the standard-specific parts of these responses referred to the latency range in which the control-deviant tones did not significantly differ in response amplitude from the standard tones. Note that there were no significant differences in response amplitude between the deviant tones and the control-deviant tones.

Deviant type	**Latency range (ms) of differential responses**	**p**	**t**	**Latency range (ms) of standard-specific part of differential responses**
5800-Hz deviant tones	67.0-117.5	.049-.003	2.24-3.82	109.0-117.5[1]
5300-Hz deviant tones	51.5-123.0	.049-.001	2.24-4.99	117.5-123.0[2]

[1] Control-deviant vs. standard tone, t = 1.858 – 2.232, p = .093 – .05 (109.0 – 117.5ms)

[2] Control-deviant vs. standard tone, t = 1.770 – 2.195, p= .107 – .053 (117.5 – 123.0 ms)

Highlights

- Auditory cortical and hippocampal electrical activity in anesthetized rats.
- Frequency-deviant tones presented with and without standard tone context.
- Context-dependent hippocampal but not auditory cortical responses to deviants.
- Sound frequencies alone explaining auditory cortical responses.