

**This is a self-archived version of an original article. This version may differ from the original in pagination and typographic details.**

**Author(s):** Yang, Tiantian; Hämäläinen, Jarmo; Lohvansuu, Kaisa; Lipponen, Arto; Penttonen, Markku; Astikainen, Piia

**Title:** Deviance detection in sound frequency in simple and complex sounds in urethane-anesthetized rats

**Year:** 2021

**Version:** Accepted version (Final draft)

**Copyright:** © 2019 Elsevier B.V. All rights reserved.

**Rights:** CC BY-NC-ND 4.0

**Rights url:** <https://creativecommons.org/licenses/by-nc-nd/4.0/>

**Please cite the original version:**

Yang, T., Hämäläinen, J., Lohvansuu, K., Lipponen, A., Penttonen, M., & Astikainen, P. (2021). Deviance detection in sound frequency in simple and complex sounds in urethane-anesthetized rats. *Hearing Research*, 399, Article 107814. <https://doi.org/10.1016/j.heares.2019.107814>

# Journal Pre-proof

Deviance detection in sound frequency in simple and complex sounds in urethane-anesthetized rats

Tiantian Yang, Jarmo Hämäläinen, Kaisa Lohvansuu, Arto Lipponen, Markku Penttonen, Piia Astikainen



PII: S0378-5955(19)30270-9

DOI: <https://doi.org/10.1016/j.heares.2019.107814>

Reference: HEARES 107814

To appear in: *Hearing Research*

Received Date: 11 June 2019

Revised Date: 4 October 2019

Accepted Date: 9 October 2019

Please cite this article as: Yang, T., Hämäläinen, J., Lohvansuu, K., Lipponen, A., Penttonen, M., Astikainen, P., Deviance detection in sound frequency in simple and complex sounds in urethane-anesthetized rats, *Hearing Research*, <https://doi.org/10.1016/j.heares.2019.107814>.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2019 Elsevier B.V. All rights reserved.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24

**Deviance detection in sound frequency in simple and complex sounds in urethane-anesthetized rats**

Tiantian Yang, Jarmo Hämäläinen, Kaisa Lohvansuu, Arto Lipponen, Markku Penttonen, Piia Astikainen \*

Affiliation of all authors: Department of Psychology, University of Jyväskylä, Jyväskylä, Finland;

Address: Department of Psychology, P.O. Box 35, 40014 University of Jyväskylä, Finland

\*Corresponding author:

Piia Astikainen

Phone: +358408053480

facsimile: +358142601021

e-mail: piia.astikainen@jyu.fi

Tiantian Yang: tiantian.t.yang@student.jyu.fi

Jarmo Hämäläinen: jarmo.a.hamalainen@jyu.fi

Kaisa Lohvansuu: kaisa.lohvansuu@jyu.fi

Arto Lipponen: arto.j.lipponen@jyu.fi

Markku Penttonen: markku.penttonen@jyu.fi

25 **Abstract**

26

27 Mismatch negativity (MMN), which is an electrophysiological response demonstrated in  
28 humans and animals, reflects memory-based deviance detection in a series of sounds.

29 However, only a few studies on rodents have used control conditions that were sufficient in

30 eliminating confounding factors that could also explain differential responses to deviant

31 sounds. Furthermore, it is unclear if change detection occurs similarly for sinusoidal and

32 complex sounds. In this study, we investigated frequency change detection in

33 urethane-anesthetized rats by recording local-field potentials from the dura above the

34 auditory cortex. We studied change detection in sinusoidal and complex sounds in a series of

35 experiments, controlling for sound frequency, probability, and pattern in a series of sounds.

36 For sinusoidal sounds, the MMN controlled for frequency, adaptation, and pattern, was

37 elicited at approximately 200 ms onset latency. For complex sounds, the MMN controlled for

38 frequency and adaptation, was elicited at 60 ms onset latency. Sound frequency affected the

39 differential responses. MMN amplitude was larger for the sinusoidal sounds than for the

40 complex sounds. These findings indicate the importance of controlling for sound frequency

41 and stimulus probabilities, which have not been fully controlled for in most previous animal

42 and human studies. Future studies should confirm the preference for sinusoidal sounds over

43 complex sounds in rats.

44

45

46 **Keywords:** auditory cortex, change detection, local-field potentials, mismatch negativity, rat

Journal Pre-proof

48 **Abbreviations**

49 CSAS: Cascade ascending control condition

50 MMN: Mismatch negativity

51 MST: Many-standards control condition

52 NMDA: N-methyl-D-aspartate

Journal Pre-proof

53

54

## 1. Introduction

55

Detecting changes in sensory environment is important for adaptive behavior. Brain

56

responses that reflect change detection are studied using so-called oddball stimulus condition

57

in which a rare “deviant” stimulus is interspersed with a frequent “standard” stimulus.

58

Recordings of the brain’s electrical responses to oddball stimuli elicit the mismatch negativity

59

(MMN) component (Näätänen et al., 1978). The MMN in the auditory domain has been well

60

documented in both humans and animals (Harms et al., 2016; Näätänen et al., 2010), and it

61

has the potential for clinical applications (Näätänen, 2000; 2003).

62

63

MMN is a response to a rare stimulus that violates the regularity formed by a repetitive

64

standard stimulus (Näätänen et al., 2005). According to the memory-comparison hypothesis

65

the brain compares an incoming (deviant) stimulus with the memory trace formed by a

66

standard stimulus. When these two are found to differ, MMN is elicited (Näätänen et al.,

67

2005). Another possible explanation for differential responses to deviant stimuli (in

68

comparison to standard stimuli) is the different levels of adaptation in the neural populations

69

responding to frequent and rare sounds because the neurons that respond to standard sound

70

features are more frequently activated than are the neurons that respond to deviant sound

71

features (May & Tiitinen, 2010).

72

73

Different control conditions have been developed to study whether mere adaptation can

74

explain differential responses elicited in the oddball condition (Harms et al., 2016). Currently,

75 the most frequently used control condition is the many-standards condition, which is also  
76 termed the equal probability condition (Ruhnau et al., 2012; Schröger & Wolff, 1996). It  
77 contains several other stimuli in addition to the deviant and standard stimuli in the oddball  
78 condition. In the many-standards condition, stimuli are presented otherwise in a random order,  
79 but without consecutive repetitions of the same sound. Also, the probability of each sound is  
80 the same as the probability of the oddball-deviant sound. Therefore, the oddball-deviant  
81 stimulus and control stimulus differ only by the context in which they are presented:  
82 regularity formed by a standard stimulus in the oddball condition vs. continuously changing  
83 stimuli without regularity in the many-standards condition. Importantly, because the  
84 oddball-deviant stimulus and its control sound have the same probability of occurrence, the  
85 responses they elicit include the same level of adaptation. In humans, in the many-standards  
86 control condition, it has been demonstrated that the differential response found in the oddball  
87 condition cannot be explained by adaptation only (Jacobsen & Schröger, 2001; Jacobsen &  
88 Schröger, 2003; Jacobsen et al., 2003; Lohvansuu et al., 2013; Maess et al., 2007) because  
89 oddball-deviant sound elicits larger responses than the physically identical control sound.

90

91 Another appropriate but less used control condition is the cascade condition, which comprises  
92 a fixed pattern of stimuli (Ruhnau et al., 2012). When frequency change detection is  
93 investigated, the stimuli are ordered according to ascending and/or descending frequency, and  
94 the repeated pattern allows the upcoming stimulus to be predictable. This feature is  
95 comparable to the oddball condition in which repeated standard stimuli are predictable.

96

97 Rodent MMN studies using frequency changes in stimuli have applied either the  
98 many-standards (e.g. Astikainen et al., 2011; Farley et al., 2010; Fishman & Steinschneider,  
99 2012; Nakamura et al., 2011; Polterovich et al., 2018; Shiramatsu et al., 2013) or cascade  
100 (Harms et al., 2014; Parras et al., 2017) control conditions. A potential confounder in some  
101 studies is that smaller frequency differences were applied between the sounds in the control  
102 condition than between the stimuli in the oddball condition (Astikainen et al., 2011; Fishman  
103 & Steinschneider, 2012; Jung et al., 2013; Kurkela et al., 2018; Nakamura et al., 2011). Thus,  
104 the results may have been confounded because of different levels of across-frequency  
105 adaptation, a mechanism that reduces responses to the consecutively presented sounds of  
106 nearby frequencies (Taaseh et al., 2011), between the oddball and control condition sounds.  
107 Moreover, the control conditions in previous rodent studies were different from those used in  
108 the original human studies. In previous human studies (Jacobsen & Schröger, 2001; Maess et  
109 al., 2007; Ruhnau et al., 2012; Wiens et al., 2019), the control sounds were at one end of the  
110 frequency range. In previous rodent studies, the control sounds were positioned in the middle  
111 of other sounds (Astikainen et al., 2011; Farley et al., 2010; Fishman & Steinschneider, 2012;  
112 Harms et al., 2014; Nakamura et al., 2011; Parras et al., 2017; Taaseh et al., 2011). It is  
113 possible that the across-frequency adaptation on the responses differed from sounds that were  
114 assigned to the end of the frequency range compared to those in the middle position.

115

116 The aim of the present study is to investigate change detection of sound frequency in  
117 anesthetized rats. The MMN responses were recorded in the dura above the auditory cortex  
118 for two sound frequencies: 4000 Hz and 4600 Hz. Both sound frequencies were assigned as

119 standard and deviant sounds in two reversed oddball series, i.e. the “flip-flop” condition (Fig.  
120 1A). Importantly, the MMN was calculated by comparing the responses to physically  
121 identical sounds presented in the two flip-flop and oddball conditions (Nakamura et al.,  
122 2011).

123

124 Two separate conditions were used to control for adaptation: many-standards and cascade  
125 conditions. The latter also controlled for a predictive pattern in sound series. In addition, the  
126 frequency difference between the control stimuli was the same as the frequency difference  
127 between the standard and deviant stimulus in the oddball condition. Similar to previous  
128 human studies (Jacobsen & Schröger, 2001; Maess et al., 2007; Ruhnau et al., 2012; Wiens et  
129 al., 2019), in the many-standards control condition, the control sounds were positioned to one  
130 end of the frequency range. The controlled MMN reflecting deviance detection (i.e., deviant -  
131 control) and estimation of the repetition suppression (i.e., standard - control) was calculated  
132 as in previous studies (Auksztulewicz & Friston, 2016; Parras et al., 2017).

133

134 Change detection was tested using sinusoidal sounds and intensity-matched complex sounds  
135 consisting of three different frequencies. Previous studies on humans suggested that simple  
136 and complex sounds are processed in different areas of the supratemporal cortex (Alho et al.,  
137 1996; Novitski et al., 2004). Therefore, it was expected that we would observe the MMN  
138 differently as a response of sinusoidal and complex sounds.

139

## 140 **2. Methods**

## 141 2.1. Subjects and surgery

142 The experiments were approved by the Finnish National Animal Experiment Board. The  
143 procedures followed in the care and the use of animals were in accordance with Directive  
144 2010/63/EU.

145

146 Thirteen Sprague-Dawley male rats were used as subjects. They were housed in groups of  
147 four rats in cages with water and feed ad libitum and kept under a 12 h light-dark cycle with  
148 lights on at 7:00 a.m. and off at 7:00 p.m. The rats weighed  $417.6 \pm 26.0$  g (mean  $\pm$  SD) on  
149 the day of the recording. For the surgery and acute recordings, the rats were anesthetized with  
150 urethane (Sigma-Aldrich, St. Louis, MO, USA; 24 g/100 ml-solution, 1.2 g/kg, i.p.). The  
151 level of anesthesia was controlled by regular testing of the pedal withdrawal reflex, and if  
152 required, extra doses of urethane were given (0.2 ml). When under full anesthesia, the rats  
153 were placed in a stereotaxic instrument using blunt ear bars. The temperatures of the rats  
154 were maintained at 38.2 °C using a small animal temperature controller (ATC2000), a rodent  
155 rectal temperature probe, and a heating pad (World Precision Instruments, USA). Before  
156 removing the skin and muscle tissues above the skull over the electrode's target area, local  
157 anesthesia was applied s.c. (bupivacaine, Bicain 5 mg/ml, Orion, Finland). The reference  
158 electrode was a 28 gauge stainless steel needle (outer diameter 0.36 mm, BD Lo-Dose  
159 syringe, USA) inserted into the right side of the cerebellum through a hole drilled 1.5 mm  
160 posterior to lambda and 2.0 mm lateral to midline. The tip of the electrode was lowered 1.0  
161 mm below the brain surface and the electrode was then glued to the skull. The ground  
162 electrode was a similar needle inserted subcutaneously into the left side of the neck.

163 For the auditory epidural recording, a craniotomy was drilled on the squamosal bone of the  
164 skull exposing the dura over the left auditory cortex; from the bregma anterior-posterior (AP):  
165 (- 4.5) – (-6.5) mm, and dorsoventral (DV): 3–5 mm. The recording electrode was a 0.25 mm  
166 diameter silver wire (A-M Systems, WA, USA) positioned on the surface of the dura.

167

168 A stainless-steel screw (1.2 mm in diameter, 3.0 mm in length) was implanted in the right  
169 prefrontal skull and fixed with a plexiglass holder to the stereotaxic instrument by dental  
170 acrylic to stabilize the rat's head and allow the removal of the right ear bar.

171

172 After the experiment, the anesthetized animals were administered an overdose of  
173 pentobarbital by i.p. injection (Mebunat 60 mg/ml, Orion, Finland).

174

## 175 2.2. Stimulation

176 Sinusoidal and complex sounds were applied as stimuli.

177

178 Eleven sinusoidal sounds at frequencies of 2630 Hz, 3024 Hz, 3478 Hz, 4000 Hz, 4600Hz,  
179 5290 Hz, 6084 Hz, 6996 Hz, 8045 Hz, 9252 Hz, and 10640 Hz were applied as stimuli in the  
180 sinusoidal sound experiments. The frequency step between the tones closest to frequency was  
181 15%,  $\Delta f = (f_2 - f_1) / f_1$ . All of the tones were 100 ms in duration (10 ms fade-in and fade-out),  
182 and the sampling frequency was 44100 Hz.

183

184 The eleven complex sounds consisted of three sinusoidal tones. The highest frequency was  
185 the same as the corresponding sinusoidal sound. The other two frequencies of each tone were  
186 calculated as 50% and 20% of the highest frequency. For example, the sinusoidal sound at  
187 4000 Hz determined the complex sound (complex 4000 Hz) in which the highest frequency  
188 was 4000 Hz, and the two sub-tones were 2000 Hz and 600 Hz in frequency. The complex  
189 tones were matched with the sinusoidal sounds in intensity: the sound pressure level in both  
190 was 76.71 dB.

191

192 Oddball conditions at two frequencies, 4000 Hz and 4600 Hz, were applied in a  
193 counterbalanced manner in each animal under the many-standards and cascade-ascending  
194 control conditions (Figure 1). The probability of the standard sound in the oddball condition  
195 was 87.5%. The probability of the deviant sound and each control sound was 12.5%. The  
196 tones in the oddball condition were delivered in a pseudo-random fashion with the restriction  
197 that the consecutive deviant sounds were separated by at least three standard sounds. In the  
198 many-standards control condition, eight tones with frequencies ranging from 4000 Hz to  
199 10640 Hz were presented in a random order. The control tones of 4000 Hz and 4600 Hz had  
200 the lowest frequency of all tones in the sequence. In the cascade-ascending sequence, the  
201 frequencies of eight tones ranged from 2630 Hz to 6996 Hz and were delivered in ascending  
202 order by frequency. The control tones at 4000 Hz and 4600 Hz were presented in the middle  
203 of the sequence.

204

205 Each sequence contained 1200 tones that were presented by a 400-ms stimulus-onset  
206 asynchrony. In addition to these stimulus conditions, an oddball sequence of high frequency  
207 sounds (9252Hz and 10640 Hz) was presented to the animals. These data are not presented  
208 here because the stimuli were not optimal for the electrode location, because there were no  
209 clear responses to these sounds.

210

211 The stimuli sequences were delivered by E-prime software (E-prime 1.1, Psychology  
212 Software Tools, Inc., USA). In the complex sound conditions, the same sequences were  
213 presented in a similar manner as the sinusoidal sounds were. The only difference was that the  
214 sinusoidal tones were replaced by the corresponding complex tones, which had the same  
215 highest frequency as in the sinusoidal sounds.

216

### 217 2.3. Recording

218 Before the recording was conducted, the right ear bar was removed. The sounds were  
219 amplified using an SA1 Stereo Power Amp with 6 dB attenuation and MF1 Multi-Field  
220 Magnetic Speakers (Tucker-Davis Technologies, USA) were used to present them. The  
221 loudspeaker was positioned at a distance of 30 cm from the animal's right pinna.

222

223 A continuous electrocorticogram was amplified at two stages. First it was amplified 10-fold  
224 using an MPA8 preamplifier (MultiChannel Systems MCS GmbH, Germany) and then it was  
225 amplified 50-fold using a FA64I filter amplifier (MultiChannel Systems MCS GmbH,

226 Germany). The signals were high-pass filtered at 1 Hz and low-pass filtered at 5 kHz. The  
227 signals were digitized (USB-ME-64, MultiChannel Systems MCS GmbH, Germany) and  
228 recorded using MCRack software (MultiChannel Systems MCS GmbH, Germany) at a  
229 sampling rate of 20 kHz.

230

231 The tip of the silver wire electrode touched the dura, and its position was selected based on  
232 the largest response to test sounds. The test sound was a sinusoidal sound of 1661 Hz at  
233 frequency of 50 ms in duration and 88.44 dB in intensity. It was repeated at onset-to-onset  
234 intervals of 3 s. The sinusoidal and complex sounds and the different types of sequences were  
235 presented randomly and counterbalanced among the subjects.

236

#### 237 2.4. Data analysis

238 The recorded data were analyzed offline using Brain Vision Analyzer (Brain Products,  
239 Gilching, Germany) and MatLab (version 2016b, the MathWorks, Inc, USA). The data were  
240 further filtered at a low bandpass of 30 Hz (24dB / octave, Butterworth filter), down-sampled  
241 to 2 kHz, and then segmented from -50 ms to 350 ms according to the stimulus onset of the  
242 deviant and standard sounds immediately preceding the deviant sounds in oddball conditions  
243 and the corresponding stimuli in the many-standards and cascade-ascending conditions. The  
244 data segments were baseline corrected against the mean of the signals during the 50 ms  
245 pre-stimulus period and then averaged for each stimulus type and animal.

246

247 The responses to the deviant sounds and those to the same frequency tone that served as the  
248 standard in the oddball sequence were compared using a paired, two-tailed *t*-test. The  
249 sample-point-by-sample-point pairs were compared from the stimulus onset to the end of the  
250 sweeps. To counteract type 1 errors due to multiple comparisons, an alpha level of 0.05 was  
251 required in at least 20 consecutive data points (10 ms) (Ruusuvirta et al., 2015). If such robust  
252 differential responses were found in the oddball condition, the responses to the deviant tone  
253 and standard tone were further compared to the same tones in the many-standards and  
254 cascade-ascending conditions. A 10-ms period of significant difference was again set as the  
255 minimum for a robust amplitude difference between the deviant and control sound responses.

256

257 PLEASE INSERT FIGURE 1 APPROXIMATELY HERE

258

259 **3. Results**

260 All the sounds elicited the typical waveform reported in studies using urethane-anesthetized  
261 rats (e.g., Astikainen et al., 2011), where a positive polarity deflection peaked at  
262 approximately 35 ms, and a negative-polarity deflection peaked at approximately 80 ms after  
263 the stimulus onset. In the present study, the MMN was calculated between the deviant and  
264 standard responses elicited by the same frequency sounds. However, the responses to the  
265 standard and deviant responses obtained separately from each oddball condition comparing  
266 different frequency standard and deviant responses are reported in the supplementary material  
267 (Supplement 1).

268

269

## 270 3.1. Responses to sinusoidal sounds

271 The 4000 Hz deviant sound shifted the responses toward negative polarity in comparison to  
272 the 4000 Hz standard sound at 204.5–253.5 ms post-stimulus latency (Figure 2, Table 1). In  
273 this time range, the responses to the 4000 Hz deviant sound were more negative than to the  
274 corresponding control sound in the cascade condition. The same result was found in the  
275 comparison between the 4000 Hz deviant and the corresponding control sound in the  
276 many-standards condition, but the difference began to appear at 215.5 ms after stimulus  
277 onset.

278

279 Regarding the sinusoidal 4600 Hz tones, the responses to the deviant and the standard sounds  
280 differed at 56.5–86.5 ms latency (Figure 2, Table 1). The deviant sound shifted the responses  
281 toward negative polarity compared with the responses to the standard sounds. There were no  
282 differences between the responses to the deviant and control sounds during the same time  
283 period. The response to the 4600 Hz standard tone was smaller (i.e., more positive) than the  
284 response to the same tone in the cascade control condition at 67.5–84.5 ms latency.

285

286 PLEASE INSERT FIGURE 2 APPROXIMATELY HERE

287

## 288 3.2. Responses to complex sounds

289 The response to the 4000 Hz deviant sound was larger toward negative polarity than the  
290 response to the same frequency standard tone at 64.5–84.5 ms latency (Figure 3, Table 1).  
291 The responses to the deviant sound were more negative in amplitude than the responses to the  
292 control sound in the many-standards condition at 64.5–84.0 ms latency, but no difference was  
293 found between the responses to the deviant and the control sounds in the cascade condition.

294

295 Regarding the analysis of repetition suppression, there was no difference between the  
296 responses to the 4000 Hz standard sound and the corresponding sound in the control  
297 conditions.

298

299 No differential response was found to the complex 4600 Hz sound.

300

301

302 PLEASE INSERT FIGURE 3 APPROXIMATELY HERE

303

304 3.3. Comparison of the differential responses to sinusoidal and complex sounds

305 The differential responses between the deviant and standard sounds of the same frequency  
306 were compared using point-by-point t-tests (Figure 4). The differential responses to the 4000  
307 Hz sounds were larger (i.e., greater negative polarity) for the sinusoidal sounds than for the  
308 complex sounds at 209–238 ms after the stimulus onset. The differential responses to the  
309 4600 Hz sounds were also larger (i.e., greater negative polarity) in the sinusoidal than in the  
310 complex sounds at 55.5–85 ms latency.

311

312 PLEASE INSERT FIGURE 4 APPROXIMATELY HERE

313 Table 1. Latencies of the significant differences (in ms).

	Differential response in Oddball	Genuine MMN (MST)	Genuine MMN (CSAS)	Repetition suppression (MST)	Repetition suppression (CSAS)
Sinusoidal 4000 Hz	204.5–253.5	215.5–253.5	204.5–253.5	n.s.	n.s.
Sinusoidal 4600 Hz	56.5–86.5	n.s.	n.s.	n.s.	67.5–84.5
Complex 4000 Hz	64.5–84.5	64.5–84	n.s.	n.s.	n.s.
Complex 4600 Hz	n.s.	n.a.	n.a.	n.a.	n.a.

314 *Note.* Differential response (deviant-standard) was calculated as a difference between the responses to the same

315 frequency sounds obtained in the two oddball conditions. Genuine MMN = Genuine mismatch negativity

316 latency (i.e., when the deviant stimulus responses were larger than the responses to the control stimulus). In the

317 repetition suppression analyses, standard responses were compared with the same frequency sounds of the

318 many-standards (MST) and cascade-ascending (CSAS) control conditions: n.s. = non-significant; n.a. = not

319 applicable (not calculated because there was no differential response in the oddball condition).

320

#### 321 **4. Discussion**

322 The present study was designed to investigate change detection of sound frequency as  
323 reflected by a genuine MMN defined here as a differential response to a rare deviant sound  
324 that reflects the detection of regularity violations. The adaptation effect, which can affect the  
325 responses because of lower probability and thus smaller neural adaptation related to rare than  
326 frequent sounds (May & Tiitinen, 2010), was controlled by applying two control conditions:  
327 the many-standards condition and the cascade ascending condition.

328

329 In this study, the MMN was calculated as the difference between the standard and deviant  
330 responses elicited by the same frequency sounds (e.g., deviant 4000 Hz - standard 4000 Hz).  
331 Sound frequency affected the differential responses, however. When the differential response  
332 was calculated for the 4600 Hz sounds, an early latency differential response (starting at 56.5  
333 ms post-stimulus) was found. However, it was not a genuine MMN because the response to  
334 the deviant stimulus in the oddball condition did not differ from the response to the same  
335 frequency sound in the control conditions. Therefore, the differential response in the oddball  
336 condition cannot be considered a genuine MMN. Instead, the differential response could be  
337 explained by different amounts of neural adaptation to standard and deviant stimuli due to  
338 their different repetition rates in the stimulus series. Furthermore, repetition suppression was  
339 found in this condition: the responses to standards were more adapted than the responses to  
340 the cascade control sounds. No difference was found between the responses to the standard  
341 sounds and those to the control sounds in the many-standards condition. However, a different  
342 result was found for the differential response calculated between the 4000 Hz sinusoidal

343 sounds. The differential response between deviant and standard responses was elicited  
344 starting at 204.5 ms post-stimulus latency, and it reflected the detection of regularity  
345 violations (i.e., a genuine MMN) because the amplitude was higher for the responses to the  
346 deviant sounds than for the responses to the control sounds.

347

348 The pattern of the results differed regarding the complex sounds, which elicited an early  
349 (starting at 64.5 ms post-stimulus) genuine MMN to the 4000 Hz sounds. Similar to the  
350 sinusoidal sounds, the complex sounds elicited no differential response to the 4600 Hz sounds.

351 Regarding the 4000 Hz complex sounds, the oddball-deviant responses and control sound  
352 responses differed only when the control sound was obtained in the many-standards control  
353 condition but not when the control sound response was obtained in the cascade condition.

354 These results indicated that a genuine MMN was not found when the predictive pattern of the  
355 sound sequences was controlled.

356

357 In summary, a genuine MMN was found for both sinusoidal and complex sounds of 4000 Hz,  
358 but not of 4600 Hz frequency. Therefore, the stimulus frequency had a significant effect on  
359 the MMN elicitation, which was observed in the recording electrode on the dura above the  
360 auditory cortex. This finding aligns with previous findings in studies on

361 urethane-anesthetized rats, which showed differential responses to deviant sounds of some  
362 but not all sound frequencies (Harms et al., 2014; Nakamura et al., 2011; Ruusuvirta et al.,

363 2015). In Ruusuvirta et al. (2015), eight different deviant sound frequencies were interspersed

364 with a standard sound. The statistical model also showed that the sound frequency, not the

365 stimulus probability or context, explained the differential responses to the oddball-deviant  
366 sounds (Ruusuvirta et al., 2015). In the present study, the sound frequency was controlled by  
367 applying the flip-flop condition, and the MMN was calculated between the same frequency  
368 standard and deviant sounds. The MMN was elicited to 4000 Hz sounds but not to the 4600  
369 Hz sounds. This result could be explained by the position of the recording electrode, which  
370 could have been more favorable for the mismatch response to the 4000 Hz sounds than to the  
371 4600 Hz sounds. The reason for this could be that the frequency of the test sound guiding the  
372 electrode position was 1661 Hz. A lower frequency test sound comparing to the sinusoidal  
373 sound frequencies applied in the oddball condition was used because the aim was to make the  
374 recording location suitable for both sinusoidal and complex sounds. The latter contained  
375 lower frequencies than the ones applied in the sinusoidal sounds. However, because we did  
376 not record responses from different auditory cortical areas simultaneously (e.g., Shiramatsu et  
377 al., 2013), the ultimate reason for differential responses to 4000 Hz, but not to 4600 Hz,  
378 deviant sounds remains unclear. In future research, the measurement of local-field potentials  
379 with multiple electrodes over the auditory cortex should be combined with an analysis in  
380 which MMN is calculated as the difference between the responses to the same frequency  
381 standard and deviant sounds.

382

383 The results showed that the complex sounds elicited the controlled MMN in early (i.e., before  
384 100 ms) latency and the sinusoidal sounds in later (i.e., after 200 ms) latency. The earlier  
385 response (MMN before 100 ms latency) has been frequently reported in rats and mice as a  
386 genuine MMN controlled for adaptation to speech sounds (Ahmed et al., 2011) and

387 sinusoidal sounds (Astikainen et al., 2011; Kurkela et al., 2018; Nakamura et al., 2011; Parras  
388 et al., 2017; Polterovich et al., 2018). However, some previous results may have been  
389 confounded by the sound frequency because the flip-flop condition was not applied (Ahmed  
390 et al., 2011; Astikainen et al., 2011; Kurkela et al., 2018) or the same frequency standard and  
391 deviant responses were not compared (Parras et al., 2017; Polterovich et al., 2018). Similar to  
392 our study, Nakamura et al. (2011) compared the responses to the same frequency sounds  
393 obtained in the two flip-flop conditions in rats. They found genuine MMN for high (3600 Hz)  
394 frequency sounds but not for low (2500 Hz) frequency sounds. In contrast, our results showed  
395 genuine MMN in lower but not in higher frequency sounds. This difference can be explained  
396 by the electrodes' position, which could have been more optimal for one frequency than the  
397 other.

398  
399 The later response at 200 ms latency, which was a genuine MMN, was elicited only by 4000  
400 Hz sinusoidal sounds; that is, not by 4600 Hz sinusoidal sounds or complex sounds of either  
401 frequency. Late MMN latencies have been observed less often in rodents, but in our previous  
402 mice study, a genuine MMN with a large effect size was found until 255 ms latency in the  
403 descending deviant frequency and 185 ms latency in the ascending deviant frequency  
404 (Kurkela et al., 2018). In a previous study that investigated stimulus-specific adaptation (SSA)  
405 in rats to oddball sounds, a late response at 200–400 ms latency was found in the excitatory  
406 neurons (Chen et al., 2015). Importantly, this late response shared some of the defining  
407 features of the MMN: it was larger for oddball sounds than for control sounds, and it was  
408 reduced when the intracellular N-methyl-D-aspartate (NMDA) receptors were inhibited. Our

409 result showing a late MMN at over 200 ms latency thus extends the previous similar findings  
410 of local-field potentials in mice and single cell responses in rats to local-field potentials in  
411 rats. Further studies are required to elucidate the possible functional roles of early and late  
412 latency MMN in rodents.

413

414 The direct comparison of the differential responses to sinusoidal and complex sounds (Fig. 4)  
415 showed that the sinusoidal sounds elicited larger differential responses than the complex  
416 sounds did before 100 ms latency (4600 Hz sounds) and after 200 ms latency (4000 Hz  
417 sounds). This finding was surprising because previous studies on humans showed that  
418 complex sounds elicited larger brain responses than sinusoidal sounds did (Tervaniemi,  
419 Schröger et al., 2000; Tervaniemi, Ilvonen et al., 2000). The discrepancy between the current  
420 rat study and earlier human studies is likely due to the methodological differences in these  
421 studies. In the present study, the brain responses were measured directly from the dura of the  
422 auditory cortex. Neural generators of complex sound responses are different from those of  
423 sinusoidal sound responses (Alho et al., 1996; Novitski et al., 2004). Therefore, our recording  
424 electrode could have been better placed to detect the responses to the sinusoidal sounds rather  
425 than the complex sounds. Further studies using multiple recording sites in the auditory cortex  
426 in rats are required to clarify this issue. Another reason for the contradictory results could be  
427 that the human brain responds better to complex sounds than to sinusoidal sounds because it  
428 is specialized and/or more exposed to speech sounds, but there is no such preference in the rat  
429 brain.

430

431 The methods applied in the present study included controls that were more stringent than  
432 those used in previous animal studies. However, genuine MMN was elicited by both  
433 sinusoidal and complex sounds in the anesthetized rats. The pattern of results indicate the  
434 importance of controlling for sound frequency, which may be particularly important when a  
435 single intracranial recording electrode is used to study frequency change detection. Although  
436 local-field potentials recorded in the dura capture activity in a large area, the tonotopic  
437 organization of the auditory cortex can affect MMN responses, especially when it is  
438 calculated as the difference between the deviant and standard responses of different  
439 frequencies. Therefore, the most valid way to define the MMN is to calculate the difference  
440 in the responses to the standard and deviant stimuli that are of the same frequency. However,  
441 only a few human MMN studies have applied this calculation method (Jacobsen & Schröger,  
442 2001; Maess et al., 2007). It is possible that human scalp-recorded event-related potentials are  
443 not as sensitive as the intracranial local-field potentials to sound frequency because the signal  
444 that is recorded over the scalp reflects activity of a large cortical area. However, similar  
445 control conditions and analyses used to detect physically identical deviant and control sounds  
446 would increase the comparability of rodent and human MMN studies.

447

448 In the present study, the pattern of the results for the MMN response to sinusoidal sounds was  
449 the same regardless of whether the many-standards or the cascade-ascending condition was  
450 applied. Repetition suppression in comparison to standard sounds was found only in  
451 comparison to the 4600 Hz control sound in the cascade condition but not in the  
452 many-standards condition. Regarding the complex sounds, the control responses obtained in

453 the many-standards condition, but not in the cascade condition, were different from the  
454 oddball-deviant responses. Thus, the results were not identical in the two control conditions.  
455 Regarding the complex sounds, the cascade condition appeared to be a more stringent control  
456 than the many-standards condition was. This result was similar to a previous finding in awake  
457 rats (Harms et al., 2014), but it differed from the results of other previous studies on rats and  
458 mice (Parras et al., 2017) and humans (Wiens et al., 2019), which did not report any  
459 differences between the two controls.

460

461 The cascade-ascending or -descending condition is an effective choice when the objective is  
462 to control for the pattern in sound series. The cascade mountain condition (Ruhnau et al.,  
463 2012) is not the best choice because stimulus probabilities cannot be the same for all stimuli.  
464 Namely, the control stimuli that are presented at the ends of the frequency range have half of  
465 the probability of the other sounds (Harms et al., 2014; Ruhnau et al., 2012). It has been  
466 suggested that the cascade condition might be too complex for rat cognition (Harms et al.,  
467 2014). However, previous studies on anesthetized rats showed that regularity violations in  
468 complex patterns in sounds elicited MMN in these animals (Astikainen, Mällo et al., 2014;  
469 Astikainen, Ruusuvirta et al., 2014), which suggests that rat brain is capable of complex  
470 probabilistic calculations. Furthermore, the present study demonstrated that genuine MMN in  
471 the cascade control was similar to the many-standards control for sinusoidal sounds.

472

473 It is notable that similar to adult human studies, the present results demonstrated a negative  
474 polarity MMN. However, our results differed from previous results for urethane-anesthetized

475 rats that demonstrated a positive polarity MMN (Astikainen et al., 2011). It is difficult to  
476 determine whether this discrepancy was due to differences in the stimulus conditions or other  
477 methodological aspects. In our earlier study, in which urethane-anesthetized rats were  
478 presented with ascending or descending tone pairs, some animals showed negative polarity  
479 MMN and others showed positive polarity MMN (Ruusuvirta et al., 2007). In Ruusuvirta et  
480 al. (2015), urethane-anesthetized rats demonstrated differential responses of positive polarity  
481 to ascending deviant frequencies, but a negative polarity difference was found in some  
482 descending frequencies. The polarity of MMN in rats clearly warrants further research.

483

#### 484 **Conclusions**

485 The brains of urethane-anesthetized rats exhibited genuine MMN under stringent controls.  
486 Sound frequency itself significantly affected the MMN response – most probably because the  
487 recording location was more optimal for one than the other frequency. Therefore, future  
488 studies should apply multiple recording electrodes and study designs that enable controlling  
489 for frequency. Genuine MMN to complex sounds was found in early latency, and genuine  
490 MMN to sinusoidal sounds was found in late latency. The differential responses to the  
491 sinusoidal sounds were larger than to the complex sounds. Further research is required to  
492 elucidate the possible functional differences in early and late MMNs. Future studies should  
493 also be conducted to confirm the preference for sinusoidal sounds over complex sounds in  
494 rats with multiple electrode sites placed over auditory cortices. Overall, the results of the  
495 present study suggest that the epidural recording of MMN in rats is feasible in studying  
496 change detection in sinusoidal and complex sounds.

497

498

499

500 **Acknowledgements**

501 The authors thank Dr. Jari Kurkela for his help with study design and data analysis, Mr. Petri  
502 Kinnunen for his help in constructing stimulus conditions, Dr. Miriam Nokia for helping with  
503 surgeries and Professor Fengyu Cong for applying the personal grant for TY.

504

505 **Competing interests:** None

506

507 **Funding:** This work was supported by the China Scholarship Council (personal grant for  
508 Tiantian Yang) and the Academy of Finland (grant number 322114 for Kaisa Lohvansuu).

509

510 **References**

- 511 Ahmed, M., Mällo, T., Leppänen, P. H., Hämäläinen, J., Äyräväinen, L., Ruusuvirta, T., &  
512 Astikainen, P. (2011). Mismatch brain response to speech sound changes in rats.  
513 *Frontiers in Psychology*, 2, 283.
- 514 Alho, K., Tervaniemi, M., Huotilainen, M., Lavikainen, J., Tiitinen, H., Ilmoniemi, R. J., . . .  
515 Näätänen, R. (1996). Processing of complex sounds in the human auditory cortex as  
516 revealed by magnetic brain responses. *Psychophysiology*, 33(4), 369-375.
- 517 Astikainen, P., Mällo, T., Ruusuvirta, T., & Näätänen, R. (2014). Electrophysiological  
518 evidence for change detection in speech sound patterns by anesthetized rats. *Frontiers in*  
519 *Neuroscience*, 8, 374.
- 520 Astikainen, P., Ruusuvirta, T., & Näätänen, R. (2014). Rapid categorization of sound objects  
521 in anesthetized rats as indexed by the electrophysiological mismatch response.  
522 *Psychophysiology*, 51(11), 1195-1199.
- 523 Astikainen, P., Stefanics, G., Nokia, M., Lipponen, A., Cong, F., Penttonen, M., &  
524 Ruusuvirta, T. (2011). Memory-based mismatch response to frequency changes in rats.  
525 *PloS One*, 6(9), e24208.
- 526 Auksztulewicz, R., & Friston, K. (2016). Repetition suppression and its contextual  
527 determinants in predictive coding. *Cortex*, 80, 125-140.

- 528 Buzsáki, G., Anastassiou, C. A., & Koch, C. (2012). The origin of extracellular fields and  
529 currents—EEG, ECoG, LFP and spikes. *Nature Reviews Neuroscience*, *13*(6), 407.
- 530 Chen, I., Helmchen, F., & Lütcke, H. (2015). Specific early and late oddball-evoked  
531 responses in excitatory and inhibitory neurons of mouse auditory cortex. *Journal of*  
532 *Neuroscience*, *35*(36), 12560-12573.
- 533 Farley, B. J., Quirk, M. C., Doherty, J. J., & Christian, E. P. (2010). Stimulus-specific  
534 adaptation in auditory cortex is an NMDA-independent process distinct from the sensory  
535 novelty encoded by the mismatch negativity. *Journal of Neuroscience*, *30*(49),  
536 16475-16484.
- 537 Fishman, Y. I., & Steinschneider, M. (2012). Searching for the mismatch negativity in  
538 primary auditory cortex of the awake monkey: Deviance detection or stimulus specific  
539 adaptation? *Journal of Neuroscience*, *32*(45), 15747-15758.
- 540 Harms, L., Fulham, W. R., Todd, J., Budd, T. W., Hunter, M., Meehan, C., . . . Hodgson, D.  
541 M. (2014). Mismatch negativity (MMN) in freely-moving rats with several experimental  
542 controls. *PLoS One*, *9*(10), e110892.
- 543 Harms, L., Michie, P. T., & Näätänen, R. (2016). Criteria for determining whether mismatch  
544 responses exist in animal models: Focus on rodents. *Biological Psychology*, *116*, 28-35.
- 545 Jacobsen, T., & Schröger, E. (2001). Is there pre-attentive memory- based comparison of  
546 pitch? *Psychophysiology*, *38*(4), 723-727.

- 547 Jacobsen, T., & Schröger, E. (2003). Measuring duration mismatch negativity. *Clinical*  
548 *Neurophysiology*, *114*(6), 1133-1143.
- 549 Jacobsen, T., Schröger, E., Horenkamp, T., & Winkler, I. (2003). Mismatch negativity to  
550 pitch change: Varied stimulus proportions in controlling effects of neural refractoriness  
551 on human auditory event-related brain potentials. *Neuroscience Letters*, *344*(2), 79-82.
- 552 Jung, F., Stephan, K. E., Backes, H., Moran, R., Gramer, M., Kumagai, T., . . . Tittgemeyer,  
553 M. (2013). Mismatch responses in the awake rat: Evidence from epidural recordings of  
554 auditory cortical fields. *PloS One*, *8*(4), e63203.
- 555 Kurkela, J. L., Lipponen, A., Kyläheiko, I., & Astikainen, P. (2018). Electrophysiological  
556 evidence of memory-based detection of auditory regularity violations in anesthetized  
557 mice. *Scientific Reports*, *8*(1), 3027.
- 558 Lohvansuu, K., Hämäläinen, J. A., Tanskanen, A., Bartling, J., Bruder, J., Honbolygó, F., . . .  
559 Leppänen, P. H. (2013). Separating mismatch negativity (MMN) response from auditory  
560 obligatory brain responses in school- aged children. *Psychophysiology*, *50*(7), 640-652.
- 561 Maess, B., Jacobsen, T., Schröger, E., & Friederici, A. D. (2007). Localizing pre-attentive  
562 auditory memory-based comparison: Magnetic mismatch negativity to pitch change.  
563 *NeuroImage*, *37*(2), 561-571.
- 564 May, P. J., & Tiitinen, H. (2010). Mismatch negativity (MMN), the deviance- elicited  
565 auditory deflection, explained. *Psychophysiology*, *47*(1), 66-122.

- 566 Näätänen, R. (2000). Mismatch negativity (MMN): Perspectives for application.  
567 *International Journal of Psychophysiology*, 37(1), 3-10.
- 568 Näätänen, R. (2003). Mismatch negativity: Clinical research and possible applications.  
569 *International Journal of Psychophysiology*, 48(2), 179-188.
- 570 Näätänen, R., Astikainen, P., Ruusuvirta, T., & Huotilainen, M. (2010). Automatic auditory  
571 intelligence: An expression of the sensory-cognitive core of cognitive processes. *Brain*  
572 *Research Reviews*, 64(1), 123-136.
- 573 Näätänen, R., Gaillard, A. W., & Mäntysalo, S. (1978). Early selective-attention effect on  
574 evoked potential reinterpreted. *Acta Psychologica*, 42(4), 313-329.
- 575 Näätänen, R., Jacobsen, T., & Winkler, I. (2005). Memory- based or afferent processes in  
576 mismatch negativity (MMN): A review of the evidence. *Psychophysiology*, 42(1), 25-32.
- 577 Nakamura, T., Michie, P. T., Fulham, W. R., Todd, J., Budd, T. W., Schall, U., . . . Hodgson,  
578 D. M. (2011). Epidural auditory event-related potentials in the rat to frequency and  
579 duration deviants: Evidence of mismatch negativity? *Frontiers in Psychology*, 2, 367.
- 580 Novitski, N., Tervaniemi, M., Huotilainen, M., & Näätänen, R. (2004). Frequency  
581 discrimination at different frequency levels as indexed by electrophysiological and  
582 behavioral measures. *Cognitive Brain Research*, 20(1), 26-36.

- 583 Parras, G. G., Nieto-Diego, J., Carbajal, G. V., Valdés-Baizabal, C., Escera, C., & Malmierca,  
584 M. S. (2017). Neurons along the auditory pathway exhibit a hierarchical organization of  
585 prediction error. *Nature Communications*, 8(1), 2148.
- 586 Polterovich, A., Jankowski, M. M., & Nelken, I. (2018). Deviance sensitivity in the auditory  
587 cortex of freely moving rats. *PloS One*, 13(6), e0197678.
- 588 Ruhnau, P., Herrmann, B., & Schröger, E. (2012). Finding the right control: The mismatch  
589 negativity under investigation. *Clinical Neurophysiology*, 123(3), 507-512.
- 590 Ruusuvirta, T., Koivisto, K., Wikgren, J., & Astikainen, P. (2007). Processing of melodic  
591 contours in urethane- anaesthetized rats. *European Journal of Neuroscience*, 26(3),  
592 701-703.
- 593 Ruusuvirta, T., Lipponen, A., Pellinen, E., Penttonen, M., & Astikainen, P. (2015). Auditory  
594 cortical and hippocampal local-field potentials to frequency deviant tones in  
595 urethane-anesthetized rats: An unexpected role of the sound frequencies themselves.  
596 *International Journal of Psychophysiology*, 96(3), 134-140.
- 597 Schröger, E., & Wolff, C. (1996). Mismatch response of the human brain to changes in sound  
598 location. *Neuroreport*, 7(18), 3005-3008.
- 599 Shiramatsu, T. I., Kanzaki, R., & Takahashi, H. (2013). Cortical mapping of mismatch  
600 negativity with deviance detection property in rat. *PLoS One*, 8(12), e82663.

- 601 Taaseh, N., Yaron, A., & Nelken, I. (2011). Stimulus-specific adaptation and deviance  
602 detection in the rat auditory cortex. *PLoS One*, 6(8), e23369.
- 603 Tervaniemi, M., Ilvonen, T., Sinkkonen, J., Kujala, A., Alho, K., Huutilainen, M., &  
604 Näätänen, R. (2000). Harmonic partials facilitate pitch discrimination in humans:  
605 Electrophysiological and behavioral evidence. *Neuroscience Letters*, 279(1), 29-32.
- 606 Tervaniemi, M., Schröger, E., Saher, M., & Näätänen, R. (2000). Effects of spectral  
607 complexity and sound duration on automatic complex-sound pitch processing in  
608 humans—a mismatch negativity study. *Neuroscience Letters*, 290(1), 66-70.
- 609 Wiens, S., Szychowska, M., Eklund, R., & van Berlekom, E. (2019). Cascade and  
610 no-repetition rules are comparable controls for the auditory frequency mismatch  
611 negativity in oddball tasks. *Psychophysiology*, 56(1), e13280. doi:10.1111/psyp.13280
- 612
- 613
- 614

615 **Figure 1.** The illustration of the stimulus conditions. Here, sinusoidal conditions are used as  
616 an example, but all the stimulus conditions were also applied to complex sounds. A) Oddball  
617 descending condition: a 4000 Hz tone is a deviant stimulus, and a 4600 Hz tone is a standard  
618 stimulus. B) Oddball ascending condition: the assignment of the standard and deviant  
619 stimulus is reversed compared to the oddball descending condition. C) Many-standards  
620 control condition: in consistently changing stimuli, the control stimuli were 4000 Hz and  
621 4600 Hz tones. D) Cascade-ascending control condition: fixed pattern of stimuli, control  
622 stimuli were 4000 Hz and 4600 Hz tones. In both control conditions, the probability of each  
623 tone was 0.125 (as for the deviant in the oddball condition), and similar to the oddball  
624 condition, there was a 15% frequency difference between the tones.

625  
626 **Figure 2.** Responses to the sinusoidal sounds (above 4000 Hz and below 4600 Hz). The  
627 shadings of the waveforms represent 95% confidence intervals (CI). The stimulus onset was  
628 at time 0. The left column: standard (STD) and deviant (DEV) responses to sounds of the  
629 same frequency (responses obtained in the flip-flop condition) and a differential response  
630 (deviant–standard). The black horizontal bars in the bottom of the left column figures mark  
631 the time window of the significant difference between the deviant and standard responses: 95%  
632 CI is presented for the standard and deviant stimulus responses. The middle column:  
633 responses to the deviant stimulus and the same frequency stimulus in the many-standards  
634 (MST) and cascade-ascending control conditions (CSAS): 95% CI is presented for the MST  
635 and CSAS responses. The green and yellow horizontal bars mark the time window of the  
636 significant difference between the responses to the deviant sounds and each control sound.

637 The right column: responses to the oddball standard and the same frequency stimulus in the  
638 many-standard and cascade control condition: 95% CI is presented for the MST and CSAS  
639 responses. The yellow horizontal bars mark the time window of the significant difference  
640 between the deviant and each control sound response. Oddball and control responses were  
641 compared only when there was a significant difference between the responses to the standard  
642 and deviant stimuli in the oddball condition.

643

644 **Figure 3.** Responses to the complex sounds (above 4000 Hz and below 4600 Hz). The  
645 shadings of the waveforms represent 95% CI. The stimulus onset was at time 0. The left  
646 column: standard and deviant responses to sounds at the same frequency (responses obtained  
647 in the flip-flop condition) and a differential response (deviant–standard): 95% CI is presented  
648 for the standard and deviant stimulus responses. The black horizontal bars at the bottom of  
649 the left column figures mark the time window of significant difference between the deviant  
650 and standard responses. The middle column: responses to deviant stimulus and to the same  
651 frequency stimulus in the many-standards (MST) and cascade-ascending (CSAS) control  
652 conditions: 95% CI is presented for the MST and CSAS responses. The green horizontal bar  
653 marks the time window of the significant difference between deviant responses and each  
654 control sound response. The right column: responses in the standard, many-standards, and  
655 cascade control conditions to the same frequency sounds: 95% CI is presented for the MST  
656 and CSAS responses. The oddball and control responses were compared only when there was

657 a significant difference between the responses to the standard and deviant stimuli in the  
658 oddball condition.

659

660 **Figure 4.** Differential responses (deviant–standard) to sinusoidal and complex sounds.

661 Differential responses to 4000 Hz (left) and 4600 Hz (right) sounds with shading show 95%

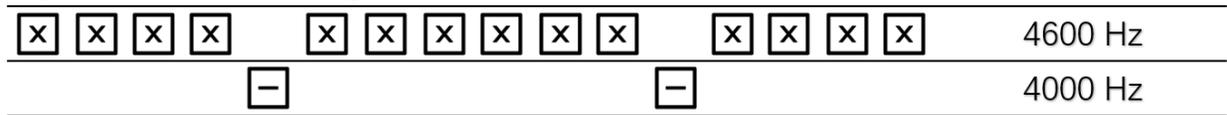
662 CI. The stimulus onset was at time 0. The black horizontal bars show the latency of the

663 significant difference between the differential responses to the sinusoidal and complex

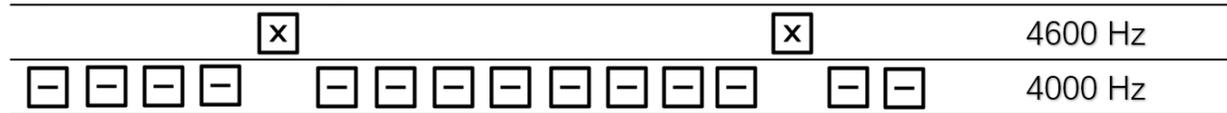
664 sounds.

665

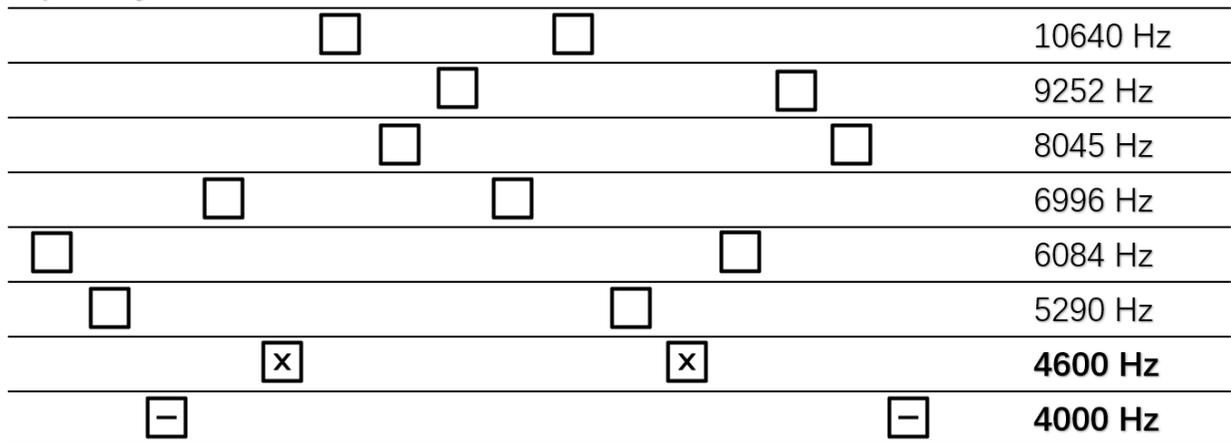
## A) Oddball descending



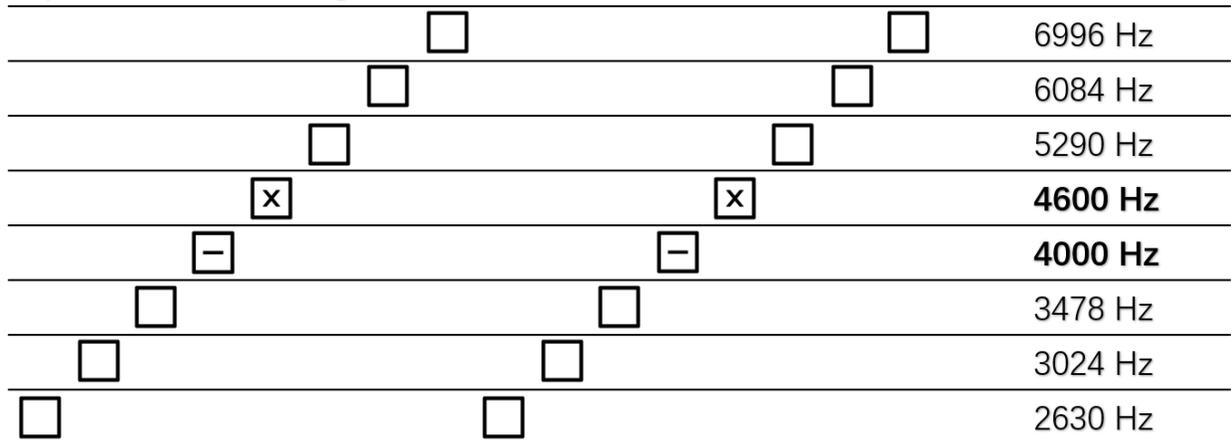
## B) Oddball ascending



## C) Many-standards

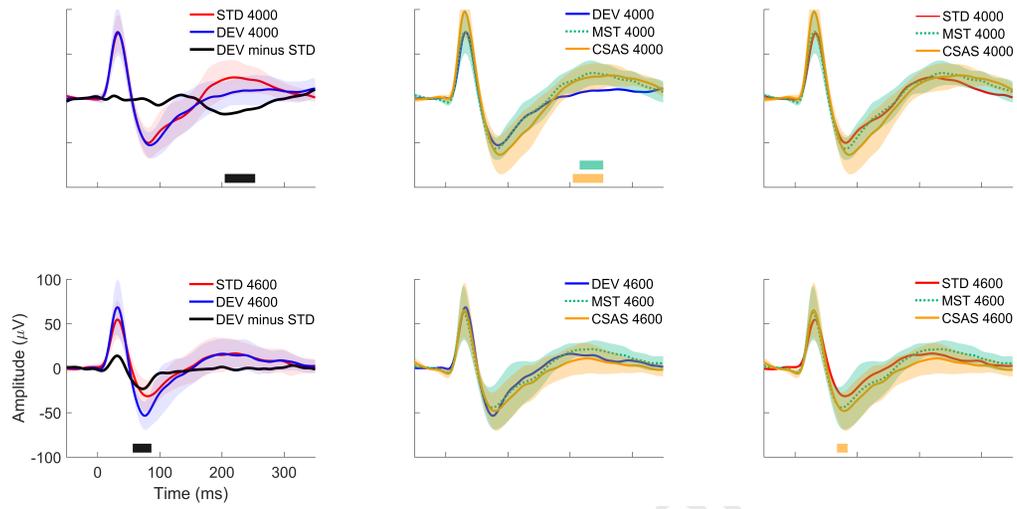


## D) Cascade ascending

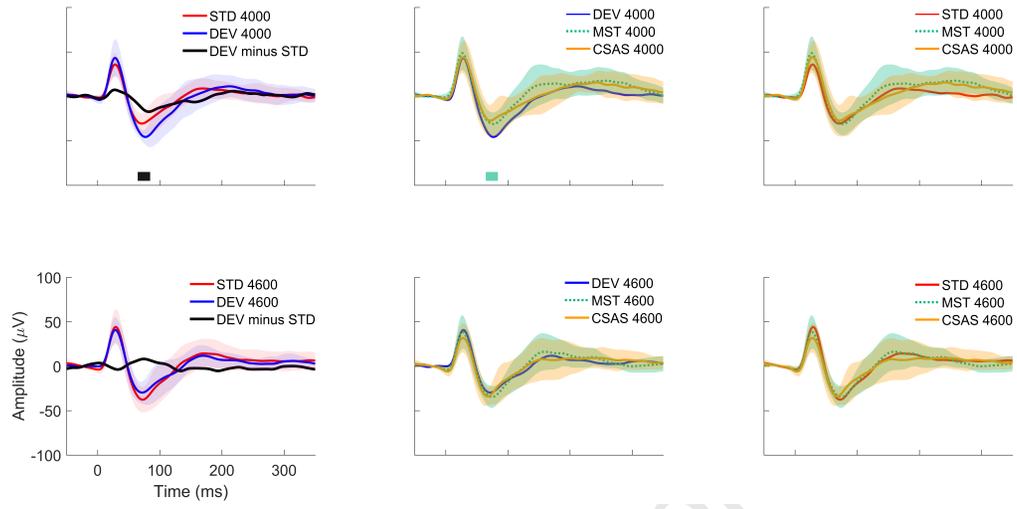


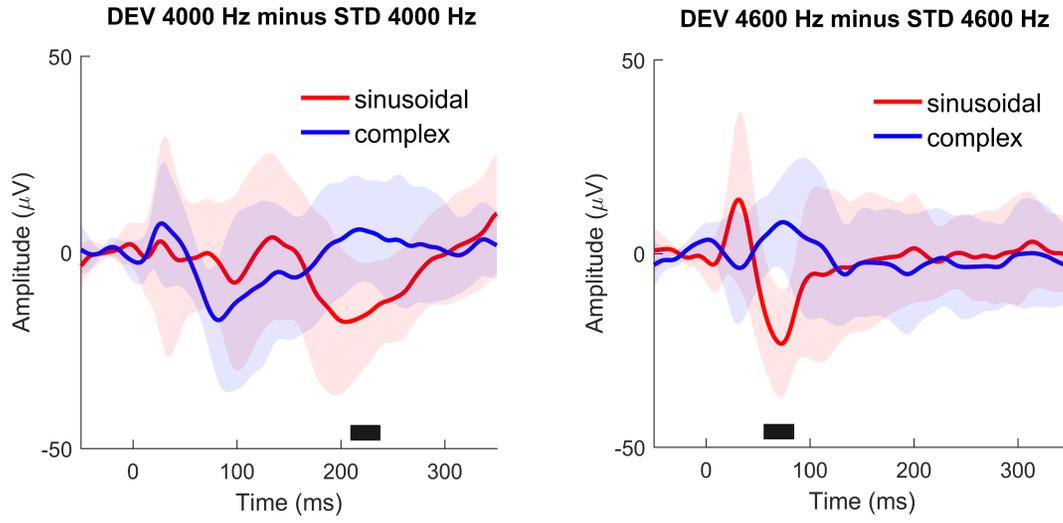
Time →

## Sinusoidal sound condition



## Complex sound condition





Journal Pre-proof

## Highlights

- Controlled MMN was elicited for frequency changes in urethane-anesthetized rats
- Sound frequency affected the differential responses to deviant sounds
- Complex sounds elicited an early and sinusoidal sounds a late MMN
- MMN amplitude was larger for sinusoidal sounds than complex sounds
- Rat model is suitable to study auditory cognition with simple and complex sounds

Journal Pre-proof