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

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Abstract

Mismatch negativity (MMN), which is an electrophysiological response demonstrated in humans and animals, reflects memory-based deviance detection in a series of sounds. However, only a few studies on rodents have used control conditions that were sufficient in eliminating confounding factors that could also explain differential responses to deviant sounds. Furthermore, it is unclear if change detection occurs similarly for sinusoidal and complex sounds. In this study, we investigated frequency change detection in urethane-anesthetized rats by recording local-field potentials from the dura above the auditory cortex. We studied change detection in sinusoidal and complex sounds in a series of experiments, controlling for sound frequency, probability, and pattern in a series of sounds. For sinusoidal sounds, the MMN controlled for frequency, adaptation, and pattern, was elicited at approximately 200 ms onset latency. For complex sounds, the MMN controlled for frequency and adaptation, was elicited at 60 ms onset latency. Sound frequency affected the differential responses. MMN amplitude was larger for the sinusoidal sounds than for the complex sounds. These findings indicate the importance of controlling for sound frequency and stimulus probabilities, which have not been fully controlled for in most previous animal and human studies. Future studies should confirm the preference for sinusoidal sounds over complex sounds in rats.

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

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48 **Abbreviations**

49 CSAS: Cascade ascending control condition

50 MMN: Mismatch negativity

51 MST: Many-standards control condition

52 NMDA: N-methyl-D-aspartate

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1. Introduction

Detecting changes in sensory environment is important for adaptive behavior. Brain responses that reflect change detection are studied using so-called oddball stimulus condition in which a rare “deviant” stimulus is interspersed with a frequent “standard” stimulus. Recordings of the brain’s electrical responses to oddball stimuli elicit the mismatch negativity (MMN) component (Näätänen et al., 1978). The MMN in the auditory domain has been well documented in both humans and animals (Harms et al., 2016; Näätänen et al., 2010), and it has the potential for clinical applications (Näätänen, 2000; 2003).

MMN is a response to a rare stimulus that violates the regularity formed by a repetitive standard stimulus (Näätänen et al., 2005). According to the memory-comparison hypothesis the brain compares an incoming (deviant) stimulus with the memory trace formed by a standard stimulus. When these two are found to differ, MMN is elicited (Näätänen et al., 2005). Another possible explanation for differential responses to deviant stimuli (in comparison to standard stimuli) is the different levels of adaptation in the neural populations responding to frequent and rare sounds because the neurons that respond to standard sound features are more frequently activated than are the neurons that respond to deviant sound features (May & Tiitinen, 2010).

Different control conditions have been developed to study whether mere adaptation can explain differential responses elicited in the oddball condition (Harms et al., 2016). Currently,

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

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

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

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

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

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

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

2. Methods

2.1. Subjects and surgery

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

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

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

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

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

2.2. Stimulation

Sinusoidal and complex sounds were applied as stimuli.

Eleven sinusoidal sounds at frequencies of 2630 Hz, 3024 Hz, 3478 Hz, 4000 Hz, 4600Hz, 5290 Hz, 6084 Hz, 6996 Hz, 8045 Hz, 9252 Hz, and 10640 Hz were applied as stimuli in the sinusoidal sound experiments. The frequency step between the tones closest to frequency was 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), and the sampling frequency was 44100 Hz.

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

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

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

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

2.3. Recording

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

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

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

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

2.4. Data analysis

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

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

PLEASE INSERT FIGURE 1 APPROXIMATELY HERE

3. Results

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

3.1. Responses to sinusoidal sounds

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

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

PLEASE INSERT FIGURE 2 APPROXIMATELY HERE

3.2. Responses to complex sounds

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

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

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

PLEASE INSERT FIGURE 3 APPROXIMATELY HERE

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

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

PLEASE INSERT FIGURE 4 APPROXIMATELY HERE

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.

Note. Differential response (deviant-standard) was calculated as a difference between the responses to the same frequency sounds obtained in the two oddball conditions. Genuine MMN = Genuine mismatch negativity latency (i.e., when the deviant stimulus responses were larger than the responses to the control stimulus). In the repetition suppression analyses, standard responses were compared with the same frequency sounds of the many-standards (MST) and cascade-ascending (CSAS) control conditions: n.s. = non-significant; n.a. = not applicable (not calculated because there was no differential response in the oddball condition).

4. Discussion

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

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

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

The pattern of the results differed regarding the complex sounds, which elicited an early (starting at 64.5 ms post-stimulus) genuine MMN to the 4000 Hz sounds. Similar to the sinusoidal sounds, the complex sounds elicited no differential response to the 4600 Hz sounds. Regarding the 4000 Hz complex sounds, the oddball-deviant responses and control sound responses differed only when the control sound was obtained in the many-standards control condition but not when the control sound response was obtained in the cascade condition. These results indicated that a genuine MMN was not found when the predictive pattern of the sound sequences was controlled.

In summary, a genuine MMN was found for both sinusoidal and complex sounds of 4000 Hz, but not of 4600 Hz frequency. Therefore, the stimulus frequency had a significant effect on the MMN elicitation, which was observed in the recording electrode on the dura above the auditory cortex. This finding aligns with previous findings in studies on urethane-anesthetized rats, which showed differential responses to deviant sounds of some but not all sound frequencies (Harms et al., 2014; Nakamura et al., 2011; Ruusuvirta et al., 2015). In Ruusuvirta et al. (2015), eight different deviant sound frequencies were interspersed with a standard sound. The statistical model also showed that the sound frequency, not the

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

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

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

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

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

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

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

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

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

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

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

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

Conclusions

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

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

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

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

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

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

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

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

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

significant difference between the differential responses to the sinusoidal and complex

sounds.

A) Oddball descending

B) Oddball ascending

C) Many-standards

D) Cascade ascending

Time

Sinusoidal sound condition

Complex sound condition

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