Fetal auditory evoked responses to onset of amplitude modulated sounds. A fetal magnetoencephalography (fMEG) study

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**Abstract**

The human fetal auditory system is functional around the 25th week of gestational age when the thalamo cortical connections are established. Fetal magnetoencephalography (fMEG) provides evidence for fetal auditory brain responses to pure tones and syllables. Fifty five pregnant women between 31 and 40 weeks of gestation were included in the study. Fetal MEG was recorded during the presentation of an amplitude modulated tone (AM) with a carrier frequency of 500Hz to the maternal abdomen modulated by low modulation rates (MRs) - 2/s and 4/s, middle MR - 8/s and high MRs - 27/s, 42/s, 78/s and 91/s. The aim was to determine whether the fetal brain responds differently to envelope slopes and intensity change at the onset of the AM sounds.

A significant decrease of the response latencies of transient event-related responses (ERR) to high and middle MRs in comparison to the low MRs was observed. The highest fetal response rate was achieved by modulation rates of 2/s, 4/s and 27/s (70%, 57%, and 86%, respectively). Additionally, a maturation effect of the ERR (response latency vs. gestational age) was observed only for 4/s MR.

The significant difference between the response latencies to low, middle, and high MRs suggests that still before birth the fetal brain processes the sound slopes at the onset in different integration time-windows, depending on the time for the intensity increase or stimulus power density at the onset, which is a prerequisite for language acquisition.

1. **Introduction**

During the pregnancy, the fetus is exposed to a large variety of sounds including maternal voice, other voices and sounds coming from the surrounding environment. It has been established that the human fetus learns specific sound features in utero and reacts after the birth to known and unknown sounds differently. Several studies reported that newborns prefer the maternal voice immediately after birth, ([DeCasper and Fifer, 1980](#_ENREF_3); [Ockleford et al., 1988](#_ENREF_33)) and that newborns show an altered behavioral response to a story recited by the mother during the pregnancy than to a novel story not recited by the mother ([DeCasper and Spence, 1986](#_ENREF_4)) ([Kisilevsky et al., 2009](#_ENREF_22)). These studies suggest, that the fetus is able to memorize prosodic sound properties in order to discriminate foreign from native language additionally to their sensitivity to the acoustic properties of the mother voice.

Behavioral studies provided evidence for the functional maturity of the fetal auditory system to external non-speech stimuli (sinusoidal tone) starting at 19 to 27 week of gestation ([Hepper and Shahidullah, 1994](#_ENREF_18); [Shahidullah and Hepper, 1994](#_ENREF_44)). Additional advanced brain imiging thechniques were used to investigate neuronal mechanisms of the fetal hearing and learning. Fetal magnetoencephalography (fMEG) and functional magnetic resonance imaging (fMRI) allow the investigation of functional brain development and plasticity before the birth. In particular fMEG and fMRI were largely used for the investigation of the developing auditory system, ([Anderson and Thomason, 2013](#_ENREF_1)). Most fMEG studies investigated the responses to pure tones ([Eswaran et al., 2000](#_ENREF_8); [Eswaran et al., 2002](#_ENREF_9); [Holst et al., 2005](#_ENREF_19); [Schleussner and Schneider, 2004](#_ENREF_42); [Schneider et al., 2001](#_ENREF_43); [Wakai et al., 1996](#_ENREF_51)) and possible alterations during gestation or in different disease states ([Fehlert et al., 2016](#_ENREF_11); [Linder et al., 2015](#_ENREF_24); [Morin et al., 2015](#_ENREF_30)). fMRI studies confirmed the fetal cortical activation during pure tone stimulation ([Jardri et al., 2008](#_ENREF_21)) and to recorded spoken nursery rhyme by the mother, ([Hykin et al., 1999](#_ENREF_20)). In addition fetal auditory event related fields were recorded to white noise, ([Muenssinger et al., 2013](#_ENREF_31)) and syllables, ([Hartkopf et al., 2016](#_ENREF_17)). The studies clearly show that the fetal brain processes speech and non-speech sounds at least during the last trimester of gestation. Early sensory experience in utero was shown to be a prerequisite for initiation of auditory learning and neuronal plasticity during gestation and after the birth. Evidence for experience-developed plasticity in the auditory cortex in newborns was provided by Webb et al. ([Webb et al., 2015](#_ENREF_52)). They investigated preterm newborns exposed to recordings of maternal sounds before full term brain maturation, showing that after the birth the auditory cortex is more adaptive to the maternal sounds than to environmental noise. Recently, Partanen et al. demonstrated that fetal exposure to pseudo-word stimuli in the last trimester of pregnancy creates neural memory traces ([Partanen et al., 2013a](#_ENREF_34)). They reported a specific discriminative brain evoked response to a change in pitch in trained words only for the newborns presented to these stimuli in utero. In another study the authors played a simple melody to fetuses (trained group) during the last trimester of pregnancy 5 times per week, ([Partanen et al., 2013b](#_ENREF_35)). After the birth, they presented the same melody to the newborns in which either some of the notes was changed or not. Event-related responses to changed and not changed tones were recorded in the learning group and in the control group newborns, who were not exposed to the melody during the pregnancy. They reported increased responses to the unchanged tones in the melody in the trained group in comparison to the control group ([Partanen et al., 2013b](#_ENREF_35)). Additionally, the authors reported a correlation between the number of times the fetuses in the trained group were exposed to the melody and the response amplitudes of the infants to the changed and unchanged tones in the melody up to 4 months after birth. The authors concluded that the exposure of melodies to the fetuses had a long lasting effect and led to a specific response pattern after birth. These studies provided clear evidence that memory traces for speech and melodic streams are created already in early stages of development with a long-lasting effect after birth.

Acoustic features of speech and melodies are associated with pitch and spectral coding but temporal structure and onset cues play an important role in prosody and melodic recognition. Acoustic stimuli especially language and melodies can be classified at different time scales. The intonation contour being an element of prosody is expressed by acoustical features over hundreds or thousands of milliseconds and are similar to musical melodies ([Phillips and Farmer, 1990](#_ENREF_36)). But prosody is also related to attributes like pitch envelope, energy contour, speech rhythm and modulation. In addition speech rhythm plays a role in language acquisition contributing to the development of speech discrimination ability and speech perception, ([Ramus et al., 2000](#_ENREF_39)). In a recent study ([Granier-Deferre et al., 2011](#_ENREF_15)), 15s streams of speech with only temporal variations in amplitude or with rapid and slow amplitude and spectral variations over time and piano melodies with slow amplitude and spectral variations were presented to fetuses. The authors investigated the effect of the different stimuli by comparing second-by-second heart rate (HR) changes. The authors reported that the near-term fetuses react to the onset of the stimulus and the HR response contour accelerates or decelerates differently over the time dependent on the stimuli (melody or speech stream). This provides evidence that that fetuses process music melodies and speech differently. Recently, Moon et al. discussed their hypothesis that the birth is not the starting point for perception and processing of phonetic units, ([Moon et al., 2013](#_ENREF_29)). For example during phoneme changes, an acoustic event with a temporal resolution of about 20ms is regarded as shortest detectable feature. Studies in infants and newborns using non speech stimuli, demonstrated simultaneous electrophysiological (EEG) and hemodynamic (NIRS) activation and lateralization in the newborn and infant brain corresponding to differences in temporal sound features, ([Telkemeyer et al., 2009](#_ENREF_47); [Telkemeyer et al., 2011](#_ENREF_48)). These studies confirmed the ability of the newborns and infants to process and use the temporal information.

Simple intensity temporal changes present in language and music are sound envelope fluctuations, mostly associated with amplitude modulations or sound pulsations. The onset slope of this signal is determined by the modulating frequency, higher modulation rates have a steeper rising, whereas low modulations are characterized by slower rising. In an adult MEG study ([Engelien et al., 2000](#_ENREF_7)) used an one-second long tone modulated by 40 Hz and reported that this stimuli elicits simultaneously transient ERR (involving N1 component) and Steady-state response (SSR). The late transient response reflects only the onset processing of the stimulus ([Fellman and Huotilainen, 2006](#_ENREF_12)). Therefore, the ERR should be sensitive to different rising times at the AM sound onset introduced by the different modulation frequencies in the time window up to hundreds of milliseconds.

Based on this preliminary knowledge, we aimed to establish paradigms and protocols for appropriate auditory stimulation in relation to prosody and short temporal changes like consonants and to investigate fetal auditory evoked brain responses to these stimuli. Amplitude modulated stimuli are appropriate for the investigation of the auditory temporal processing. Their structure resembles phonetic elements and modulation rates in speech and could be manipulated in different speech relevant time scales. Our hypothesis is that fetal ERR latency is coupled to a certain level of sound onset energy corresponding to slow or fast enhancement of the envelope and thus showing that already in an early development phase, the fetal brain is tuned to multiple time-scales in perception. Our goal is to describe the transient fetal brain responses to stimulus energy related rising times (from 0 to 250ms). We used amplitude modulated AM tones with a constant carrier frequency (500 Hz) modulated with different frequencies (2 Hz, 4 Hz (low modulation rates - MRs), 8 Hz – middle MR and 27 Hz, 42 Hz, 78 Hz, and 91 Hz – high MR’s). These modulation rates correspond to rise time in the sound energy from 0 to 250ms.

1. **Materials and methods**
	1. **Subjects**

Fifty five pregnant women between 31 and 40 weeks of gestation were included in the study.

The study was approved by the local ethical committee from the Medical Faculty at the University of Tübingen. Each mother signed an informed consent.

* 1. **Stimulation**

The acoustic stimulation set consisted of seven different AM tones with carrier frequency of 500 Hz, separated in 3 stimulation categories dependent on the amplitude modulation frequency. In the low modulation rate (MR) category, a 500 Hz tone (duration of 10s) was modulated by modulation frequency of 2 Hz or 4 Hz. The middle MR category was represented by a 500 Hz tone (duration of 5s) modulated by frequency of 8 Hz, and in the high MR category the 500 Hz tone (duration of 1s) was modulated by 27 Hz, 42 Hz, 78 Hz or 91 Hz. The different modulation rates were determined by the human auditory perception of different amplitude modulated frequencies. The low amplitude modulation rate - 2/s corresponds to perception of loudness (slow increasing intensity), the modulation rate - 4/s corresponds to the perception of the syllables (syllabic rhythm). The modulation rate 8/s corresponds to perceived articulation or higher rate syllabic rhythm. The high modulation rates correspond to perception of roughness characterizing phonetic segments like consonants.

The stimuli were adjusted in order to be able to evoke not only the transient response but also a steady-state response. In order to achieve at least 20 periods in the stimulus length for the lowest modulation rate (2/s),which is recommended for reliable calculation of the steady-state response, and to keep the length for the higher modulation rates equal, we chose different stimulus length, 10s, 10s, 5 s, for 2/s, 4/s and 8/s, respectively and 1s for four higher rates (27/s, 42/s, 78/s and 91/s), Table1. In addition, we selected a constant stimulation block of 10 minutes for all stimuli. This time is best suited to ensure a stable fetal state during the recording. Based on this we had to use one or two stimuli per stimulation block. The sound onset asynchrony (SOA) was also adjusted for each modulation rate in order to avoid a habituation and to achieve appropriate inter-stimulus interval (ISI).

The stimuli were presented in two trials and dependent on the modulation rate either alone or in combination of two tones in the trial. In the current paper we consider only the transient responses elicited by the AM sounds.

Tones modulated at low and middle frequency range (2, 4 or 8 Hz) were presented always alone in the trial. The choice of the modulation frequency of the stimulus was assigned for each fetus randomly. The AM stimuli with high modulation frequencies (27 Hz, 42 Hz, 78 Hz, 91 Hz) were presented in pairs of two into one trial. For each trial one of the combinations (27 and 42 Hz ) or (78 Hz and 91) were used and each of the stimulus was presented with a percentage of 50 and a maximum of 5 identical stimuli in a row to avoid habituation. It has to be stressed, that single subject (fetus) was stimulated only in two stimulation trials. Each trial contains either one stimulus from the low or the middle MRs or two different tones from the high MRs. Table 1 represents all measurement sessions with their parameters.

***Place Table 1 around here***

Stimulus delivery was controlled by the *Presentation software* (*www.neurobs.com*). The sound was generated by a speaker located outside the magnetically shielded room and delivered to the maternal abdomen through plastic tubing. The distal end of the tubing was attached to an inflated air-filled balloon directly placed over the maternal abdomen. The low and middle AM tones were presented at 78 dB and the high AM with 86 dB. The higher sound pressure was selected based on pilot studies showing a clearer response for the high AM. The stimulus intensity was measured on the bag surface which was positioned on the mother’s abdomen, using A-weighted filter of the intensiometer. The difference between the intensities of the sounds to different modulation rates is based on the following fact: the A-weighted filters adjust the sound loudness as perceived by the typical human. This is valid only for loudness measurement for a single tone. In complex tones, the perceived loudness in humans depends on whether the spectral components are closely or widely spaced in frequency. In our case, that means, sounds with low and high modulation rates should be perceived differently even that the measured loudness was kept constant. This was the criteria for intensity selection to achieve similar perception of the different stimuli. So different stimuli were presented with slightly different intensities but the perceived loudness on the surface of the bag was the same in all measurements, independent from the modulation rate. Based on the reported attenuation of the sound pressure through the maternal abdomen ([Gerhardt et al., 1996](#_ENREF_14); [Querleu et al., 1988](#_ENREF_38)), we assume an intensity of approximately 58 - 66 dB reaching the fetus.

* 1. **Fetal Magnetoencephalography**

The fetal magnetoencephalography system (fMEG) contains 156 primary magnetic sensors (first order gradiometers), spaced approximately 3 cm apart over an area of 850 cm2. The noise level of primary sensors is below 5 fT/√Hz. The sensors are arranged in a concave array covering the maternal abdomen. The mother sits and leans forward against the smooth surface of the array. During the stimulation, a self-chosen CD was played through inserted ear plugs to the mother or her ears were occluded with foam inserts if she was not comfortable to hear music.

To attenuate the influence of external magnetic fields, the fMEG system is installed in a magnetically shielded room (Vakumschmelze, Germany). In order to determine the position of the fetal head in relation to the fMEG sensors we utilized a fiduciary marking system consisting of four coils. The fetal head position was determined by ultrasound before and after the recording. After the first ultrasound, the mothers were seated on the fMEG device in the shielded room. Three coils were attached to mother’s left and right side and the back. The fourth coil was positioned over the fetal head whose location was determined by ultrasound before the fetal measurements. Before each recording, the location of the coils was determined by activating them at a certain frequency to compute their coordinates in relation to the SQUID sensors. Our experience showed that the fetuses between 30 and 40 week of gestation do limited gross movements of the head in a short time window. However, in the case when the fetus was moving and the second ultrasound measurement showed different position of the head, the fetus was excluded for further analysis. Seventeen fetuses were excluded from the analysis, based on changes in head position during recording or artifacts in the recording data.

* 1. **Magnetic field analysis**

The magnetic field data were recorded with a sampling rate of 610.352 Hz. Each AM stimulus was marked by a trigger for classification in the corresponding conditions, AM 2 Hz, AM 4 Hz, AM 8 Hz, AM 27 Hz, AM 42 Hz, AM 78 Hz and AM 91 Hz. The artifacts from the maternal and fetal magnetocardiogram signals were attenuated by using an orthogonal projection algorithm ([McCubbin et al., 2006](#_ENREF_26); [Vrba et al., 2004](#_ENREF_50)). The magnetic signals were filtered between 1 Hz and 10 Hz and separated in trials based on the triggers (pre- and post-trigger time were 100ms and 800ms, respectively). Amplitude sensitive threshold detection was applied to the data and trials with amplitude higher than 2 pT were rejected. By additional visual inspection trials with breathing artifacts were also rejected. Breathing artefacts can be distinguished by a large wave into the single trial with high amplitude (over the individual event-related brain activity) and very clear dipolar distribution. Averages were computed for responses to each condition after rejection of all artifact trials. Auditory ERR were determined by averaging across trials with respect to the stimulus onset (- 100ms to 800ms). The number of the averaged trials ranged between 38 to 46 trials for the low frequency AM, 65 to 75 trials for the middle frequency AM and 55 to 86 trials for the high frequency AM. Following criteria must have been fulfilled to include the response in the analysis: (i) The differences detected between no response and discernible response were determined by a noise estimate through plus-minus averaging. A criterion of signal-to-noise ratio of at least 2:1 was applied. (ii) The magnetic field distribution showed the activity in the area around the head-coil location. Channels that showed a response around the location of the head coil placed on the mother’s abdomen by ultrasound determination were picked for further analysis. By a combined ultrasound and fMEG study we already showed that the evoked responses are generated in the fetal brain ([Micheli et al., 2010](#_ENREF_28)).

Event-related responses were quantified by calculating the root-mean squares (RMS) of the channels with the highest amplitudes. The latency of the RMS maximum of the response component to each MR was used for statistical analysis.

* 1. **Statistical analysis**

The response latencies for each condition were compared using One-Way ANOVA (SPSS Software). We used Hochberg’s GT2 post hoc test because of the different sample sizes for each condition. Because of the violation of the data homogeneity, assessed by Levene’s test, we also applied Games-Howell procedure and non-parametric (Mann-Whitney) test comparing pairwise the response latencies between the conditions. Finally, linear regression analysis was applied on response latencies for each modulation rate in the period from 31 to 40 week gestational age in order to assess possible maturation effects. All analysis with p<0.05 were regarded as statistical significant.

1. **Results**
	1. **Fetal response rates**

Transient event-related responses could be extracted for each of the AM sounds. Examples of fetal ERR for each of the different AM sounds are given in Fig. 1A, B. The mean and standard deviation of the fetal response latencies for each modulation frequency is provided in Table 2. The number of successful recordings of fetal evoked fields is unequally distributed between the conditions. The total number of measured fetuses for each condition is presented in Table 2. The highest percentage of detectable responses was observed for condition AM 27 Hz (86%), followed by condition AM 2 Hz (70%) and AM 4 Hz (57%). The smallest was gained for highest MR (91 Hz – 31%).

***Place Fig. 1A,B and Table 2 around here***

* 1. **Fetal response Latencies**

There was a significant main effect (One Way ANOVA) of MR on the response latencies, F(6,73)=25.77, p<0.0001 (Fig. 2). A significant decrease in latency of the response to middle MR (8/s) in comparison to low MR (2/s) was observed, assessed by Hochberg post-hoc comparison between conditions, (p=0.012). Further, the significant latency decrease was found for the high modulation rates 27/s, 42/s, 78/s and 91/s compared to middle MR of 8/s, (p<0.0001, p=0.014, p=0.003, p=0.041), respectively and between each low MR (2/s , 4/s) and the high MRs (27/s, 42/s, 78/s and 91/s),(p<0.0001).

 ***Place Figure 2 around here***

* 1. **Maturation effects**

A linear regression analysis was performed on the data (response latencies vs. gestational age) for each condition separately. We observed a significant latency decrease from 31 to 39 week GA only for the fetal responses to AM 4 Hz (R2 = 0.283, p=0.017), represented in (Fig 3). All other regressions were not significant.

***-----------------------Place Figure 3 around here-----------------------------***

This group was presented by one of the highest number of investigated fetuses and responders (see Table 2). The smaller numbers of responders in the fetal group expose to the higher modulation rates (78/s and 91/s) were insufficient to reach a significant level in fitting the regression curves.

1. **Discussion**

In the current study we found a continuous decrease of the fetal response latencies with an increase of the stimulus modulation rate. There was a significant difference for response latencies to the middle MR compared to low MR’s, and between the response latencies to the high MR’s in comparison to the low and middle MR’s. This confirms our hypothesis that the fetal brain reacts not only to spectral differences of the stimulus but also recognizes different slopes of the stimulus energy rise at the onset. It is known that the detectability of a stimulus is triggered by a certain threshold of its intensity and is dependent on stimulus duration and frequency, ([Luce and Green, 1974](#_ENREF_25); [Terhardt, 1974](#_ENREF_49)). We assume that fetal ERRs to slow modulation rates corresponding to 2 and 4 Hz modulation frequency were elicited due to sound energy increase of the stimulus envelope i.e. at a certain intensity level. That means that the large-latency response to the low MR’s can be triggered by the slow rising of the signal envelope.

Different studies of our group reported that the averaged fetal response latencies to 500 Hz sinusoidal burst varied from 220ms to 264ms ([Draganova et al., 2007](#_ENREF_6); [Eswaran et al., 2002](#_ENREF_9); [Holst et al., 2005](#_ENREF_19)). A fetal MEG study using a combined odd-ball paradigm, demonstrated that there were no significant amplitude differences in fetal response to differences in spectral wide comparing white noise vs. sinusoidal tone, ([Muenssinger et al., 2013](#_ENREF_31)). Even that the white noise has wider spectrum than the sinusoidal stimulus, both are constant in amplitude in the time. However, in the study of Muenssinger et al., latency differences to pure tones and white noise were not compared. Our study demonstrated that the response latencies to the high MRs fall in a timescale range between 150ms - 184ms and to the middle and low MRs between 301ms and 397ms, respectively. Thus, response latencies to sinusoidal tones were larger in latencies, than what we found for the latencies of the responses to the high modulation rates in current study. As it was already reported by the studies, ([Fellman and Huotilainen, 2006](#_ENREF_12); [Telkemeyer et al., 2009](#_ENREF_47)), the late transient ERR reflects only the onset processing of the stimulus but rapid changes in ongoing stimuli could be traced by auditory Steady-state responses and evoke following oscillatory responses, ([Poulsen et al., 2009](#_ENREF_37)). Moreover, a modulation in high frequency range produces a wider spectral content around the carrier frequency. For instance, a modulation frequency at 27 Hz, produced side-band spectral lines around the 500 Hz carrier frequency and thus wider spectrum than those of the 500 Hz sinusoidal tone. Therefore, we assumed that the shorter latencies of the ERR to higher MRs might reflect the signal energy at the onset of the stimulus in a time-window from 0 to 100 ms being more robust to elicit a response than the constant in energy sinusoidal tone. Further studies are required in order to investigate the fetal brain response to rapid changes over rate of 27/s, reflected by auditory Steady-State response.

We observed also different response success rate to AM tones in this study and to pure tone-bursts reported in our previous study ([Draganova et al., 2007](#_ENREF_6)). The AM tones with low MR’s of 2/s and 4/s revealed a response rate above 50 %, (70% and 57%, respectively) in the whole range of gestation. Significant decrease in response latency along the GA was calculated only for responses to 4 Hz modulation frequency. We assume that these results are mainly driven by the fact that the standard deviation of the latencies was smaller for the response to MR 4/s compared to the response for MR of 2/s. The fetuses respond with less variability to the MR of 4/s. It is known that fluctuation strength of a sound at 4 Hz is better perceived than sound fluctuation at other frequencies, ([Fastl and Zwicker, 2007](#_ENREF_10)). In fact, these authors claimed that a good understandable spoken language contains 4 syllables per second, which modulated the speech at 4 Hz. Thus, based on earlier studies hypothesized that the syllable rhythm is one of the prosody elements and very important for language discrimination and that infants are able to discriminate different languages using the syllabic rhythms ([Mehler et al., 1988](#_ENREF_27); [Ramus et al., 2000](#_ENREF_39); [Ramus et al., 1999](#_ENREF_40)), we assumed that fetal audition is influenced by the modulated spoken language and thus becomes more sensitive to a sound modulated in rate close to best syllables rhythm (4/s). Taking into account that the fetuses are exposed to maternal speech and to other external speeches during the gestation, we assumed that the event-related response to MR 4/s reflects: (i) a faster adaptation to known spoken rhythm and (ii) response latency maturity due to the longer exposure to the spoken language in later GAs.

The larger standard deviation across fetuses in the very low frequency modulation rate of 2/s could be caused by time delays in achieving sound intensity threshold eliciting event related response due to different fetal head positions to the sound source. Fetal state, ([Nijhuis et al., 1982](#_ENREF_32)), might be also play a role in the response latency variance in different measurements. The inclusion of fetal state data in our analysis would probably improve our results by reducing the variance in latencies. However the 10 min recording are not well suited to provide a reliable state estimate. Further studies may include longer recording sessions to extract fetal state information.

The highest response rate however was found for modulation frequency at 27 Hz, whereas the smallest response rate was found to the rest of the high modulation rates, especially to MR 91/s. Hz. Indeed, the 86% response rate of the response to 27 Hz modulation frequency is above the observed response success rate to pure tone-bursts reported in our previous studies, ([Draganova et al., 2005](#_ENREF_5); [Eswaran et al., 2002](#_ENREF_9); [Holst et al., 2005](#_ENREF_19)). Rubel, ([Draganova et al., 2005](#_ENREF_5); [Rubel, 1978](#_ENREF_41)) reported that fetuses between 29 and 32 week of GA responded more easily to complex tones than to pure tones. Further, he concluded that even in the early stages, the auditory system is prepared and set up to analyze natural complex tones rather than pure tones. The fetus hears sounds in a noisy amniotic fluid environment. The fetus’s middle ear and the external auditory canal are also filled with fluid and hence the sounds entering this system lose their energy. Gerhard et al., and Sohmer et al., ([Gerhardt et al., 1996](#_ENREF_14); [Sohmer et al., 2001](#_ENREF_46)) performed studies in human and animal fetuses and confirmed that fetuses hear the sounds coming from outside rather by bone conduction than by the ear (middle ear and auditory canal). They hypothesized that sounds entering through the maternal abdomen in the amniotic fluid surrounding the fetal head causes bone vibrations. These vibrations excite the fetal inner ear mainly by fluid pathway ([Freeman et al., 2000](#_ENREF_13); [Sohmer et al., 2000](#_ENREF_45); [Sohmer et al., 2001](#_ENREF_46))(Freeman et al. 2000). Additionally, Sohmer et al., ([Sohmer et al., 2001](#_ENREF_46)) reported that because the fetal head is immersed in water the intensity of the sound increases. Thus the bone conduction mechanism seems to be very effective. Finally, Groome et al., reported that pulsed sounds elicit significantly greater fetal cardiac orientation reflex then the continuous sounds and concluded that temporal characteristic is more effective then spectral one ([Groome et al., 2000](#_ENREF_16)). This leads to the conclusion that periodical envelope fluctuations at higher frequencies, in our case 27 Hz might be better transmitted by the bone conduction than constant in energy pure sinusoidal tones, which could explain the higher response rate of the responses to middle and higher modulation rate at 27/s. This may contribute to the fact that fetuses respond better to complex tones than to pure tones.

According to ([Kuhl, 2004](#_ENREF_23)) newborns are prepared to detect differences between phonetic contrasts. In another study ([Bertoncini et al., 1987](#_ENREF_2)) demonstrated that the onset of 44ms in syllable stimuli carries sufficient information for infants at 3-4 days of age in order to discriminate place of articulation differences in stop consonants. They assumed that this does not require an exposure to the speech prior to the stimulation. However, presenting native and non-native vowels to newborns after birth, Moon et al. found that newborns react differently to both groups of vowels, ([Moon et al., 2013](#_ENREF_29)). They suggested fetal language learning at a phonetic level. The fetal neuronal plasticity also in learning of frequency, intensity and duration cues in a pseudo-word was demonstrated in the study of Partanen et al. ([Partanen et al., 2013a](#_ENREF_34)). Hence, due to the prenatal formation of neuronal memory traces to specific sound cues attributed to language, ([Moon et al., 2013](#_ENREF_29); [Partanen et al., 2013a](#_ENREF_34); [Partanen et al., 2013b](#_ENREF_35)), newborns are able to recognize these cues after birth. Our results demonstrated a considerable significant difference between fetal response latencies to different slopes at the envelope onset of the AM tones in time-windows from 0ms to 250ms (low and middle MRs) and from 0ms to 100ms (high MRs). These results demonstrate the fetal neuronal tuning to different rise-time fluctuations of the stimulus intensity at the onset from 0 to 250ms. The significant difference between the response latencies to low, middle, and high modulation rates suggests that fetal auditory cortex processes the sound intensity change at the onset in different integration time-windows. We supposed that the large time scale of the response latencies to different MR’s indicate that fetuses can differentiate between slow intensity onset enhancement and steep rise of the onset signal intensity. This could be referred to the hypothesis that except spectral cues (changes in frequency), fetuses use other sound features related to sound onsets and changes in sound energy in recognizing and memorizing sounds.

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**Conflict of interests:** We declare that there is no conflict of interest.

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