Automated auditory brainstem response hearing screening in NICU graduates
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Citation for published version (APA):

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Based on:

Fetal Hearing.

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2.1 Introduction

The phenomenon that the sensory development of the prenatal is a quiet process of gradually responding to the extraterine world has been anticipated by mythological and religious stories, artists, writers and many philosophers since ancient times. Aristotle argued that the individual first acquires sensation during pregnancy and might experience the extraterine environment. However, doubts remained about prenatal sensory capabilities. Jean Jacques Rousseau referred to the fetus as a "witless tadpole" and even the first scientific approaches by Preyer in 1885 led to doubtful conclusions about fetal hearing capacities.

Hearing is one of the primary modalities of human beings for communicating and prerequisite for the development of language. Serious efforts to explore prenatal recognition began in the 1920s and 30s. Peiper performed sonic stimulation with the aid of a car horn to study movement responses of the fetus. Forbes and Forbes were able to acknowledge the amodal link between movement and sound by stimulating a pregnant mother lying in a water bath, which was struck with a metal object. Ray attempted to measure fetal reactions to the smacking of two boards together. All these authors reported habituation responses following the stimulus presentations.

Habituation and conditioning became more focal as researchers attempted to tighten scientific controls for analysis. Sontag and Wallace attempted to experiment with increased numbers and greater control of the involved variables orientated to a stimulus-response method by measuring physical responses towards external stimuli. It became obvious that auditory maturation does not solely function in isolation but is part of an integrated development. The hypothesis was put forward that intrauterine conditioning accounts for certain behavioural characteristics of the newborn and the refractory times of the habituation-dehabituatin processes were studied. Fetal movement (FM) responses to sound stimulation were observed as one major focus for fetal conditioning. New methods of perinatal medicine such as Doppler registration of fetal heart rate (FHR) and FM, as well as ultrasound, became extremely helpful to objectify fetal responses to external stimuli.

The unborn is involved in a perceptual world with increasing specification and differentiation. During the first trimester self-generated movements are combined with somatosensory awareness resulting in co-ordination of early FM-patterns. Later sensory challenges may derive from two environments. The "intrauterine world" includes the emotional state and daily rhythms of the mother, touch with the umbilical cord, the uterine wall and acoustic stimuli caused by maternal circulation, digestion, movements, breathing and, finally, her voice. As birth approaches, stimuli from the "extraterine world" are increasingly responded to. Neurological development allowing preparatory interaction involves a considerable number of genetic actions, which might be
autoregulatory as well as activated or inhibited by positive- or negative feedback mechanisms.

Different disciplines have therefore become interested in the field of prenatal maturation of the auditory system such as embryology, genetics, neurophysiology, psychoanalysis and developmental psychology. Since pregnant mothers and most of the current methods used to register immediate responses are in the hands of obstetricians, research and clinical investigations in perinatal medicine have been directed towards this field.

2.2 Embryology and developmental anatomy

The special status of the ear is supported by the observation that the ossicles in the middle ear are of adult size by 8 months of gestation, and thus the only bones to attain their final form in the prenatal period. Conventionally the ear is described in terms of the outer, middle and inner ear.

Knowledge about the complex development of the ear helps in understanding its integrated function and disturbances of the auditory system.

*External ear and tympanic membrane*

The external ear is divided into the pinna and the external auditory canal. From 4 weeks’ gestation onwards the mesoderm from the first and second branchial arch gives rise to six outgrowths, the hillocks of His, condensing by 12 weeks to form the pinna. From 10 weeks onwards the future ear can be visualized. The external auditory canal develops from the first branchial groove. At 8 weeks the cavum concha deepens, growing towards the middle ear, which can be demonstrated by ultrasound in advanced pregnancy. Adult size of the external auditory canal is reached only by 9 years of age. The tympanic membrane develops from structures associated with both the external and the middle ear. The completed tympanic membrane has three layers: an outer epithelial layer, a middle fibrous layer and an inner mucosal layer, continuous with the lining of the tympanic cavity. The tympanic membrane inserts into the tympanic ring, which is complete at 16 weeks, and can be visualized by ultrasound at the end of the external auditory canal. The maximal diameter is of adult size in the term fetus.

*Middle ear, temporal bone and facial nerve*

The middle ear consists of the tympanic cavity, three ossicles (malleus, incus and stapes), the tensor tympani and the stapedius, tendons and the Eustachian tube. The ossicles develop between 4 and 6 gestational weeks from the mesenchyme of the mandibular and the hyoid arches; full size is reached by 18 weeks. Only then do they become ossified and might be visualized located cranially of the mandible. Extension of
the first and second pharyngeal pouch, lined with endoderm, forms the tubotympanic recess. At week 7, constriction of the midportion leads to the formation of the Eustachian tube and the tympanic cavity. During further development the tympanic cavity is filled with mucoid mesenchymal tissue becoming vacuolated. By 30-34 weeks the tympanic cavity is pneumatised.

The temporal bone derives from four separate elements: the tympanic bone, the squamous portion, the styloid process and the petromastoid. Only the first three parts have formed and ossified at birth. Postnatally there is still no complete bony ear canal and no mastoid process. The facial nerve derives from the second branchial arch. At the end of the 3\textsuperscript{rd} gestational week the acousticofacial ganglion can be identified; by the end of the embryonic period its neuroblasts form the main trunk of the facial nerve and the chorda tympani nerve. The fully developed facial nerve transverses the internal acoustic meatus, the middle ear and, finally, exits superficially from the stylomastoid behind the tympanic membrane, where it can be injured by obstetric manipulations.

\textit{Inner ear and auditory nerve}

The inner ear lies in the petrous portion of the temporal bone consisting of a membranous labyrinth inside a bony labyrinth. The bony labyrinth includes the cochlea, three semicircular canals, the vestibule enclosing the utricle, the saccule and part of the cochlear duct, as well as the perilymphatic spaces. Development of the bony labyrinth occurs in three stages: cartilage formation, the formation of the perilymphatic spaces, the calcification and ossification. By 24 weeks, all centres have fused to form a complete bony capsule. Ossification of the inner ear does not occur until each portion has attained adult size. The configuration of the membranous labyrinth is recognizable by 10 weeks and completed at around 24 weeks. Its neurosensory elements develop from the ectodermal otic placode in the 23-day-old human embryo giving rise to the otocyst. The differentiation of the vestibular and cochlear end organs takes place during the second month of pregnancy.

Light microscopy demonstrates that the first sign of differentiation of the organ of Corti in the wall of the cochlear duct starts at 10 gestational weeks. At 14 weeks, rows of inner and outer hair cells can be observed. At around 20 weeks, the human cochlear morphology is similar to what is considered the stage corresponding to the onset of cochlear function. The organ of Corti contains sensory and supporting cells. Ultrastructural development includes hair cell differentiation of inner and outer hair cells, synaptogenesis and ciliogenesis.

The eighth nerve ganglion also derives from the otocyst. After 4 weeks these cells form the auditory ganglion, dividing later into superior and inferior branches of the vestibular nerve and the cochlear nerve. The nerve cells remain bipolar throughout life, the peripheral processes terminating in the sensory areas of the inner ear and the central processes in the brainstem.
Auditory pathways
In the auditory system the second neuron is the brainstem. Structures in the human auditory pathway, from the proximal end of the cochlear nerve to the inferior colliculus, undergo myelination between 26 and 29 gestational weeks. The density of myelination increases in all pathways up to 1 year of age. Though code transmission is improved in myelinated pathways, myelination is not prerequisite for transmission. Primary and secondary crossed and uncrossed pathways ensure that each ear is represented on both sides of the brain.

In mammals the pathway proceeds to the auditory center in the cerebral cortex. The auditory pathway in human beings transforms the code represented by a mechanical response of the ear into a signal, which can be utilized by higher centers. The codes are changed from level to level. Through maturation infants develop an information-processing capacity, the development of which has not yet been sufficiently studied. This knowledge, however, is essential for understanding how speech and language develop, and may have implications concerning auditory behaviour, reaction to sound and diagnostic testing.

2.3 Physiologic basis of hearing, encoding and transmission

Sound is created by a vibratory source that causes molecules to be displaced. It is designed for gaseous and liquid media, while oscillation in solids is mostly referred to as vibration. The quantification of sound requires measurement of the amplitude and the frequency. The amplitude is measured in units of sound pressure called Pascal’s (Pa) proportional to the acoustic intensity or loudness. The measurement of sound pressure levels is given in decibels (dB), which are logarithmic numbers favoured due to the inherent compression of the linear scales. The frequency of sound is measured in cycles/s, namely Hertz (Hz). The range of 20-20 000 Hz is accepted as the bandwidth of human hearing. Beyond these limits high sound pressure levels are required to evoke auditory responses and there is also decreased discrimination ability. Frequency and magnitude components of sound form what is called spectrum analysis.

Physiologic acoustics
Knowledge of physiological acoustics can be obtained from selected references, whereby most information is drawn from invasive recordings in non-human species. The overall role of the outer ear is to collect sound energy and to shape it by the resonances of the concha and the external auditory canal towards the tympanic membrane. Acoustic energy is transformed into mechanical energy as vibrations of the tympanic membrane and the ossicular chain in the middle ear, providing an energy gain of around 30 dB due to area differences between the tympanic membrane and the
footplate of the stapes. The middle ear also protects the inner ear from high sound pressures.
The role of the cochlea and structures of the inner ear is to couple the vibratory energy delivered to the oval window by the stapes footplate to the hair cells. It was postulated in 1965 by Davis\textsuperscript{35} that the binding of the sensory hairs of the organ of Corti depolarises the hair-cell membrane by altering its resistance. The basis of the frequency-related regional displacement of the entire cochlear partition was revealed by the classic Nobel laureate von Bekesy\textsuperscript{36} who described it as "travelling waves"; this was verified later by sophisticated methods.\textsuperscript{37} The links between hair-cell receptors and the auditory system are primary auditory neurons with selective sensitivities for frequencies. With the entry of the acoustic nerve into the brainstem auditory neurons multiply. The frequency-to-place code is equally representative for the organization of central nuclei and the auditory cortex.\textsuperscript{38,39} Many auditory abilities are attributable to subcortical processing. Decorticated animals are capable of detecting the intensity and frequency of sounds;\textsuperscript{40-42} anencephalic fetuses demonstrate behavioural reactions to external stimuli (Fig 1). Central neurons are sensitive to frequency modulation and sound features.\textsuperscript{43} An efferent auditory pathway varies the input sensitivity at the level of the hair cells.\textsuperscript{44}

**Special conditions relating to the fetus**

Although perinatologists can occasionally observe gestures familiar from adult life, intrauterine hearing conditions are specific for the prenate. Information on fetal hearing has been obtained from invasive experiments on sheep or measurements in pregnancies with ruptured membranes.

![Fig 1](image-url)  
*Fig 1*  
Fetal heart rate (FHR, above) / fetal movement (FM, below) tracing after vibroacoustic stimulation in an anencephalic fetus of 34 weeks. There is a long reaction (>60 s) with increased FM and FHR after the first stimulation (1\textsuperscript{st} arrow) and a weaker reaction after the second stimulation (2\textsuperscript{nd} arrow)
Fetal Hearing

Sound conduction
Vibrational direct excitation of skin and tissue is more effective for developing an acoustic field than transmission across the air-skin interface.\textsuperscript{45,46} The external auditory canal and the fetal middle ear are filled with amniotic fluid; the interface at the stapes footplate is a fluid medium prenatally as compared to an air medium postnatally. Sound pressures with the same phase are present at the two windows, which may reduce hair-cell activity. On the other hand, impedance similarities may cause pressure variations to be fairly well transformed.\textsuperscript{47}
A bone conduction route is assumed for prenatal hearing based on measurements with round-window electrodes implanted after open fetal surgery in sheep. Cochlear microphonic recordings were registered towards broad-band noise delivered by a loudspeaker and compared to input / output functions in the same lamb after delivery.\textsuperscript{48} Sound energy is slightly diminished (10-20 dB) for frequencies <250 Hz, yet significantly reduced for frequencies > 500 Hz (40-50 dB).\textsuperscript{48,50} One reason for this "sound isolation" is the route of sound energy "underwater", described by bone conduction for adult and fetus.\textsuperscript{49,51}
The effectiveness of sound transmission (outer and middle ear versus bone transmission) was tested by sheep experiments, proving that when the fetal head is covered with sound attenuating material, even though the pinna and the ear canal remain uncovered, sound levels must be greater than those necessary to evoke the same response from the bare head.\textsuperscript{51} Both cochleae are equally stimulated, so that only one auditory image is likely to be formed.\textsuperscript{52,53} This might have implications for lateralisation in speech development.\textsuperscript{54}

Sound attenuation
A second reason for sound isolation is the sound pressure attenuation described for externally delivered pure tones.\textsuperscript{55} A loudspeaker in a rubber annulus was attached to the maternal abdomen, a microphone placed in utero near the cervix. Transmission losses ranged from 39 dB at 500 Hz to 85 dB at 5000 Hz,\textsuperscript{56} and were 70 dB for frequencies above 2000 Hz.\textsuperscript{57} Since an impedance mismatch may bias the results, hydrophones (underwater microphones) were used in humans\textsuperscript{58,59} and animals.\textsuperscript{46,60-63} It was shown that the mother's voice in the sheep uterus is louder when picked up by a hydrophone than by a microphone placed by the abdominal wall\textsuperscript{62} and that sound attenuation decreases during the last weeks of gestation.\textsuperscript{61,62} Further results were obtained by placing a pregnant ewe in a sound field produced by stereo speakers\textsuperscript{46,63} recording simultaneous measurements with a microphone in the air and a hydrophone within the uterine cavity. There was sound enhancement of broadband noises in utero for frequencies below 250 Hz; transmission loss increased to an average of 20 dB at 4 kHz. The overall transmission loss was 6.7 dB for broadband noise, and 10-15 dB for pure tone at 1-10kHz. In humans, exterior sound of at least 65-70 dB was transmitted to
the intra-uterine cavity with attenuation of 30 dB reduction in sound pressure levels for tones up to 12 kHz, frequencies below 200 Hz were even enhanced. All in all results of hydrophone experiments were comparable in sheep and humans.

Sound environment
The sound environment in utero might have an "imprinting" and a masking effect on external sounds as studied in humans and in sheep. Measurements in sheep have demonstrated that the basal noise is increased during labor. Data in humans, all gathered after rupture of membranes, might underestimate the emergence of external sounds or overestimate background noise described as pulsations of uterine vessels, interstitial injections, or sounds associated with digestion, maternal body movements and with the mother's breath and voice (see below). The intrauterine sound environment is dominated by frequencies below 500 Hz with mean sound pressures of 90 dB at 250 Hz.

Sound intelligibility
Intelligibility is based on the ability to distinguish complex sounds and thereby provide speech communication and musical abilities. The auditory sensitivity and prenatal sound attenuation characteristics are similar in sheep and humans. Language signals are comparable as proven by simultaneous recordings from microphone, fetal cochlear microphone and intrauterine hydrophone recordings in sheep. Music and voices are distinguishable from the basal noise by 8-12 dB for exterior voices and 24 dB for the mother's voice, if all had an intensity of 60 dB. The male voice with an average frequency of 125 Hz is better transmitted, but emerges in the range where internal noise is highest. Female voices with an average frequency of 220 Hz receive a greater attenuation, but emerge in a range where internal noise is low. The fetus might detect speech and music, ideally low-frequency components below 500 Hz, when the airborne signals exceed 60 dB. Intrauterine sound levels of the mother's voice were enhanced by an average of 5.2 dB. Differences are not dependent on maternal abdominal-wall thickness or amniotic-fluid volume. Intelligibility of directly transmitted maternal voice compared to airborne maternal, female or male voices did not differ if tapes from intrauterine devices were offered to adult observers. While consonants are indistinguishable, vowel sounds, rhythms and melodic timbre of voices are recognizable. Though attenuation varies with frequency, a variety of music is easily recognized from intrauterine recordings.
2.4 Prenatal auditory responsiveness under normal conditions

To demonstrate the existence of prenatal sensory perception, one can empirically study electrophysiological, motor and cardiac auditory responsiveness. Studies of fetal response to sound have chosen a variety of stimuli not always with apparent rationale such as car horns, bicycle bells, electric toothbrushes, or pure tones of 100-2000 Hz, sound pressure levels from 80 to 120 dB measured in air and with various durations. Studies using airborne sounds should be differentiated from studies using vibroacoustic stimuli providing vibration and airborne sound. More recent studies have used the electronic artificial larynx with a vibrating disc attached to the maternal abdomen. It is portable and designed to propagate sound pressure more efficiently, matching impedances between tissue and fluid. The electronic artificial larynx output spectrum was measured by hydrophone fixed near to a lambs’ ear in utero. Most frequencies were between 0.5 and 1 kHz with multiple harmonics up to 15 000 Hz in air. When the device was placed directly over the hydrophone, sound pressure levels averaged 135 dB, which was greater than predicted in humans. In lambs with bilateral cochlear ablation no reactions towards vibroacoustic stimulation could be registered even with high intensities, indicating that in fetal sheep the auditory apparatus is necessary for the FHR and FM responses.

Prenatal responsiveness by means of electrophysiological methods

Recordings of electrical potentials from levels of the auditory system by means of non-invasive techniques are based on compound potentials representing the activity of many cells. Non-invasive methods to investigate the effects of early auditory stimulations are the stimulus-related electroencephalographic (EEG) and magnetoencephalographic (MEG) methods. Human EEG responses towards acoustic signals have been performed, with one exception, only after ruptured membranes using scalp electrodes. In MEG recordings sensitive magnetic-field detectors are used to measure neuromagnetic auditory brainstem responses, which can be performed prenatally with intact membranes. Short auditory stimuli like clicks of 1 kHz and a duration of 100 ms up to 100 dB are performed, in a room guaranteeing electrical radio-frequency shielding, to evoke the activity of electromagnetic sources located in nuclei of the brainstem. In an experimental setting, we succeeded in recording stimulus-related auditory-evoked neuromagnetic fields through the mother’s abdomen at 34 weeks by using a one-channel superconducting quantum interference device (SQUID) magnetometer (Fig 2). The tracings were comparable with postnatal recordings. Latency shifts of brainstem components are proposed to reflect early brain maturation and decrease with advancing age. Although electromagnetic methods are more precise than indirect methods in reflecting the occurrence and latency of reactions, at this moment, the methods are not yet sufficiently developed to allow systematic conclusions to be drawn.
Fig 2  Typical waveforms obtained from brainstem after click stimulation at 34 weeks by one-channel magnetoencephalography. TL1, temporal lateral; TL2, temporal lateral; Ft, fentotesla

Prenatal behavioural responsiveness
The association of evoked potentials with heartbeat and motor responses implies the reception of the auditory signals up to subcortical levels (Fig 1).\textsuperscript{79,80} Due to the present lack of routine technology to record neural activity directly in the womb, fetal sensory abilities are examined by observing behavioural reactions. Appropriate methodology has to be used to ensure that stimulus and response are correlated. Problems arise when the fetus does not react, since we cannot say that the stimulus is not sensed.

Onset of responsiveness
Relating to the onset of immediate fetal responses it was supposed that reactions towards acoustic stimuli do not occur before 24 weeks.\textsuperscript{74,83} Using ultrasound, blink responses were first detectable between 24 and 25 weeks, becoming consistent after 28 weeks.\textsuperscript{74} Recently, responsiveness to sound was even described at 16 weeks of gestation.\textsuperscript{1,84,85} Developmental origins were systematically examined using 80-2000
broad-band stimuli between 15 and 25 gestational weeks. When changing the stimulus from a single sound to a series of ten 2-s pulses, an increased number of FM was found after stimulation at 20 weeks. Interpretation refers to hypotheses about the developing neural system prior to the formation of specific receptor cells as described for first reactions towards touch. Ultrasound observations also allow definition to motor responses such as body, head, arm and leg movements. The methodology can be simplified by using Doppler services for the detection of FM which whilst unable to differentiate qualities of responses can differentiate quantities of FM responses with a high sensitivity and specificity. Using this method to record fetal responses and external artificial larynx for stimulation we observed a first fetal reaction (FM) in singletons at 25 weeks and simultaneous reactions of FM of both members of a twin pair at 27 weeks.

Developmental aspects
FM and FHR responses may be classified in immediate reactions such as startles, twinkling, accelerations or decelerations ("reflexes") as well as long-term changes of either FM, FHR baseline or variability ("changes of behavioural patterns"). FHR/FM patterns including breathing movements were studied during 1 h after vibroacoustic stimulation. Close company changes the development of reactions towards vibroacoustic stimuli in twins compared to singletons. Reactions of longer duration become more frequent in twins compared to singletons (Fig 3). With increasing gestational age an increasing number of FM combined with FHR accelerations is observed in singletons and twins. Among reactions of long duration (>60 s) FHR-baseline changes more dramatically than FHR variability. We also found an increasing number of extreme changes from a very passive to a very active FHR/FM pattern in twin pregnancies. This is in accordance with findings in singletons that after vibroacoustic stimulation unusual changes of state 1F-4F occur, which rarely occur spontaneously. More "physiologic" state changes from state 1F to 2F were found using a 100-Hz square-wave vibratory stimulus. In any case, vibroacoustic stimulation with the external artificial larynx must have some quite dramatic influence on the fetus, since some gynaecologists have even recommended it to induce a change of fetal position to improve visualization of fetal echocardiography (we do not!).
Chapter 2

Gestationa ll week s

II  I  Short , <  60  s ; i ü  Long , >  60  s ; r~~ J  N o  reactio n

Fig 3  Distribution of short (<60 s) and long (> 60 s) behavioural reactions towards vibroacoustic stimuli in 74 healthy singletons (a) and 64 healthy twins (b) obtained by longitudinal weekly measurements between 26 and 36 weeks. Note that there are significant changes with gestational age, Pearson $\chi^2$ test $p<0.0005$, and differences between twins and singletons, Pearson $\chi^2$ test $p<0.0005$

Quality of stimulus

Both the onset and development of auditory responsiveness also depend on qualities of the stimulus. Fetuses from 19 weeks onwards were presented with pure-tone frequencies of 100-3000 Hz. First responsiveness was detected at 23 weeks at 500 Hz, by 27 weeks responsiveness was found to 100, 250 and 500 Hz and only at 31 weeks were responses observed to 1000 and 3000 Hz. It was interpreted that the delayed responsiveness to high frequency is related to the development of phase-locked firing describing a cooperation of nerve fibres to encode high-frequency sound. The sound pressure level required to elicit a response at 35 weeks is 20-30 dB less than at 23 weeks, indicating that the fetal auditory system becomes more sensitive with prenatal age. However, changes in attenuation, maturing behaviour and sensomotor neural connections might also have an impact. Even fetal intelligibility has developmental aspects. Using a habituation-dishabituation technique, fetuses aged 35 weeks could discriminate between
frequencies of 250 and 500 Hz and between different speech sounds, whereas fetuses of 27 weeks were unable to make this differentiation, although they were able to differentiate between a 250-Hz tone and 80-2000 Hz broad-band sound.

Specific or momentary disposition
Additional factors influence fetal acoustic responsiveness such as behavioural state before the stimulus, gender, position in utero and individual disposition (own data). Fetal responses to speech stimuli consisting of syllables ('ee' or 'ah') were studied in healthy pregnancies at 26-34 weeks gestation. During periods of low FHR variability, a decrease in FHR and an increase in the standard deviation of heart rate were found. This is the only demonstration of prenatal responses to speech stimuli whereby the response is dependent on FHR variability which is the primary determinant of fetal state. Female fetuses seem to respond earlier than males. In our experience twins are ideal models to differentiate further the simultaneous influence of activity state before stimulation, individual disposition (zygosity), position and sex of the prenates by analysing intertwin differences of FHR/FM-patterns towards external artificial larynx (Fig 4). Similar reactions towards external artificial larynx were significantly increased in twins with the same sex and differences between sex-alike male compared to female groups can be calculated. If all parameters are analysed by multivariate analysis, age-dependent determinants of fetal responsiveness can be evaluated.

2.5 Postnatal auditory responsiveness under normal conditions

After delivery sound conduction and environment change significantly and the newborn is more directly accessible to behavioural and electrophysiological examinations.

Postnatal responsiveness assessed by electrophysiological methods
Auditory-evoked potentials reflect electrical activity from different anatomic levels of the auditory system: the earlier components from peripheral and brainstem levels, the later ones from midbrain and cortical levels. The cochlear (0-4 ms) and early components (4-12 ms) are used for assessing peripheral hearing in children by electrocochleography and auditory brainstem response. Whereas electrocochleography can only be used under anesthesia, auditory brainstem response has become the method of choice to estimate hearing sensitivity in newborns since it was first described. It reflects the integrity of the outer, middle and inner ear and the auditory pathway, as described by Davis, and is free from the effects of medication or state. Via headphones click stimuli are performed and short-latency electroencephalographic waveforms are recorded by skull electrodes (Fig 5). The detection of cortical potentials and auditory brainstem response as early as 25 weeks indicates functional maturity of the auditory pathway.
Electrophysiological responses from the cochlea, the eighth nerve and the auditory brainstem are similar to those in the adult by 32-36 gestational weeks. The responses continue to develop in wave components, shape and latency with myelinization until the 2nd year. The auditory brainstem response does not necessarily depend on the same neural events that are essential for perception of auditory capabilities. Von Bekesy postulated that some sound energy must be emitted from the cochlea back to the external auditory canal ("feedback from the inner ear"). Transiently evoked otoacoustic emissions can be measured, e.g. sounds generated and emitted by the outer hair cells of the cochlea in response to acoustic stimulation. A small probe is placed in the external auditory canal, and click stimuli are presented. Otoacoustic emission methods are candidates for application in the newborn permitting the acquisition of frequency-specific information, which can be drawn later from audiology.

Fig 4  Distribution of combinations of 390 fetal heart rate (FHR)/fetal movement (FM) reactions towards vibroacoustic stimuli within 32 twin pairs. (a) Classified relating to first short reactions, (b) classified relating to duration of reactions (0, no reaction; Ac, Acceleration; long, > 60 s; short, < 60 s). Note the significantly higher intertwin differences (lighter columns) in twin pairs of different sex. Pearson χ² test p<0.0001
Hearing screening in a preterm infant at the NICU with an automatic version (ALGO-1E automated auditory brainstem response infant hearing screener, Natus Medical Inc.) detecting auditory brain stem response

Postnatal behavioural responsiveness

Behavioural reactions towards acoustic stimuli are less suitable for preterm neonates, but they are of value to learn about what babies hear and about possible positive or negative influences. Responses and alertness to voices, bells and rattles, or the neonate’s ability to shut down aversive reactions due to habituation towards repeated stimuli are used to score infants according to their "abilities." Postnatal behavioural responsiveness

Broad-band sounds (speech) are more likely than narrow-band sounds to elicit responses which also depend on physiological states of hunger and sleep/wakefulness. A newborn infant can differentiate bandwidth, duration, inter-stimulus interval, frequency and sound pressure level. Signals <4 kHz evoke responses three times more often than signals in the higher range. Lower frequencies generally evoke gross motor activity, high frequencies evoke freezing reactions. The newborn can respond to gross right-left changes, although sound localization is not purely auditory. Even soon after birth a blind infant is less likely to localize sound than a sighted infant. After 2 months infants integrate auditory and visual space (e.g. voice and facial movements). Some methods have been introduced to objectify behavioural responsiveness. The auditory response cradle compares reactions with and without sound by a pressure sensitive mattress and a monitor for head movements. As in prenatal life high intensities of up to 85 dB are needed to elicit motor responses. The observer-based psychoacoustic procedure controls for observer bias by documenting behavioural changes towards acoustic stimuli while the observer is "blinded" by earphones for all signals. Using the observer-based psychoacoustic procedure infants of 2-5 weeks showed thresholds at 500 and 4000 Hz of 55- and 60-dB sound pressure levels respectively.
2.6 Effects of prenatal hearing on postnatal development

By correlating morphological and neurofunctional results with perinatal observations, conclusions might be drawn for sensomotory, cognitive and emotional development.

**Recognition or "memorization"**

Considerable attention is given by parents to the possibility that the fetus forms memories of speech and music that may influence the abilities postnatally. Habituation has primarily been described by Peiper who reported a decrease in FM after repeated stimulation with a car horn. Since then a number of studies have demonstrated a decrement in response to repeated stimulation while using FHR/FM or ultrasound documentation. Habituation may be distinguished from adaptation or fatigue by several criteria, the most important being the recovery of response on presentation of a new stimulus ("dishabituation") and faster habituation upon re-presentation of the stimulus. The intensity of the stimulus influences habituation time (faster habituation to more intense stimuli).

While habituation reflects short-term memory, there is also proof of long-term memory from pre- to postnatal life. To demonstrate the existence of prenatal sensory perception, one can indirectly study behavioural modifications of a neonate presented with stimuli she or he has been confronted with during prenatal life ("memorization"). Prenatal sound experience might have an influence on postnatal sound preference and fine tune the developing auditory system. Neonates have been taught that if they produce a specific sucking pattern they could listen to their mother's voice. Newborn babies showed a strong preference for the voice of their mother over that of other male or female talkers. Using the specially designed baby's-dummy connected to a tape recorder, newborns are even able to distinguish between their mother speaking in her native versus in an unfamiliar language (sucking with higher frequency). Using this "high-amplitude sucking procedure", 3-day-old newborns prefer a lullaby read twice a day by the mother during the last weeks of pregnancy to a new story. All this suggests the possibility of prenatal acquisitions and antenatal discrimination, however elementary it might be.

Prenatal stimulation has an impact on temperament behaviour. Settings of talking, music and meditation were performed during pregnancy and correlated with behavioural outcome. Talking had a 65%, music a 34% and meditation a 31% correlation with all behaviours. For talking only 58% of behavioural variables were identified as positive (e.g. the child being easily comforted, contentment), 16% as ambiguous and 26% as negative (e.g. crying for obscure reasons, needing constant supervision). Music and meditation however, correlated in 90% and 100%, respectively, with positive attitudes. Talking is known not only to express care, but also irritability, anger or anxiety experienced in pregnancy. It is speculated that not only variations of timbre, but also hormonal or
physiological changes associated with the content of speech, may evoke associations in the infant. This hypothesis is supported by a study of infants whose excessive crying was in association with their mothers' depression scores. Implications for language development and musical expression

Hearing has a close relation to the kinetic system, i.e. "auditory-vocal-kinetic channel". From embryology we know that the origins and innervation of hearing and language function are in close proximity, suggesting that there is also "crying in utero" - only we have not yet become sensitive for it. Vocal expression can be heard in fetuses from around 20 weeks onwards. Uterine crying has been proposed following unpleasant manoeuvres such as attaching electrodes, versions or catheter insertions. The newborn's phonation is based on laryngeal co-ordination reflecting maturation processes and training. Newborn "cryprints" are as unique as fingerprints, and characterized by specific features such as fundamental frequency and variation in time ("vibrato") using voice-signal recording.

We suppose influences of nature and nurture on prespeech development. Monozygotic twins have synchronous cry patterns at birth. By listening to the mother's speech the fetus obviously stores her speech features. Early spectrography of the first cries proved that even newborns born at 28 weeks had similar voice performance features with their mothers. How far genetic influences or experience of the same sound environment is responsible for first hearing and cry patterns remains to be determined. Babies as young as 12 h old react to rhythms of human speech. This also might imply that learning of language begins soon after or even before birth. Newborns are able to distinguish between phones in any language and make all sounds of any language. By 6 months linguistic experience has resulted in language-specific phonetic prototypes. Adults can no longer distinguish between phones used in unfamiliar languages. In this context, experience seems paradoxically to diminish auditory skills. Somehow, babies even stimulate adults to speak to them in a stylised way.

Music exists in the passage of time and cross-culturally. It is supposed that musicality is structured by an ensemble of protorhythms of biological inheritance. The hypothesis that music has prenatal origins does not explain the survival value of music, but might clarify its nature with cross-cultural features of rhythm and dancing comparable to rhythmic elements in utero, e.g. movements of maternal vessels felt and heard by the prenate.

2.7 Prenatal acoustical responsiveness as a test for fetal well-being

The understanding of pathophysiology has led to improvements in interpretation of behavioural and hemodynamic mechanisms. Fetal hypoxia is the condition studied most
intensively. An arrest of muscular activity to reduce oxygen consumption and a redistribution of fetal blood flow to maintain oxygen delivery to essential organs were primarily described in sheep experiments\textsuperscript{135,136} and later in humans.\textsuperscript{137-139} Meanwhile correlations with antenatal blood gases have been performed.\textsuperscript{140-141} Fetal activity and blood-flow redistribution have an impact on FHR patterns. FHR variability and the presence of FHR accelerations with FM are recognized as indicators of fetal health by non-invasive and invasive studies.\textsuperscript{142,143} In this context, (vibro)acoustic stimulation was proposed mainly for the assessment of fetal well-being and to discriminate between "non-reactive" non-stress tests (NSTs) due to hypoxia or just to quiet state.\textsuperscript{144,145} Using Medline we found >150 studies on vibroacoustic stimulation as a test of fetal well-being from 1980 to date; here we focus only on selected aspects.

*Increase of "efficiency" of non-stress testing and biophysical profile*

To compare results, the frequency, duration and intensity of the stimulus as well as gestational age, FHR/FM criteria, habituation time and behavioural states in association with the response have to be analysed. In general, a 3-s sound stimulus is adequate for a shift to an "awake" state.\textsuperscript{146} In a review of 61 studies, the ability of vibroacoustic stimulation to elicit FHR accelerations has been established, decreasing false-positive rates of non-reactive NSTs.\textsuperscript{147} Pregnancies with early-onset intrauterine growth retardation (IUGR) were studied after vibroacoustic stimulation between 26 and 32 weeks' gestation. Accelerations, FHR variability and FM were reduced compared with age-matched normal fetuses.\textsuperscript{148} The conclusion that nutritional deprivation is associated with delayed sensory maturation is not necessarily true, since similar FHR and FM patterns are recognizable in early NSTs. The result only proves that there is no reaction, though the stimulus might well be received. Vibroacoustic stimulation was performed in patients with low biophysical profile scores after 15 min. The results of patients whose biophysical profile score improved to normal were compared to normal scores without stimulation. Vibroacoustic stimulation was found to "improve" an abnormal or equivocal biophysical profile score to normal in 82% of cases without increasing obstetric or neonatal complication rates or the false-negative rate of biophysical profile score.\textsuperscript{149}

*Comparative observational studies and clinical trials*

Some observational studies compare the vibroacoustic stimulation test concurrently with other tests. In most studies the prediction of poor outcome was comparable with results of the NST.\textsuperscript{150,151} In the prediction of an abnormal contraction stress test (CST), a non-reactive vibroacoustic stimulation had a sensitivity of 100% and a specificity of 91%; therefore vibroacoustic stimulation was slightly better able to diagnose fetal distress compared to the CST in pregnancies with a non-reactive NST at term.\textsuperscript{152} After the introduction of Doppler velocimetry of fetal redistribution it has become possible to differentiate ambiguous results of NSTs non-invasively. Thus we compared the clinical
value of NST, Doppler ratio of cerebral versus umbilical blood flow, vibroacoustic stimulation test and CST to predict poor outcome using different cut-off values for all tests separately for IUGR and post-term pregnancies. Doppler velocimetry and NSTs had better prognostic capacities than vibroacoustic stimulation tests and CSTs\textsuperscript{153,154} (Fig 6). We therefore do not use CST or vibroacoustic stimulation tests in clinical routine, while Doppler studies, computerized FHR analysis, or real-time ultrasound of fetal behaviour and amniotic fluid analysis can be performed without "unnatural stress" for mother and fetus. We admit that in units where skills in ultrasound examinations or sophisticated equipment are not available, vibroacoustic stimulation or CST might be of value. Clinical trials demonstrate that the introduction of vibroacoustic stimulation in combination with the NST\textsuperscript{155} or with amniotic fluid assessment\textsuperscript{156} has not led to an increased rate of mortality. Since in the Western world all of the currently used forms of antenatal surveillance are combined with low mortality rates, it is unlikely that vibroacoustic stimulation will ever lower perinatal mortality in any randomised or historic trial.\textsuperscript{157} In the only controlled clinical trial, where vibroacoustic stimulation test was compared to NST\textsuperscript{145} false-positive tests were slightly lowered and performance reduced from a mean of 27 to 23 min. Although it has been proven that vibroacoustic stimulation does at least not increase catecholamine release\textsuperscript{158} or meconium passage in healthy fetuses,\textsuperscript{159} the question remains whether 4 min justify frightening - or even awakening - an innocently sleeping fetus (it is recommended that vibroacoustic stimulation is performed during quiet sleep!). In other words: which parents would appreciate this postnatally?

Fig 6

Receiver-operator characteristics of antepartum tests to predict fetal distress requiring operative delivery (measurements 1-3 days antepartum, CTS, contraction stress test; VAS, vibroacoustic stimulation; Doppler ACI/UA, resistance index ratio of arteria carotis communis/umbilical artery; NST, non-stress-test) in 103 intrauterine growth retarded (IUGR) (a) and 110 post-term (b) pregnancies.\textsuperscript{142,143}
Vibroacoustic stimulation during labor

The same holds true for studies during labor, where vibroacoustic stimulation was primarily applied to test attenuation via hydrophones. Data suggest that an intrauterine threshold of 94-dB sound pressure level is necessary to produce a consistent FHR response during active labor.\textsuperscript{160}

At the first stage of labor vibroacoustic stimulation was studied possibly to improve interpretation of ominous spontaneous FHR testing. Undoubtedly non-reactive responders to vibroacoustic stimulation were at significantly greater risk of subsequent abnormal FHR-patterns, meconium staining and fetal distress; however, transient FHR decelerations occurred in 25\% where fetal outcome was not impaired.\textsuperscript{161} Although it was concluded that vibroacoustic stimulation differentiated compromised from non-compromised fetuses, we think that it might also confuse inexperienced staff. At the second stage of labor fetal blood sampling (FBS) has been correlated with vibroacoustic stimulation testing: vibroacoustic stimulation had a sensitivity of 100\%, a specificity of 59.6\% and a positive predictive value of 27.6\% for the detection of fetal acidosis.\textsuperscript{162}

Although it was evident that mean fetal blood pH values obtained within 30 min of vibroacoustic stimulation were higher in reactive compared with decelerative or non-responders, acidotic pH values were also found in fetuses with reactive FHR patterns after vibroacoustic stimulation.\textsuperscript{163} In recent studies it was concluded that the FHR response after vibroacoustic stimulation does not predict neonatal outcome and might not replace FBS.\textsuperscript{164,165} There are even warning hints about using vibroacoustic stimulation as a routine procedure, such as an unknown influence on cerebral blood flow in quiet sleep,\textsuperscript{166} false-negative results in fetal sepsis,\textsuperscript{167} activation of swallowing\textsuperscript{168} or micturation\textsuperscript{169} and provocation of unnecessary pathological FHR patterns in cases with nuchal cord.\textsuperscript{170}

We conclude that many studies do not address the physiological changes of fetal responsiveness or habituation time by gestational age or standardize FHR/FM patterns before or after vibroacoustic stimulation. Appropriate implementation of vibroacoustic stimulation tests requires appropriate questions. In twin studies we have found that prenatal reactiveness -either to touch of the co-twin or to external stimulation- is combined with larger interindividual variations than spontaneous behaviour. For the time being, we recommend vibroacoustic stimulation as a challenge test only under controlled conditions, but not for routine use just to simplify antenatal surveillance. This has been proposed by other authors.\textsuperscript{171}
Table 1  
Indications associated with sensorineural and/or conductive hearing loss as provided in the Joint Committee on Infant Hearing position statement for examination of neonates (birth to 28 days) when universal screening is not available.179

- Family history of congenital sensorineural hearing impairment
- Congenital perinatal infections (Syphilis, TORCH)
- Craniofacial anomalies
- Birth weight < 1500 g
- Hyperbilirubinaemia exceeding a level needing exchange transfusion
- Use of ototoxic medication in potential toxic dosage (e.g.: aminoglycosides, diuretics)
- Bacterial meningitis
- Severe birth asphyxia (Apgar < 4 at 1 min or ≤ 6 at 5 min)
- Mechanical ventilation ≥ 4 days
- Syndromes associated with sensorineural hearing loss

2.8 Early hearing disturbances

Compared with the vestibular part, the auditory system is phylogenetically young with a greater demand for oxygen and glucose and can therefore be selectively damaged. Early hearing is prerequisite for speaking and for intellectual, social and emotional development.172 Even a relatively mild hearing loss of 35-40 dB near hearing level means that a child misses approximately 50% of normal conversation.173 Severe congenital hearing impairment affects 0.1% of live-born infants104,174 and 1-4% of graduates of neonatal intensive care units (NICU’s).174,175 The prognosis is improved when the diagnosis is made early.176 The fitting of hearing aids within the first 6 months improves speech and language development compared with placement at a later age. However, the age at diagnosis of hearing impairment is 18-30 months,177 even in countries with a nationwide behavioural screening programme starting at 9 months of age. The American Joint Committee on Infant Hearing proposed risk criteria for hearing loss in the neonatal period (Table 1). Using high-risk registers 50-75% of infants with hearing loss are identifiable.178 Based on long-term cost-efficiency studies, the Joint Committee concluded in 1993 that all infants admitted to NICU’s should be screened prior to discharge and universal screening should be implemented for all infants within the first 3 months of life.179 Similar efforts are being made in Europe.180

Electrophysiological measurements for screening
The conventional auditory brainstem response is considered to be the gold standard in detecting the neonatal hearing threshold,105,181-182 though it is not yet widely used because it is cost- and time-consuming.
An automated version (ALGO-1 automated auditory brainstem response infant-hearing screener) has been introduced as an alternative. Based on a statistical model for the detection of a response (auditory brainstem response algorithm) and a dual artefact rejection system for environmental noise and myogenic artefacts, the equipment provides a pass/fail outcome. Stimulation via headphones with 35 dB near hearing level clicks with frequencies of 700-5000 Hz can be performed even under NICU conditions. No special training is necessary. Clinical trials proved the concordance between an automated version and conventional auditory brainstem response screening, whereby a sensitivity of 100% and a specificity of 98.7% was detected. Follow-up results support the value of automated auditory brainstem response screening. The mean time needed to perform a screening has been reduced from 20 to 8 min. To avoid ambiguous results in preterms, screening is advised from a post-conceptional age of 30 weeks onwards. In transiently evoked otoacoustic emissions (TEOAE) measurements, a "pass" has been defined as the presence of emitted energy of at least 3 dB signal/noise ratio between 1.6 and 4 kHz, but until now no objective pass/fail criteria have been established. The response is frequency specific and does not provide an indication of audiometric threshold. Depending on the stimulus, TEOAE can be detected in up to 98% of humans with normal hearing and are absent in hearing impairment of 20-40 dB. TEOAE takes 7-9 min and can be administered by a trained nurse. In NICU infants screened with TEOAE at 3 months, there is a sensitivity of 93% and a specificity of 84% to detect absent auditory brainstem response. Hypoxia or infection may result in a reversible reduction of the TEOAE spectrum. Children with hearing loss primarily due to involvement of the auditory pathway or CNS may have normal evoked otoacoustic emissions as a result of a normal functioning cochlea. Otoacoustic emissions (OAE) screening is less suitable for use on a NICU because of the ambient noise and the difficulty of placing the ear probe in preterms. With future research and automated analysis, OAE will become increasingly available for hearing screening, especially in term neonates.

**Behavioural screening**

Behavioural hearing screening methods such as the auditory response cradle and the crib-o-gram have been developed for term infants. Although easy to perform, they are not suitable for screening preterm or sick neonates and detect only severe hearing impairment. The sensitivity (75%) and specificity (71%) are too low compared to neurofunctional methods. Behavioural observations remain the basis to the investigation of the harmful influences of high-intensity noise in neonatal intensive care units: prematures wearing earmuffs spent more time in quiet sleep states and had higher mean oxygen saturation levels compared to controls. Information about pediatric auditory research projects is available on Internet.
Fetal Hearing

Possible damage from environmental hazards during pregnancy
Children who have been exposed in utero to vibroacoustic stimulation for the assessment of fetal well-being have been evaluated at 4 years of age by auditory tests of 20-25 dB at frequencies between 500 and 8000 Hz in Sweden (n=460)\textsuperscript{195} and the USA (n=465).\textsuperscript{196} No hearing damage or neurodevelopmental abnormalities that could be connected to the vibroacoustic stimulation were found. Fetal noise-induced hearing loss has been a matter of concern regarding the working and living conditions of pregnant women.\textsuperscript{197,198} Epidemiological and animal research suggest that noise can adversely change fetal hearing; however, studies in humans still lack control groups. Auditory brainstem response changes were examined in sheep suggesting that noise sources with low-frequency components and high-intensity impulses have temporary effects on auditory brainstem response waves, whereas long-term effects are still unknown.\textsuperscript{199} In summary, the Committee on Hearing, Bioacoustics and Biomechanics,\textsuperscript{200} attempting to protect fetal hearing, suggested that pregnant women should avoid noise exposures greater than 90 dB.

2.9 Direction of future research

The phenomenon of prenatal perception and the implications for future life represent a wealth for ongoing and future research.

Methodological aspects

Prenatal hearing reflects the complex neurological development of the early brain. Evaluation of fetal brain function is one of the most important objectives of basic and clinical research in perinatology. Imaging of the fetal brain only demonstrates a small part of its function and most functional approaches have been indirect. The introduction of magneto-encephalography in perinatal medicine was a pioneer step to evaluate prenatal brain function directly,\textsuperscript{79,80} though the single-channel magnetometer did not allow high-quality auditory-evoked signals. By using multichannel magnetoencephalography\textsuperscript{201} or detectors specifically designed for the prenate, the window of observation to early gestational age and various indications might be expected to be extended. The method might be used in newborns: examinations in twins would enhance our knowledge about hereditary or environmental influences.\textsuperscript{202} The sophisticated method might have implications for prenatal neuroscience and clinical use. Prenatal training of laryngeal co-ordination is likely to occur during mouthing or breathing movements. Doppler combined with real-time ultrasound examinations might elucidate how far this is performed at a special rhythm. Correlations with postnatal cry spectroscopy might be of interest.
Aspects of using early sensation for developmental support

Health is understood as the physical, mental and social well-being that goes further than just the absence of illness. As important as it is to teach children abilities at certain times creating integrated stimulations for the unborn might favour development or prevent sensory retardation. This should be a matter of concern of public health projects. In Venezuela a programme was introduced including weekly lectures to pregnant mothers about adequate stimulation and nutrition. Significant improvement was found at the 2nd and 25th days in orientation and autonomic stability of the infants in the experimental compared to the control group. Perinatal auditory stimulation programmes need the knowledge of critical developmental phases. Pilot studies have demonstrated an immediate reduction of fetal breathing and an increase of FM when mothers only listen to a preferred type of music via earphones, or a reduction of fetal activity if fetuses only listen to rhythmic music. The first study is a proof that integrated programmes for mother and fetus may even be regarded as conditioning (e.g. "music, mother relaxed"). Is the unborn more affected by the mother’s preferences of listening -the same holds true for singing and talking to the unborn- than by the sound alone? Postnatal conditioning has a larger scale, e.g. sound stimulations recalling intrauterine noise can not only lull the newborn to sleep, but also serve as a reinforcer during sucking. Similarly, feeding may be enhanced by background music. The perception of music as pleasant might even be a process of conditioning from the parents. Singing lullabies and simultaneously rocking cradles have been used to soothe babies by simultaneous stimulation of the vestibular and acoustic sensory system. The experience of rhythmic stimulations by a "breathing teddy bear" adapted to the child’s individual breathing frequency has proven to facilitate neurobehavioural development. Further studies relating to supportive expression of our genetic potential, prevent unnecessary illness and to detect hearing loss perinatal conditioning are necessary.

Aspects for pathological development

Working groups in Western countries are planning protocols for the evaluation of early hearing screening. Thereby it is a major goal of Maternal Child Health grant in the USA to establish universal neonatal screening, to identify specific hearing loss by 3 months, to institute intervention by 6 months and to measure the impact of early identification on developmental outcome. Average costs were given as $25 per test, and as $43785 to identify a child with sensorineural hearing loss. Considering the given incidence of hearing impairment further programmes are needed. Future research of the use of acoustic therapy and shelter may provide measures positively to alter the critically ill newborn’s environment.
2.10 Conclusion

Encouragement and avoidance of sound have to be considered and balanced in future analytical and interventional projects according to critical developmental phases. Whether we go so far as to found prenatal universities, as in California, is of secondary importance as long as we strive to understand the physiology and pathophysiology of early hearing and to create designs of adequate stimulation suitable to induce a comprehensive as early as possible.
2.11 References


Fetal Hearing

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Chapter 2


