Fetal learning and memory

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We used fetal habituation to repeated vibroacoustic stimulation to assess fetal memory. After the initial stimulus, we assessed the fetuses 10 min later and again after 24 h. 16 of 19 fetuses habituated rapidly to the stimuli at 10 min (p=0·004) and 24 h (p=0·042) after the initial test. We conclude, therefore, that fetuses are able to learn: they have a short-term memory of at least 10 min, and a long-term memory of at least 24 h.

For many years, babies have been believed to be born without a functional memory. However, several studies on the memory of neonates that tested the infants after birth have claimed to show that infants are able to memorise acoustic stimuli in utero.1 However, whether these studies prove the existence of fetal memory is not certain, because no measurement of response was done in utero.

Our aim was to test fetal learning and memory. We used observations of fetal habituation to repeated vibroacoustic stimuli at various timepoints to study the fetal memory of the stimuli. Habituation is the decrease, and eventual cessation, of the response of an organism to a repeated application of the same stimulus. The ability to recognise and ignore harmless repeated stimulation is essential for individuals to function in a given environment. Habituation is thought to be based on learning in the same way that learning is based on memory.

We studied habituation to repeated stimulation in 25 healthy term fetuses at three timepoints. After the initial test, we tested the fetuses 10 min later and again after 24 h. All habituation tests were done under the same conditions (the mother was not allowed to smoke, drink coffee, or eat for 3 h before testing), in the same room, and by the same examiner.

The stimuli, produced by a fetal vibroacoustic stimulator (Corometrics model 146, Wallingford, CT, USA; audible sound 20–9000 Hz, vibrations 67–83 Hz, sound level 74 dB at 1 m in air), were repeatedly applied to the maternal abdomen above the fetus's legs for a period of 1 s every 30 s. The fetus's trunk was displayed by a real-time ultrasound scanner.

A general movement of the fetus's trunk within 1 s of application of the stimulus was defined as a positive response. Lack of response to four consecutive stimuli indicated habituation. After fetal habituation, stimulation was stopped. We allowed a maximum of 24 stimulus applications in each test. However, a minimum of four extra stimuli were necessary to show habituation if the fetus responded to the 21st stimulus. We therefore decided to stop stimulating if a fetus still responded to the 21st stimulus. The habituation rate was defined as the number of stimuli applied before a fetus stopped responding.

Fetal heart rate was recorded by cardiotocography, starting 10 min before the tests and continuing for 10 min afterwards.

The procedure was repeated at 10 min and 24 h after the initial series of stimuli.

The study was approved by the ethics committee of the University Medical Centre Nijmegen and all participants gave their written informed consent. Data from the three tests were compared by Friedman repeated measures ANOVA. If ANOVA indicated significance, the intermediate variances were analysed by Wilcoxon matched-pairs signed-rank test. Differences were considered significant at p<0·05.

Six fetuses were excluded from analysis because irregular movement responses to the stimuli prevented interpretation of at least two out of three tests. In two of the remaining 19 fetuses, habituation could be determined in only two tests. Data from the 17 fetuses who had results for three tests are shown in the figure. The Friedman test showed significance at p<0·001. 10 min after the initial test, 16 of 19 fetuses habituated more rapidly to stimuli (p=0·002) than to the initial series or did not respond at all (which probably indicated immediate recognition of the stimuli). Two fetuses habituated more slowly and one fetus had a persistent response in both tests. 24 h after the initial test, 16 of 19 fetuses habituated more rapidly than to the initial series on the first day, two had persistent responses on both days, and one habituated more slowly (p=0·042). Three of these 16 fetuses had persistent responses on the first day, but all three habituated 24 h later. The observed baseline fetal heart rate and fetal heart rate pattern 10 min before the tests did not affect the habituation rate.

Compared with the initial habituation test, fetuses not only habituated more rapidly 10 min later, but also after 24 h. We therefore conclude that fetuses have a short-term memory of at least 10 min and a long-term memory of at least 24 h.
Fetuses are able to memorise the stimuli, although they may need more than one stimulus to establish recognition.

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Prenatal DNA diagnosis of a single-gene disorder from maternal plasma

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Achondroplasia is a short-limb disorder caused by a point mutation in a single gene. To diagnose such a disorder prenatally requires the use of invasive procedures such as amniocentesis. However, using PCR and restriction fragment length polymorphism analysis, we were able to detect the mutation in the plasma of a woman carrying a fetus suspected of having achondroplasia. The detection of a fetus-derived mutant gene from maternal plasma may therefore permit non-invasive prenatal diagnosis of single-gene disorders.

Prenatal DNA diagnosis is usually done via invasive procedures such as amniocentesis and chorionic villus sampling. Fetal nucleated erythrocytes in maternal blood have been proposed as potential target cells for non-invasive prenatal diagnosis. However, the presence of fetal nucleated erythrocytes in maternal blood is extremely rare, and an efficient method for recovering fetal cells from maternal blood has not yet been established. Since 1997, detection of fetal cell-free DNA in maternal plasma and serum has been done by assessment of Y-chromosomal sequences in pregnant women bearing male fetuses, or the rhesus-factor gene in rhesus-negative pregnant women. Therefore, maternal plasma may have the potential to provide non-invasive prenatal diagnosis of single-gene disorders in which the mother does not have genomic alterations in the target sequence.

Although some short-limb disorders are suggested by routine ultrasonography during pregnancy, a definitive diagnosis as to whether or not the disorder is fatal is impossible. Achondroplasia is the most common genetic form of dwarfism and is inherited as an autosomal dominant disorder, although most of the cases are sporadic. DNA analysis has revealed that more than 90% of achondroplasia patients have the same mutation—a G-to-C transition at the same nucleotide. Both mutations result in the substitution of an arginine for glycine at position 380 in the transmembrane domain of the mature protein. The next most common mutation is a G-to-A transition at the same nucleotide. The analysis revealed that DNA from maternal plasma and amniotic fluid were collected at 30 weeks of gestation. DNA was extracted by conventional methods from 2 mL maternal plasma and 3 mL amniotic fluid. Maternal DNA was also obtained from maternal leucocytes to exclude the possibility of maternal inheritance. The segment of FGFR3 containing the nucleotide at which the achondroplasia mutation occurs (nt 1138) was amplified by PCR with specific primers. Restriction fragment length polymorphism analysis of PCR products was done with SfiI. The achondroplasia mutation creates an SfiI restriction site such that, upon digestion, two extra bands (of 111 bp and 55 bp) are seen.

The analysis revealed that DNA from maternal plasma and amnion cells contained the mutant allele (figure). No mutation was found in maternal leucocytes, suggesting that the mutant allele in maternal plasma DNA originated from the fetus. Direct sequencing of amnion-cell DNA confirmed the presence of a G-to-A transition at nt 1138. At 40 weeks of gestation, a girl was born by normal vaginal delivery (birthweight 2834 g, Apgar score 9/10). A radiograph of the infant showed a slight shortness of limbs.

We have successfully carried out a non-invasive prenatal DNA diagnosis of a single-gene disorder, achondroplasia, from maternal plasma. The detection of a fetus-derived mutant gene from maternal plasma could also be used in the prenatal diagnosis of single-gene disorders caused by paternally inherited genes or mutations that are distinguishable from maternally inherited ones.