Research report

Fetal habituation correlates with functional brain development

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Abstract

In this study, we divided 26 fetuses at 32–37 weeks of gestation into three groups using combined criteria of gestational age and behavioral indicators. We investigated fetal habituation to vibroacoustic stimulation (VAS). Fetuses showed habituation from at least 32 weeks of gestation. Furthermore, fetuses less developed from behavioral standpoint took significantly more trials to achieve habituation than developed fetuses even in the same gestational age. Taken together with these data, it was proved that there was a relationship between aspects of behavioral development and habituation.

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1. Introduction

Habituation is defined as “the decrement in response following repeated stimulation with the same stimulus” [28]. It is considered to be a basic form of learning, requiring an intact central nervous system (CNS) [19]. Several studies on habituation in normal human fetuses have shown that fetuses are able to habituate to stimulation [9,22,24,25,29]. However, there are few studies investigating gestational changes of habituation with regard to neurological development. Kuhlman et al. [22] defined habituation as “the cessation of all components of the startle reaction, except eye blinking, over two sequential stimuli,” and found that the mean number of stimuli required to habituate decreased in accordance with advancing gestational age. Groome et al. [9] scored the fetal response according to the nature of the fetal movement and found that the change in the rate of habituation occurred with changing gestational age. By comparison, Madison et al. [25] could not find any effect of gestational age on the rate of habituation. Thus, it is controversial whether the rate of habituation changes with gestational age.

Based on the idea that every fetal behavior could be a manifestation of the central nervous system, we have described the ontogeny of fetal behavior through observing the developmental process of each movement with advance in gestational age. In particular, eye movement pattern sensitively reflect the activity of the neural control system and the relationship to other behaviors give information on the integration of central nervous system function [2,27]. As a result, we have shown that the development of fetal behavior correlates well with that of CNS function [15,16,18,21]. Furthermore, these findings also indicated that it is possible to some extent to identify the degree of localization of functional brain impairment prenatally [17]. By assessing fetal behavioral patterns at term in gestation, it is now possible, with considerable certainty, to identify prenatally pathological lesions as long as they are localized caudal to the pons-medulla. However, if the lesions are localized in the cerebral hemisphere(s), such fetuses did not indicate any abnormal behavior patterns, as determined by using conventional parameters, during the intrauterine period [17]. In other words, it is still impossible to evaluate higher CNS function of the fetus, including that of the cerebral hemispheres, in utero.

Impaired habituation has been reported in fetuses with Down’s syndrome, in brain-injured neonates [5,6,12–14],
and in adults with disorder such as schizophrenia, mental retardation, and depression [7,11,23]. Therefore, it could be assumed that habituation may reflect higher CNS function and be used as a new parameter for fetal assessment. However, there is no research that has studied the relationship between CNS functional development and habituation. The aim of this study is to elucidate the relationship between habituation and neurological development evaluated by fetal behavior in the human fetus.

2. Materials and methods

2.1. Fetal population

Included in this study were 27 normal singleton fetuses between 32 and 37 weeks of gestation. The study was approved by the Ethical Committee of Kyushu University Medical Sciences. All mothers gave written informed consent to participate in the study. The women were followed in the Maternity Care Unit of Kyushu University Hospital. They had no medical or obstetric complications and no use of alcohol, drugs, or medication other than vitamins and/or iron.

The period of gestation was calculated from the date of the last menstrual period and was confirmed by serial ultrasonographic measurements of crown-rump length before 11 weeks of gestation. After birth, none of the neonates were found to have any abnormalities. All had birth weight above the 10th percentile.

2.2. Procedure and data analysis

2.2.1. Definition of fetal behavioral development

The mothers were placed in the semi-recumbent position on a bed. We observed fetal behavior to evaluate fetal CNS functional development using real-time ultrasound (ALOKA SSD-5500, Tokyo, Japan). For the ultrasound observation of eye and mouth movements, the standard plane was determined to be the coronal facial view [16]. The lens could be detected as a ring-like, rounded echo, originating from the margin of the lens, and was used as a landmark for detecting eye movement [18]. The ultrasound image was stored by video cassette recorder (SONY model WV-DR9, Tokyo, Japan).

To define the developmental stage of fetal CNS function, we determined three behavioral indicators: alteration of the eye movement and no eye movement periods (EM/NEM) [21], rapid (REM) and slow eye movement (SEM) [15], and concurrence of regular mouthing movement (RMM) in the NEM period [16]. A detailed account of the indicators is given below.

2.2.1.1. EM/NEM. The fetal eye was continuously observed for 60–90 min. A single eye movement was defined as that movement from one position to another. With the total observation period being divided into one minute epochs, alteration of the eye movement and no eye movement periods are defined as those having periods, sustaining the presence or absence of eye movement, emerging alternately. The 25–75% ranges for the duration of the EM and NEM periods are 2–30 and 2–25 min, respectively [21].

2.2.1.2. REM. While replaying the video tapes using the slow motion and frame freezing modes, we measured the duration the duration of each eye movement unit, in seconds, by subtracting the frame code at the onset from that at the end of each eye movement unit.

REMs and SEMs were defined as those having durations of less than, and equal to or more than, the critical value of 0.6–0.8 s, respectively. REMs coexisting with SEMs are observed in the EM period [15].

2.2.1.3. RMM. The mouth movements were defined as one unit of mouthing movements, in which the mouth opened and returned to a closed position. Repetitive mouthing movements are observed at a regular interval of 300–600 ms during the NEM period [16].

2.2.2. Fetal developmental groups

Based on gestational age at the time of testing, we divided the fetuses into two groups; 32–34 weeks of gestation, and 35–37 weeks of gestation, because it is known that the neurologically normal fetus shows RRM at or above 35 weeks of gestation [17]. Furthermore, we classified the fetuses in terms of the positive or negative of three behavioral indicators; EM/NEM, REM, RMM. Using these criteria, fetuses were classified into three groups (Table 1).

2.2.3. Fetal habituation

Vibroacoustic stimulation (VAS) was presented to the fetus. The stimulus was produced by a commercially available vibroacoustic stimulator (Corometrics Model 146; Wallingford, CT, USA; manufacturer specifications: 75 ± 10%, 74 dB at 1 m in air). Each fetus was tested only once. All studies were performed between the hours of 2 p.m. and 4 p.m. Stimuli of 0.3 s duration were repeatedly applied every 10 s to the maternal abdomen above the fetal hip. The timing of the first stimulus was chosen to occur when the fetus was not moving. Fetal movements were observed using real-time ultrasound (ALOKA SSD-5500, Tokyo) with

<table>
<thead>
<tr>
<th>Group</th>
<th>GA in weeks</th>
<th>Number of subjects</th>
<th>EM/NEM</th>
<th>REM</th>
<th>RMM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>32–34</td>
<td>8</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>2</td>
<td>32–34</td>
<td>9</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>3</td>
<td>35–37</td>
<td>9</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
</tbody>
</table>

GA: gestational age. See text for abbreviations.

Table 1

Definition of fetal population divided by gestational age and fetal behavioral indicators.
Table 2
Characteristics of the 26 fetuses

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case (n)</td>
<td>26</td>
<td>8</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Gestational age at delivery (weeks)</td>
<td>39.4 (37–41)</td>
<td>39.4 (37–41)</td>
<td>40.3 (38–41)</td>
<td>39.3 (38–41)</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3158 (2544–3808)</td>
<td>3040 (2696–3694)</td>
<td>3380 (2728–3808)</td>
<td>2940 (2750–3670)</td>
</tr>
<tr>
<td>Sex (male/female; n)</td>
<td>10/16</td>
<td>3/5</td>
<td>3/6</td>
<td>4/5</td>
</tr>
</tbody>
</table>

Data are given in medians and ranges.

A 5.0-MHz convex transducer positioned to provide the best possible view of the upper torso, at least one extremity, and the fetal head. Movements of the fetal trunk, head, or limb within 1 s of application of the stimulus were considered to be a positive response. A lack of response to five consecutive stimuli indicated habituation [26]. The habituation rate was defined as the number of stimuli required to produce extinction of the response. Each study was recorded on videotape for later analysis. Typically, the fetal reaction to the initial stimulus was a rapid, intense full-body startle response involving extension of the extremities, trunk, and neck.

2.2.4. Statistical analysis
Each group was compared using the one-way functional ANOVA and multiple comparison test (post hoc tests using Scheffe test). Differences were considered significant at $P < 0.05$.

3. Results
Twenty-six of 27 fetuses showed a startle response to the VAS. Only one fetus (3.7%) examined at 37 weeks of gestation did not respond to the first stimulation. This fetus was excluded from this study.

All fetuses showed EM/NEM and REM SEM. Among the fetuses under 34 weeks of gestation, some fetuses showed RMM while others did not. Group 1, Group 2, and Group 3 contained eight, nine, and nine fetuses, respectively. Thus, there were two different developmental stages in the 32–34 weeks gestational group (Table 1). Clinical characters in each group are shown in Table 2. Fetus in each group did not have any difference in gestational age at delivery, birth weight, and sex.

All fetuses habituated at 2–24 stimuli. There was a significant difference in the habituation rate of each group ($F(2, 23) = 7.01, P < 0.01$); fetuses of Group 1 took longer to habituate, i.e., required more stimuli than fetuses of Group 2 and Group 3 (Fig. 1). Moreover, there was a significant difference ($P < 0.05$) in Group 1 and Group 2, and in addition, to Group 1 and Group 3. However, there was no significant difference in gestational age between Group 1 and Group 2.

4. Discussion
We classified 26 fetuses into three groups with combined criteria of gestational age and behavioral indicators. We investigated fetal habituation to repeated VAS in 32–37 weeks of gestation and showed that the fetuses habituated at least at 32 weeks of gestation. Furthermore, fetuses of Group 1 took significantly more trials to produce habituation than fetuses of Group 2 and Group 3. Taken together with these data, it was proved that there was a relationship between behavioral development and ability to habituate, that is, between the CNS development and habituation.

We have shown that each of the behavioral indicators in previous studies has its own developmental characteristic along with advancing gestational age [15,16,18,21]. EM/NEM remain constant until 29–32 weeks’ gestation. This suggests that the neural center of EM/NEM alternation mechanism, located from the medulla oblongata to the pons, begins to function at 29–32 weeks’ gestation [8,18,21]. REM SEM becomes apparent by 33 weeks’ gestation at the latest [15]. This suggests the start of REM sleep and its related neural mechanisms, including the locus coeruleus in thepons [2]. In this study, all fetuses showed EM/NEM and REM. This suggests that the CNS was functioning up to the pons. In addition, RMM are found at the latest by 35–36 weeks’ gestation [16], thereby demonstrating functional maturation of the neural area rostral to the pons through the thalamocortical connection to the cerebral hemisphere, implying the neural center related to NREM sleep [30]. Horimoto et al. [16] showed that from 32 to 34 weeks of gestation, the patterns of mouting movement showed a transitional stage between former and later periods [15]. In...
Acknowledgements

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References


