control observers on redundant-3 condition trials would consist of three items, whereas that on mixed condition trials would consist of four items (*9*).

We conclude that V.P.'s sensitivity to a difficulty manipulation in the unprobed field reflects competition between the hemispheres for a shared pool of resources—resources that can be utilized by either hemisphere and, to the extent that the hemispheres do not have access to a common data base, that are not specialized for individual processing structures. In addition, these resources must either reside in subcortical structures or be transferred between the hemispheres via subcortical pathways, since the principal pathway for interhemispheric communication in this patient has been sectioned. If resources are distributed interhemispherically by, say, the anterior commissure, which was not sectioned, there must be appreciable latitude with which resources that are committed to one structure can be shared with another (*4*, *10*). Although our findings imply that processing resources can be distributed among different processing structures, it remains to be determined whether competition between tasks for a common structure is a consequence of time-sharing processing structures, or whether it reflects the limits in dedicated resources that subserve specific cognitive operations.

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6. For review, see M. S. Gazzaniga, *The Bisected Brain* (Appleton-Century-Crofts, New York, 1970).
7. Of the remaining commissurotomy patients with right-hemisphere language who were available for testing, P.S. was not tested because he has demonstrated the capacity to transfer linguistic information paracallosally [M. S. Gazzaniga *et al.*, *Brain* **105**, 53 (1982)], whereas J.W. had insufficient bilateral language capacity to perform the present task in the right hemisphere [J. J. Sidtis *et al.*, *J. Neurosci.* **1**, 323 (1981)].
8. A comparison of the accuracy data for each control observer and V.P. [B. J. Winer, *Statistical Principles in Experimental Design* (McGraw-Hill, New York, 1971), pp. 855–859] revealed a significant subject by condition interaction [$\chi^2(1) = 7.05$ and 17.9, $P < .01$ and $P < .001$], thus providing statistical confirmation that the redundant-3 and mixed-3 conditions had contrasting effects on the control observers and V.P.
9. An additional study confirmed that visual information lateralized to one hemisphere of V.P. does not interact with information lateralized to the other, whereas control observers combine information from the two fields. The only difference in this study was that the mixed condition consisted of a different series of three items displayed in each field, for a total of six items. In all other respects, the two studies were identical. Results of 192 trials were collected from each observer. As expected, the accuracy and average latency of V.P.'s responses in the redundant-3 condition, 73 percent correct and 1694 ± 71 msec, were comparable to those obtained in the mixed condition, 77 percent and 1860 ± 100 msec [$\chi^2(1) = .44$, not significant; $t(142) = 1.35$, not significant]. In contrast, the control observers made fewer errors in the redundant-3 condition, 96 percent versus 80 percent correct in the mixed condition, and responded faster in the redundant-3 condition, 903 ± 24 msec versus 1180 ± 44 msec in the mixed condition [each subject analyzed separately: $\chi^2(1) = 5.69$ and 16.55, $P < .05$ and $P < .001$; $t(178) = 6.81$ and $t(154) = 5.04$, $P < .001$]. In both studies, then, V.P.'s performance was virtually unaffected by interfield redundancy, whereas each control observer performed better when interfield redundancy reduced the total number of items to be remembered.
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Human Fetal Movement: Spontaneous Oscillations Near One Cycle per Minute

Abstract. *Spectral analysis of spontaneous fluctuations in human fetal movement revealed strong oscillations at frequencies between 0.24 and 0.90 cycle per minute, which are much higher than those of the cyclic alternation of quiet and active states in the fetus and neonate. Oscillations at frequencies up to 2.88 cycles per minute were also detected, but they were usually much weaker. The prominent peaks in the fetal movement spectra are in the frequency range of recently reported neonatal motor rhythms, and indicate the existence of a cyclic process controlling spontaneous motor output that oscillates near one cycle per minute and begins to function in utero.*

The brain of the full-term human neonate provides rhythmic or cyclic control of a number of behavioral and physiological processes, such as respiration and the alternation of quiet and active sleep states (*1–4*). These rhythms are unstable or nonexistent in neonates born very prematurely, and may be disrupted by central nervous system (CNS) pathology in the full-term newborn (*5*). The function of some rhythms, like respiration, is relatively clear. Others are less well understood. For example, the function of human sleep-state cycles, which may be a manifestation of a basic rest-activity cycle (*6*), is not known. However, studies of fetal behavior and physiology suggest that major rhythms such as respiration and activity cycles are present in utero, although their mature form may be established only after birth (*7*).

Recently, evidence was found for another rhythmic process in the full-term newborn (*8*). Fluctuations in spontaneous body movement, the dominant motor output of the newborn infant, were previously thought to be without temporal organization. However, spectral analysis revealed periodicity in the fluctuations of movement, with a cycle length on the order of 1 minute. This characteristic frequency is much higher than that of the sleep states (almost hourly), but much lower than frequencies of respiration or sucking.

To determine whether the cyclic fluctuations in body movement observed in the full-term neonate are present in utero or appear only as a consequence of the major physiological changes that accompany birth, we studied fetuses in the last few weeks of gestation. Spectral analysis of the data for each fetus revealed strong oscillations in movement. Dominant frequencies were between 0.24 and 0.90 cycle per minute, or within the range of neonatal motor rhythms previously reported (*8*).

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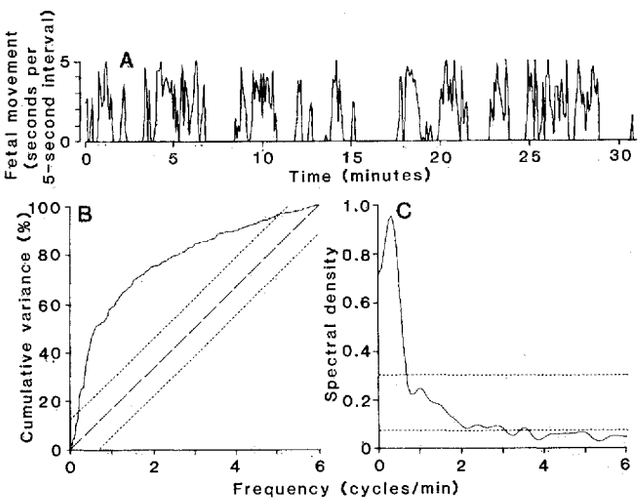

Fig. 1. Frequency analysis of fetal movement. (A) The amount of time during which fetal movement occurred in each 5-second interval in a 31-minute record. (B) Cumulative variance distribution derived from the Fourier partitioning of the time-based data into independent (harmonic) components in the frequency domain. The dashed line is the cumulative variance distribution of a theoretical white noise process. The dotted lines are the 99 percent confidence limits for the maximum deviation of sample data from the theoretical distribution, if the process generating the sample data is white noise. (C) Smoothed spectral density function computed from the time-based data. Smoothing was done with a Tukey window (*13*) with a bandwidth of 0.44 cycle/min. A linear trend, which accounted for 0.05 percent of the total variance, was removed before the spectrum was computed. The broken lines are the 99 percent confidence limits on the spectral estimates of a white noise process.

Polygraphic recordings obtained from 12 women in the last 2 weeks of gestation were examined for evidence of fetal activity. The pregnancies represented a range of obstetric risk, with Hobel antenatal risk scores (*9*) between 0 and 55 (mean ± standard deviation, 21 ± 18). Fetal (postmenstrual) ages at the time of study ranged from 38 to 42 weeks (39 ± 1 weeks). The fetuses were born between postmenstrual weeks 39 and 42 (40 ± 1 weeks) and had birth weights between 2.75 and 3.75 kg (3.33 ± 0.29 kg), appropriate for the gestational ages involved. None of the newborns had any abnormalities on physical examination.

Data collection began approximately 2 hours after the mother's morning or noon meal. Mothers were asked not to smoke or to drink beverages containing caffeine on the day they were studied, and none were taking medications other than vitamin and mineral supplements. During the 2- to 3-hour recording sessions the mothers rested in a reclining position, tilted slightly to minimize the possibility of maternal hypotension. Fetal movement was detected with two strain gauges located on the mother's abdomen over the upper and lower portions of the fetus, as determined by palpation. This technique has been validated by simultaneous ultrasonic imaging of fetal movement (*10*). The output of the two transducers, in addition to fetal electrocardiogram and event signals controlled by the mother and a technician, were recorded on paper at 5 mm/sec (*11*).

The complete analog recordings were later scanned for periods of fetal activity. Extended portions (20 to 40 minutes) of the recordings were identified that contained fetal movement and were free of major artifacts due to maternal body movement, uterine contractions, or misadjustment of recording equipment. For each fetus, the longest period that best satisfied these criteria was selected for analysis. The mean length of the resulting 12 records was 30.2 ± 7.7 minutes.

Individual records were then analyzed for the occurrence of fetal movement by comparing the outputs of the two strain gauges. Fetal body movement was inferred when the otherwise synchronous variation in the two signals (due to maternal respiration) was perturbed and the perturbation could not be attributed to fetal respiratory movements or maternal pulse (both are repeated, small deflections), or to sources such as maternal body movement or coughing (as noted by the technician). During periods when minor recording artifacts made it impossible to compare the output of the two strain gauges, fetal movement was inferred from the coded observations of the mother and technician [between 0 and 7.7 percent (mean, 0.9 ± 2.1 percent) of the movement was inferred this way]. The records were then digitized in successive 5-second intervals by measuring the amount of time (to the nearest 0.2 second) during which fetal movement occurred in each interval. These measurements constituted the raw data for the subsequent analyses (Fig. 1).

The data on the movement of each fetus were analyzed in two stages (Fig. 1) (*8*). In the first stage the time-based movement data were subjected to Fourier analysis to partition the total variance into independent components in the frequency domain. The cumulative variance distributions derived were compared to the theoretical cumulative distribution of a white noise process by means of a Kolmogorov-Smirnov test (*12*). For each fetus, the cumulative variance distribution departed significantly ($P < .001$) from that of white noise. These analyses indicate that the fluctuations in fetal movement detected during 20- to 40-minute periods of relative activity were not random, but were temporally organized.

The dominant motor rhythms of each fetus were isolated in the second stage of the analysis. For each fetal movement record, a smoothed spectral density function (power spectrum divided by total variance) was computed to obtain statistically stable estimates of the relative strength of periodic fluctuations occurring at different frequencies (*13*). The bandwidth of the spectral window was 0.44 cycle/min. With a sampling interval of 5 seconds in the time-based data, the maximum frequency that could be detected (the Nyquist frequency) was 6.0 cycle/min. Linear trends in the data, which accounted for 0 to 8.8 percent (mean, 1.4 ± 2.5 percent) of the variance, were removed before the movement spectra were computed.

For all 12 fetuses, relatively narrow frequency bands accounted for large proportions of the total variance. In 11 of the movement spectra there was a single prominent peak that exceeded the 99 percent confidence limits of a white noise process. The presence of a strong peak in each movement spectrum reflects substantial rhythmic modulation of spontaneous motor output in these fetuses. The frequency of the spectral peaks ranged from 0.24 to 0.60 cycle/min (0.39 ± 0.11 cycle/min), and their width at half-maximum was 0.52 to 1.18 cycle/min (0.79 ± 0.27 cycle/min) (*14*). The relative magnitude of the peaks and the frequency at which they occurred were not linearly related to overall activity, but there was a moderate linear dependence ($r = .69$, $P < .05$) between the frequency of the spectral peaks and the length of the records. This correlation is difficult to explain, but could result if the frequency of the dominant rhythm is related to maturational differences, since the duration of active periods tends to increase during fetal development (*15*). However, there was no relation between frequency of the spectral peaks and fetal age in this sample of near-term fetuses.

Data collected at several points in gestation would provide a stronger test of this hypothesis.

In one fetus there were two prominent peaks in the movement spectrum which were nearly identical in magnitude (*16*). Both exceeded the 99 percent confidence limits of white noise. The low-frequency peak was located at 0.24 cycle/min, the high-frequency peak at 0.90 cycle/min. Digital filtering of the time-based data to remove either peak left the other intact. The intensity (maximum spectral density) of the low-frequency peak increased markedly (95 percent) in the second half of the movement record, while the high-frequency peak was essentially unchanged (intensity decreased 14 percent). These findings suggest the existence of two distinct rhythms in this fetus, in contrast to the other fetuses, in which a single rhythm dominated motor output.

The strong peaks in all the individual movement spectra reflect significant rhythmicity in the spontaneous fluctuations of fetal movement during a period of relative activity lasting 20 to 40 minutes. However, the dominant rhythm, with a cycle of 1 to 4 minutes, did not account for all the variation in movement. Often more rapid fluctuations were apparent in the raw data. Because the presence of strong spectral peaks may obscure weaker peaks at other frequencies, all the records were reanalyzed after removing the dominant rhythms with a digital filter (*17*). Spectral analysis of the filtered data revealed significant concentrations of variance in 11 fetuses (exceeding the 99 percent confidence limits of white noise in nine cases and the 95 percent limits in two), peaking at frequencies between 1.14 and 2.88 cycle/min (mean, 1.77 ± 0.52 cycle/min).

These analyses indicate that in many fetuses there are regular fluctuations in movement occurring at relatively high frequencies. These rapid fluctuations may reflect the tendency for movement to occur in short bursts during a period of increased activity (*18*). The fluctuations are strongly modulated, however, by a slower rhythmic process oscillating at less than 1 cycle/min. The slow modulating process was clearly dominant in all 12 fetuses; it may reflect central oscillations in arousal. The double rhythm in one fetus raises the possibility that there are two sources of control that can function independently. Whether the modulating process is autonomous or is linked to an unmeasured rhythm, it could produce the observed periodicity by triggering surges in motor output or by inhibiting ongoing activity. From the present data it is not possible to distinguish between these fundamentally different mechanisms.

The frequencies of the dominant fetal movement rhythms fall within the range of neonatal motor rhythms reported previously (*8*). This accounts for the similarity of the mean spectral density functions shown in Fig. 2. The similarity of the fetal and neonatal movement spectra obtained in separate studies suggests the existence of a common rhythmic process controlling motor output, which, like the alternation of active and quiet periods, develops prenatally and is not a consequence of the physiological changes that accompany birth or of characteristics of the extrauterine environment. However, the CNS events underlying this rhythmic behavioral activation oscillate on a time scale that is an order of magnitude shorter than sleep-state cycles. Our methods provide a noninvasive means of following this aspect of neurobehavioral organization in the fetus during the last 10 weeks of gestation, when tocodynamometric recording can detect movement. The emergence of rhythmic organization in fetal movement, the developmental changes in the parameters of the movement spectra, and the possible clinical significance of variations in those parameters as manifestations of early or subtle CNS dysfunction remain to be explored.

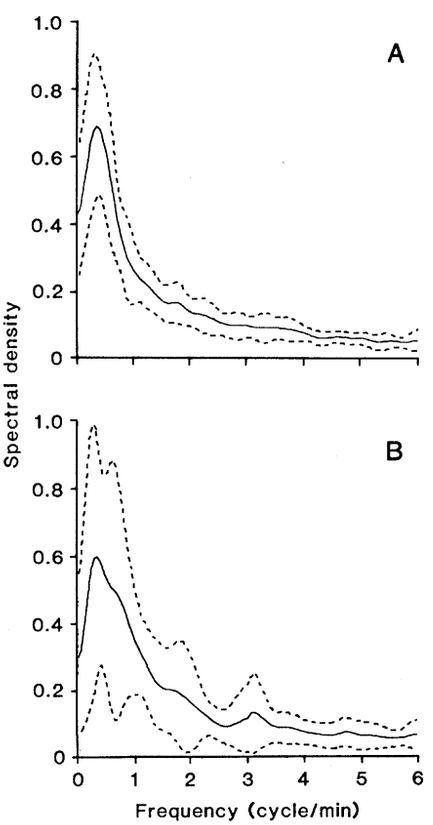

Fig. 2. Average movement spectra, computed by averaging the spectral density estimates of the individual spectra at each frequency. The dotted lines represent ±1 standard deviation. (A) Spectrum for the 12 fetuses studied in the last 2 weeks of gestation and described in this report. (B) Spectrum for 11 neonates, studied during a period of wakefulness 38 to 60 hours after birth (*8*), that were not studied as fetuses.

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10. Y. Sorokin, S. Pillay, L. J. Dierker, R. H. Hertz, and M. G. Rosen [*ibid.* **140**, 456 (1981)] found 87.8 percent agreement when ultrasound and abdominal strain gauges were used simultaneously to detect fetal movements. For 15 patients the mean difference in the number of movements detected by the two methods was 5 ± 6 with 55 ± 36 movements detected by ultrasound and 50 ± 37 movements detected by strain gauge. The correlation between the number of movements detected by the two methods was .989.
11. Details of the method are given by I. E. Timor-Tritsch, L. J. Dierker, R. H. Hertz, I. Zador, and M. G. Rosen, *Biol. Neonate* **36**, 18 (1979).
12. G. M. Jenkins and D. G. Watts, *Spectral Analysis and Its Applications* (Holden-Day, San Francisco, 1968). The Kolmogorov-Smirnov statistic is $|F(f) - \phi(f)|_{max}$, where $F(f)$ and $\phi(f)$ are the observed and expected (white noise) values of the cumulative variance distribution, expressed as a proportion of the total variance.
13. The necessary smoothing operation was done with a Tukey lag window, $w(u) = 1/2\ [1 + \cos(\pi u/M)]$, where u is the lag and M is the truncation point of the autocorrelation function (G. M. Jenkins and D. G. Watts, *ibid.*).
14. In five cases the spectral density did not reach half-maximum before zero frequency. The width of those peaks was taken to be twice the measurable half-width.

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16. The mother of this fetus had the highest antenatal risk score (55). The risk factors included a positive VDRL (serum and cerebrospinal fluid), moderate consumption of alcohol, smoking, and emotional problems. The mother was treated for neurosyphilis 2 months before delivery.
17. A high-pass filter was selected [(*12*), p. 300] so that the frequency response function was < 0.5 at frequencies below the upper half-maximum point on the dominant peak. At the frequency of maximum spectral density on the dominant peak, the frequency response of the filter was between 0.03 and 0.31 (0.16 ± 0.09).
18. The occurrence of movement in bursts is common in the behavior of neonates and young infants, including coordinated motor patterns such as sucking (*3*) and kicking [E. Thelen, G. Bradshaw, J. A. Ward, *Behav. Neural. Biol.* **32**, 45 (1981)].
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30 August 1982

Reassortant Virus Derived from Avian and Human Influenza A Viruses Is Attenuated and Immunogenic in Monkeys

Abstract. *An influenza A reassortant virus that contained the hemagglutinin and neuraminidase genes of a virulent human virus, A/Udorn/72 (H3N2), and the six other influenza A virus genome segments from an avirulent avian virus, A/Mallard/New York/6750/78 (H2N2), was evaluated for its level of replication in squirrel monkeys and hamsters. In monkeys, the reassortant virus was as attenuated and as restricted in its level of replication in the upper and lower respiratory tract as its avian influenza virus parent. Nonetheless, infection with the reassortant induced significant resistance to challenge with virulent human influenza virus. In hamsters, the reassortant virus replicated to a level intermediate between that of its parents. These findings suggest that the nonsurface antigen genes of the avian parental virus are the primary determinants of restriction of replication of the reassortant virus in monkeys. Attenuation of the reassortant virus for primates is achieved by the inefficient functioning of the avian influenza genes in primate cells, while antigenic specificity of the human influenza virus is provided by the neuraminidase and hemagglutinin genes derived from the human virus. This approach could lead to the development of a live influenza A virus vaccine that is attenuated for man if the avian influenza genes are similarly restricted in human cells.*

There is renewed interest in the development of a live attenuated influenza A virus vaccine, since current inactivated influenza virus vaccines do not provide complete protection (*1*) and they do not appear to retain their effectiveness when administered annually (*2*). Live attenuated influenza A viruses have been produced by transfer of genes from an attenuated donor virus to new epidemic influenza A viruses (*3*). Since resistance to influenza A virus is mediated by the development of an immune response to the hemagglutinin and neuraminidase glycoproteins (*4, 5*), live, attenuated, reassortant vaccine strains were selected that derived genes for these surface antigens from the epidemic virus, while the attenuating genes were derived from the attenuated parent. This process of gene exchange is readily achieved with influenza A viruses since they possess a segmented genome consisting of eight negative-stranded RNA segments that code for at least ten proteins (*6–8*). Although genes bearing temperature-sensitive mutations have been transferred to a series of new epidemic wild-type viruses and have rendered such viruses satisfactorily attenuated for man, the genetic instability of the attenuated phenotype represents an insoluble problem (*1, 9, 10*).

There is a need for stable attenuated viruses that are unable to escape their attenuated phenotype. Many of the influenza A virus genes that have evolved over a long period in birds differ significantly in nucleotide sequence from corresponding genes of human influenza A virus (*11–15*). Because of these marked differences, we would expect some avian influenza viruses to replicate inefficiently in human cells and thereby be attenuated. We would also expect such attenuated avian viruses to retain their attenuated characteristics after limited replication in man.

This concept was evaluated initially in squirrel monkeys because these primates develop illness after experimental infection with human influenza A viruses (*16*). Ten avian influenza A viruses were evaluated in squirrel monkeys, and a spectrum of virulence was observed (*17*). Although some of the avian viruses induced illness comparable to that caused by human influenza A viruses (*17*), an avian virus was identified that was infectious but avirulent for the monkeys (*17*). We now report that a virulent human influenza A virus can be attenuated for monkeys by substituting the six nonsurface antigen genes of the attenuated avian influenza virus for the corresponding genes of the human virus. This method of generating attenuated viruses by the production of a reassortant virus containing the hemagglutinin and neuraminidase genes from a human influenza virus and the remaining genes from a nonhuman virus represents a new approach to the production of viral vaccines for man.

Reassortant virus was produced by mating a human influenza A virus, A/Udorn/307/72 (H3N2), with an avian virus, A/Mallard/New York/6750/78 (H2N2), at 37°C in primary chick kidney (CK) cultures (*18*) at a multiplicity of infection of 1. Virus produced by this coinfection was next passaged at 41°C in the presence of antiserums to the avian hemagglutinin (H2) and neuraminidase (N2). Goat antiserum to avian hemagglutinin with a hemagglutination inhibition titer of 1:800 against the avian whole virus, was used at a dilution of 1:400 (fluid overlay) or 1:1000 (agarose overlay). Goat antiserum to neuraminidase, with a neuraminidase-inhibition titer of 1:4000 against the avian virus, was used at this dilution in both fluid and solid overlays. The virus harvested from the passage at 41°C was next passaged at 42°C without antiserum in the fluid overlay. The incubation temperature of 42°C was chosen because avian influenza viruses replicate efficiently at this temperature, whereas human viruses do not (*17*). Progeny virus from the CK cultures at 42°C was next plated on CK cell monolayers with an agarose (0.8 percent) and L15 medium overlay containing antiserums to the avian hemagglutinin and neuraminidase. Plaque progeny were similarly plated at 42°C and then passaged once in CK cells (37°C) maintained with a fluid overlay. Subsequently, virus was grown in 10-day-old embryonated eggs (37°C). Under these selective pressures, we recovered a virus containing the hemagglutinin and neuraminidase genes of the human virus and the six other RNA segments from the avian virus.

Antigenic analysis showed that the reassortant virus contained the surface antigens (H3N2) of the human virus. The origin of the genes at the other six loci was determined by comparing electrophoretic mobility of virion RNA segments of the parental and progeny viruses (*19*). Differences in migration of each of the corresponding genes of the two parental virion RNA's were detected with the following conditions of polyacrylamide gel electrophoresis. For RNA segment 7, a 4 percent acrylamide gel (20 cm) containing 6*M* urea was run

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